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Study of the effect of chromium on the germination parameters of Fenugreek (*Trigonella foenum-gracium* L.) and Lens (*Lens culinaris*)

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Abstract. Soil contamination by heavy metals is a global environmental problem. This contamination affects agricultural crops in the area concerned. In the present study, chromium, which is a heavy metal, is evaluated for its diverse effects on seed germination and lateral growth of fenugreek and lens seeds. A chromium solution was prepared at increasing concentrations: 0, 0.02, 0.04, 0.06, 0.08, 0.1, and 0.2 mg L^{-1} for the addition of germinating seeds in petri dishes for ten days. After two days, the germination rate is calculated. For the following days the length of radicle, stem, and number of leaves are measured. The germination rate of fenugreek varies between 100 and 73.33% for the control and 0.02 mg L^{-1} of chromium respectively. However, the germination rate of the lens varies between 100% for the control and 90% for the 0.02 mg L^{-1}. The elongation of fenugreek radicle with chromium solutions shows a significant effect. However, there is no significant difference in the lens at the different concentrations. For the growth of the fenugreek stalk, it is noticed that the concentration 0.02 shows a length of 2.83 cm compared to their control which is 2.30 cm. Consequently, chromium at 0.02 mg L^{-1} stimulates growth, but at 0.2 mg L^{-1}, it inhibits it. For lens the length of the stems shows also a significant difference compared to their control. So the effect of chromium on germination parameters depends on their concentrations, as well as on the seed response itself. For our research the response of fenugreek compared to the lens at the same concentrations is different.

Key words: chromium, germination, fenugreek, lens, toxicity.

INTRODUCTION

Fenugreek (*Trigonella foenum-graecum* L.) is an annual plant of the Leguminosae family. It is one of the oldest medicinal plants from southeastern Europe and western Asia. It is cultivated in many parts of the world, but the major growing countries are India, Egypt, Morocco, China, Pakistan, Spain, Turkey, and Afghanistan (Madhava Naidu et al., 2011). The key advantages associated with fenugreek are that it can adapt to different environments and growing conditions, also its seeds contain various health beneficial compounds steroidal saponins; diosgenin, yamogenin, tigogenin, and neotigogenin (Ganghas et al., 2021).

Lens (*Lens culinaris* L.) is an important legume in the farming systems of the Mediterranean aea because it is a source of high-quality protein in human diet and animal consumption. It offers most practical means of solving protein malnutrition. The lens is characterized by its ability to enter into a symbiotic relationship with the bactrium Rhizobium leguminosarum in the fixation of atmospheric nitrogen. It helps in reducing the amount of added nitrogenous fertilizers to the plants (Ouji et al., 2015).

Chromium is a carcinogenic pollutant, widely recognized for its unprecedented hazardous effects on the living system as well as on the environment (Jabeen et al., 2016; Singh et al., 2017; Kumar et al., 2019). Chromium is heavily released into the environment as an industrial pollutant due to its extensive use in tanning, smelting, metal plating, refractory materials, battery manufacturing, pesticides, and fertilizers (Nagajyoti et al., 2010; Huang et al., 2018). The most stable and common valences of Cr are Cr (III) and Cr (VI), the latter, is known to be highly carcinogenic and mutagenic to humans and animals (Huang et al., 2018; Farid et al., 2019).

Also, higher Cr accumulation causes irreversible physiological, biochemical, anatomical and ultrastructural alterations in plants (Sikander Pal et al., 2012; Farid et al., 2017; Kumar et al., 2019; Rahan et al., 2019). The most common symptoms are restricted seed germination, root and shoot growth, chlorophyll biosynthesis, altered photosynthesis, transpiration, respiration, key enzyme activities and carbon assimilation (Mahmud et al., 2017; Zhao et al., 2019).

In this study, Fenugreek (*Trigonella foenum-gracium* L.) and Lens (*Lens culinaris*) seeds were used to evaluate the toxicity of Cr. The parameters studied are germination rate, growth, tolerance index, root toxicity index, seeds vigor index and germination index of fenugreek and lens under increasing concentrations of Cr.

MATERIALS AND METHODS

In order to evaluate the effect of chromium on the germination process, two different species are chosen. They belong to the Fabaceae family (Fenugreek (*Trigonella foenum-gracium* L.) and Lens (*Lens culinaris*)).

Plant materials

Healthy seeds (15 seeds) of uniform size were added to Petri dishes of 9 cm diameter, containing two layers of Joseph paper. Seeds were spaced by 1 cm betwen them.

Metal treatments and germination tests

The solution used is anhydrous potassium dichromate ($K_2Cr_2O_7$) dissolved in distilled water (Suthar et al., 2014).

The different concentrations used is (0, 0.02, 0.04, 0.06, 0.08, 0.1, 0.2 mg L^{-1}). They were chosen considering the maximum accepted accumulation in water (0.1 mg L^{-1} for irrigation water) following the standards in Morocco (Order No 1276-01 of 10 Chaabane 1423, 17 October 2002) (‘moroccan-standards-for-quality-of-water-for-irrigation’ 2002).

The trial runs for 10 days, the first two days the petris dishes are put in an incubator in the dark, 25°C and 70% humidity. Afterwards, the rest of the time (8 days), the petri dishes were kept in the light 6 hours period on day, with the same conditions of 25 °C and 70% humidity. Triplicates of each treatment were studied with control using distilled water.

Germinated seeds were counted every two days. They are considered germinated when the radicle has pierced the integuments by 2 mm (Zaghdoud et al., 2018).

Measurement methods and used equipment

In this study, several parameters were measured and which are:

- Germination rate: is expressed by the ratio of the number of germinated seeds on the total number of seeds, is determined by the formula 1 (Ameziane et al., 2020)

$$TG\% = \frac{\sum n}{N} \times 100 \quad (1)$$

where n is the number of sprouted seeds and *N* is the number of tested seeds.

- Measurement of growth in length: the lengths of the radicles and stems for the two species studied were measured every two days with a caliper to determine the growth in length of the seedlings (expressed in cm).
- Tolerance index (TI)

The tolerance index is determined by the formula 2 of (El Rasafi et al., 2021)

$$TI = (\text{Root length in treatment} / \text{Root length in control}) \times 100 \quad (2)$$

- Root Toxicity Index (RTI):

The root toxicity index is determined by the formula 3 of (El Rasafi et al., 2021)

$$RTI = ((\text{Root length in control} - \text{Root length in treatment}) / \text{Root length in treatment in control}) \times 100 \quad (3)$$

- Seed vigor index

The root toxicity index is determined by the formula 3 of (El Rasafi et al., 2021)

$$SVI = ((RL + SL) \times TG) / 100 \quad (4)$$

*TG = germination rate; *RL = Root length; *SL = Shoot length.

- Germination index

The germination index is determined by the formula 5 of (Trautmann & Marianne E. Krasny, 1998)

$$IG = (GE/GT) \times (LE/LT) \times 100 \quad (5)$$

*GE = number of germinated seeds in the treatment; *GT = number of germinated seeds in the control; *LE = root length of germinated seeds in the treatment; *LT = root length of germinated seeds in the control.

Statistical analysis

The obtained results correspond to the average of three repetitions (three petri dish for each treatment). The study data were subjected to unidirectional variance analysis (ANOVA) and the average separations were made by the smallest difference (*LSD*) at the level of significance of ($p < 0.05$).

RESULTS AND DISCUSSION

Effect of chromium on the germination rate of fenugreek and lens

Seed germination is a sequence of events that involves hydration of the dried seed, activation of cellular metabolism, followed by synthesis, of hydrolytic enzymes and degradation of seed macromolecules by newly synthesized and stored hydrolases (Mabrouk et al., 2019).

For fenugreek the germination rate of the control and the 0.02 mg L^{-1} concentration is about 100%. From the concentration of 0.06 mg L^{-1} the germination rate decreases progressively until, it reaches 73% for the concentration 0.2 mg L^{-1} (Fig. 1, A).

In fact, seeds irrigated from 0.02 to 0.1 mg L^{-1} of chromium have a lower and not significant difference rate ($p > 0.05$) compared to the control, and significantly different to 0.2 mg L^{-1} (Fig. 1, A).

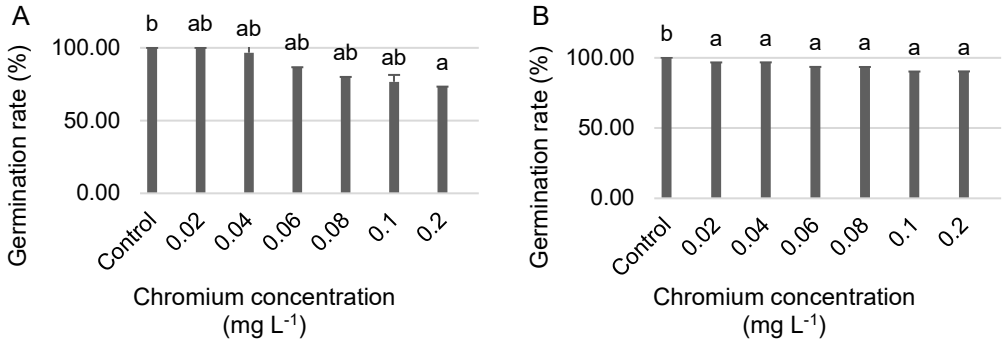

Figure 1. Effect of different chromium concentrations on the germination rate of (A) fenugreek and (B) lens (values with different letters are significantly different: $p < 0.05$).

For lens, the germination rate of the control is 100%. While for the concentrations 0.02 and 0.04 mg L^{-1} at a similar germination rate of about 97%. This rate decreases to a value of 90% for the concentrations 0.1 and 0.2 mg L^{-1}. The addition of chromium solution at different concentrations, on lens seeds are, negatively influenced the germination rate, but the latter remained significant compared to the control ($p < 0.05$) (Fig. 1, B).

These results are in agreement with the work of (El Rasafi et al., 2021) which shows a clear delay in germination of fenugreek seedlings exposed to As and Ni.

A similar effect has also been reported in other works, in the presence of Cr, Cu, Zn (Menon et al., 2016) Cr, Cd, Pb (Alaraidh et al., 2018) with different concentrations.

Effect of chromium on the growth of fenugreek and lens: radicles,stem and number of leaves

The treatment of fenugreek seeds with increasing concentrations of chromium, present a insignificant effect on radicle emergence (Table 1), only the concentration 0.2 mg L^{-1} has a significant effect on their control.

For lens, there was a significant effect on their control. Radicles exposed to higher concentrations of chromium show a brownish coloration compared to the control (Menon et al., 2016).

These results confirm the effects found, through previous studies (Menon et al., 2016; El Rasafi et al., 2021) which demonstrated that the response of these two species under chromium stress induces a significant reduction in root growth.

The decrease in root growth in the presence of Cr^{6+} can be explained by the inhibition of root cell division and/or root cell elongation, which could have the collapse of tissues and the subsequent inability of roots to absorb water and nutrients from the environment combined with the extension of the cellular cycle (Menon et al., 2016).

The elongation of fenugreek stem is about 2.83 cm for the concentration 0.02 mg L^{-1} compared to the control which is 2.30 cm. While it dies at 0.2 mg L^{-1} in chromium (Table 1).

Table 1. Effect of different chromium concentrations on the growth of fenugreek and lens radicles,stem and number of leaves (values with different letters are significantly different ($p < 0.05$)

Treatments	Fenugreek			Lens		
	Length of radicle (cm)	Length of stem (cm)	Number of leaves	Length of radicle (cm)	Length of stem (cm)	Number of leaves
Control	4.56 ± 0.16b	2.31 ± 0.2cd	2.72 ± 0.4c	4.07 ± 0.19b	5.57 ± 0.51b	6.08 ± 0.40c
0.02	2.45 ± 0.25 ab	2.83 ± 0.1 d	2.44 ± 0.08bc	1.66 ± 0.07a	3.25 ± 0.03ab	3.97 ± 0.42b
0.04	1.51 ± 0.21ab	2.22 ± 0.01cd	1.97 ± 0.14b	1.07 ± 0.11a	2.53 ± 0.20a	3.65 ± 0.50b
0.06	1.34 ± 0.04ab	2.05 ± 0.14c	2.17 ± 0.01bc	0.94 ± 0.22a	2.58 ± 0.52a	3.90 ± 0.57b
0.08	0.96 ± 0.01ab	1.60 ± 0.28bc	2.00 ± 0.01b	0.82 ± 0.17a	2.01 ± 0.39a	3.88 ± 0.17b
0.1	0.80 ± 0.19ab	1.05 ± 0.11b	2.00 ± 0.01b	0.98 ± 0.26a	2.24 ± 0.25a	3.67 ± 0.47b
0.2	- a	- a	- a	0.75 ± 0.35a	1.00 ± 0.01a	- a

Chromium at low concentration stimulates stem elongation and inhibits it when it increases.

Lens stem length was insignificant for the concentration 0.02 mg L^{-1}, and significantly different for concentrations ranging from 0.04 to 0.2 mg L^{-1} chromium compared to the control (Table 1).

The reduction in shoot and root length could be due to excessive salt accumulation in the cellular wall, which negatively alters metabolic activities and limits cell wall elasticity (Kanbar, 2014).

The number of leaves of fenugreek (Table 1) as a function of chromium concentration. For concentrations 0.02 to 0.06 mg L^{-1}, are not significantly different from the control. The number of fenugreek leaves is only significantly different from 0.08 to 0.2 mg L^{-1} compared to the control ($p < 0.05$), also the leaves of concentration 0.2 mg L^{-1} are dead.

The number of leaves of lens, have a significant effect ($p < 0.05$) from the concentrations 0.02 to 0.2 mg L^{-1} compared to their control. However, for the concentration 0.2 mg L^{-1}, the number of leaves are dead (Table 1).

Seed germination and root elongation have been proposed by the U.S. Environmental Protection Agency as phytotoxic indicators (ISTA, 2009). Both characters are widely and easily used as reliable phytotoxicity indicators for unstable chemical compounds, with many advantages; sensitivity, efficiency, simplicity, and relatively cheap as compared with another test (Osman et al., 2020).

Fig. 2 showed the development of radicles, stems and leaves of fenugreek and lens after 10 days. The reduction in these parameters is visually clear, growth in radicles, stems and leaves is proportional to the concentration of chromium used.

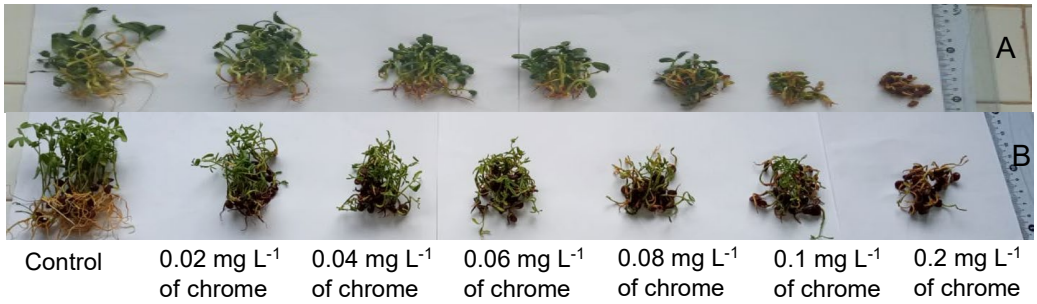

Figure 2. Development of radicles, stems and leaves of (A) fenugreek and (B) lens seedlings for different chromium concentrations.

Effect of chromium on the tolerance index of fenugreek and lens

The tolerance index (TI) was estimated for the root extension of seeds in the metal treatments and control seeds to assess the sensitivity of fenugreek and lens to different chromium concentrations (El Rasafi et al., 2021).

Fig. 3, A presents that the highest perceentage is found in fenugreek 71.76% at 0.02 mg L^{-1}. More the 0.1 mg L^{-1} the seeds of fenugreek die. The concentrations have significant effects on their control ($p < 0.05$).

For lens (Fig. 3, B), the tolerance index varies between 37.14% and 16.78% for the concentrations 0.02 and 0.2 mg L^{-1} respectively. The chromium concentration shows significant differences for lens ($p < 0.05$) compared to control.

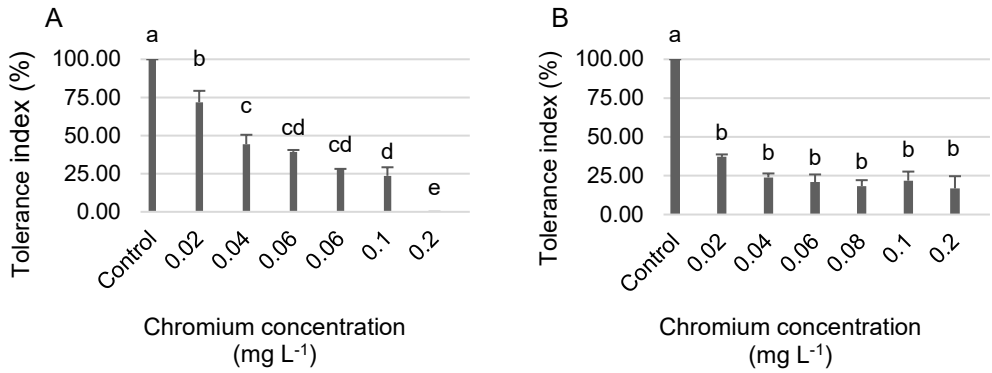

Figure 3. Effect of different chromium concentrations on the tolerance index of (A) fenugreek and (B) lens (values with different letters are significantly different: $p < 0.05$).

Effect of chromium on the root toxicity index of fenugreek and lens

The root toxicity index for fenugreek varies between 28.24 and 100% for the concentrations 0.02 and 0.2 mg L^{-1} respectively. For the latter, the root toxicity index has significant difference to their control ($p < 0.05$) (Fig. 4, A).

For lens, they vary from 63.70 to 89.05% for the concentrations measuring from 0.02 to 0.2 mg L^{-1} respectively. Also, a significant difference tends to diverge from the six chromium concentrations compared to their control ($p < 0.05$) (Fig. 4, B).

The toxic effect of chromium may be due to their competition for nutrient cation uptake at the root cellular level, direct interaction with functional proteins leading to disruption of their structure and function (Alaraidh et al., 2018). The inhibitory effect of chromium on amylase activities, resulting in delayed sugar transport (Katoch & Jit, 2016). In addition, they interfere with cell division, leading to chromosomal aberrations and mitosis (Rizwan et al., 2016; Pavlova, 2017). Their oxidative activities and the production of free radicals (reactive oxygen species) by heavy metals in the cell can cause oxidativedamage to cells of the photosynthetic and mitochondrial apparatus (Farid et al., 2017).

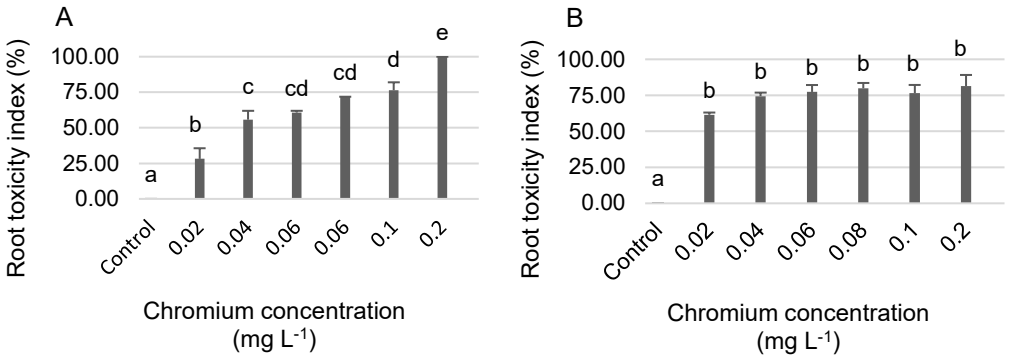

Figure 4. Effect of different chromium concentrations on the root toxicity index of (A) fenugreek and (B) lens (values with different letters are significantly different: $p < 0.05$).

Effect of chromium on the vigor index of fenugreek and lens

The fenugreek vigour index decreases from 528.00 to 141.50 for the concentrations 0.02 and 0.1 mg L^{-1} respectively. After that it is cancelled out for the chromium concentration of 0.2 mg L^{-1}. This is due to the fact that the fenugreek root does not resist to this concentration and dies after the sixth day with a vigour index of 29.08 against 283.76 for the control (Fig. 5, A).

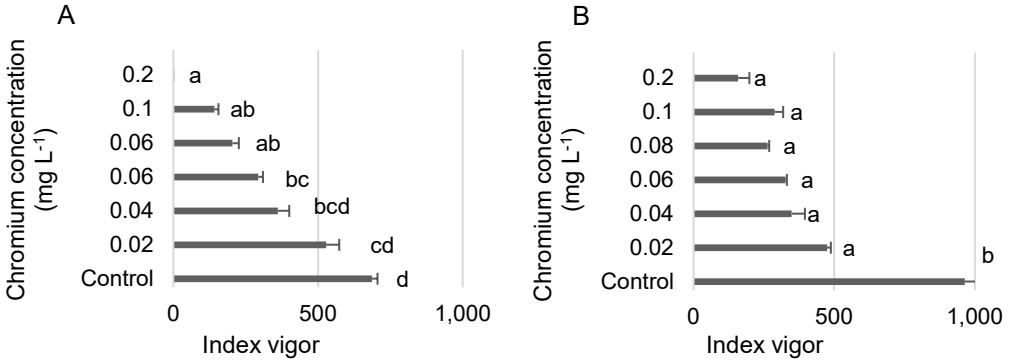

Figure 5. Effect of different chromium concentrations on the index vigor of (A) fenugreek and (B) lens (values with different letters are significantly different: $p < 0.05$).

In fact, the seeds treated with the concentrations 0.02 and 0.04 mg L^{-1}, have insigificant effect on their control. For the concentrations 0.06 to 0.2 mg L^{-1} chromium, presents a significant difference compared to the control (Fig. 5, A).

For lens, the vigour index law. It varied between 474.40 and 158.33 for the 0.02 and 0.2 mg L^{-1} chromium concentrations respectively, compared to 963.50 for the control (Fig. 5, B).

Overall, the vigour index of the lens seedlings are significantly different from the control (Fig. 5, B).

The negative effect of chromium is due to the reduction in radicle length and germination rates under this metal treatment.

Effect of chromium on the germination index of fenugreek and lens

The magnitude of phytotoxicity can be determined indirectly, since phytotoxicity decreases, the germination index increases (Komilis et al., 2005)

The addition of chromium solution on fenugreek at increasing concentrations reduces the germination index compared to the control (Fig. 6, A).

The germination index of fenugreek varies between 71.85 and 17.97% respectively for the concentrations 0.02 and 0.1 mg L^{-1} and dies at 0.2 mg L^{-1} of chromium (Fig. 6, A).

For fenugreek, the concentrations for the germination index, have a significant difference to control (Fig. 6, A). The same as, for lens, the concentrations from 0.02 to 0.2 mg L^{-1} of chromium are significantly different from the control (Fig. 6, B).

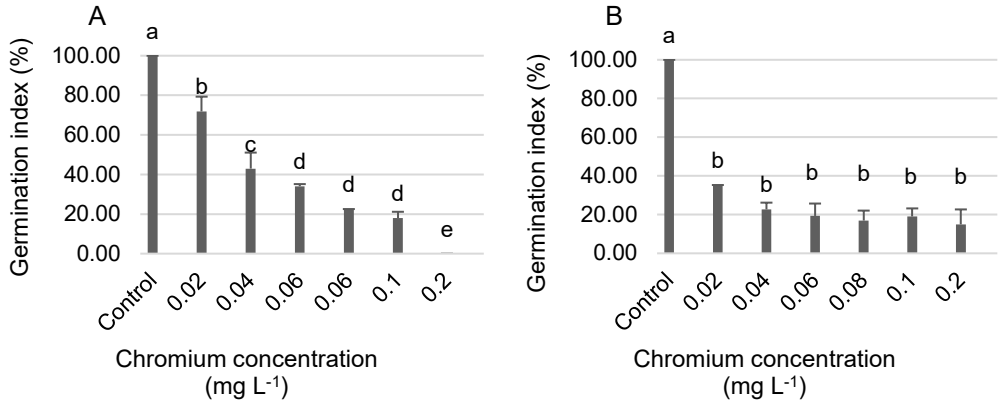

Figure 6. Effect of different chromium concentrations on the germination index of (A) fenugreek and (B) lens (values with different letters are significantly different: $p < 0.05$).

Seed germination, as the initial and critical stage of crop life cycle and the foundation of yield production (Nonogaki et al., 2018), is susceptible to chromium poisoning. And excellent seed germination is expressed in terms of both germination rate and subsequent seedling growth such as roots and shoots growth (Song et al., 2016).

Joshi et al. (2019), found that Cr stress significantly inhibited the seed vigor index, radicle length, germ length and plant fresh weight of rice (*Oryza sativa* L.) and sorghum (*High Ghum Vulgare* L.). Suthar et al. (2014) found that Cr decreased the growth of root and shoot of Mung bean (*Vigna radiata)* because the increase of the level of lipid

peroxidation which is an indication of formation of elevated level of reactive oxygen species. Mohammed et al. (2021) found that chromium also affected the germination rate of maize (*Zea mays* L.) by 20%. Lei et al. (2021), found that the germination rate, coleoptile length, radicle number per plant and radicle length of wheat under Cr stress decreased significantly and suppressed germination by 16%, radicle dry weight by 35% and coleoptile dry weight by 24%.

Similar to the above results, the present study showed that Cr toxicity significantly decreased the seed germination rate and suppressed the growth of radicles, stem and leaves of germinated seeds. Probably, the parameters studied decrease, due to the measurement methods, that based primarily on the germination rate, radicles length and stem.Thus there is a reciprocal relationship between these studied parameters and tolerance index, root toxicity index, seeds vigor index and germination index of fenugreek and lens under stress of chromium.

CONCLUSION

Our results suggest that chromium affected germination of fenugreek and lens differently and depending on the used concentrations. Seed germination, lenght of radicles, stem and number of leaves, tolerance index, root toxicity index, seeds vigor index and germination index were extremely affected under chromium stress. Suggesting that fenugreek were highly sensitive. We could also conclude that lens was able to tolerate the increase of Cr concentrations showing low decrease of the measured characters. We can consider the toxicity is maximun and lethal at concentration 0.2 mg L^{-1} (when we depassed the maximum accepted accumulation in water 0.1 mg L^{-1} for water irrigation in Morocco).

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Review of agronomic and genetic diversity of Moroccan rice varieties, and their resistance to blast disease (*Pyricularia oryzae*)

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Abstract. The study on agronomic and genetic characteristics of rice has given us scope to select varieties with desirable characteristics to mitigate various constraints. Rice (*Oryza sativa*) is the staple food for half of the world's population. However, its production is hampered by a variety of biological constraints. The Blast disease (*Pyricularia oryzae*) is an important rice disease, and one of the most effective control methods is to use resistant varieties. Study areas in Morocco include the Gharb plains. For all methods, cultural practises like soil levelling seem to be important, but biological control is not widely adopted due to cost, efficacy, and climatic conditions. The bibliographic synthesis was carried out in this context with the main goals of contributing to a better understanding of rice cultivation in Morocco; to identify and characterise the structure of the rice blast pathogen (*Pyricularia oryzae*), which will allow us to characterise the effects of rice blast; and to research on the Gharb rice field, which resulted in resistant varieties, which will potentially allow producers to have resistant varieties to overcome the diseases. The introduction and development of new rice varieties with high agronomic and socioeconomic value; the selection of lines with high yield, good grain quality, and precocity that are adapted to Moroccan conditions; as well as the development of new lines from Moroccan rice, are among the specific goals.

Key words: rice's agronomic and genetic, control methods, *pyricularia oryzae*, resistant varieties, morocco.

INTRODUCTION

Rice, being the major staple food and one of the main sources of income and employment, is an important crop all over the world. Almost 90% of the global production and consumption of rice is reported from Asia, where a considerably large part of the world's population resides (www.fao.org; accessed on January 20, 2022).

As a cereal grain, rice (*Oryza sativa* L.) is one of the most widely consumed staple foods globally (FAOSTAT, 2022).

In Morocco, the total area of rice crops reached 7,973 hectares with a production of 64,598 tonnes (FAOSTAT, 2020). Nevertheless, rice domestic consumption is

considered to be one of the lowest in the world (1.2 kg of rice per capita), which represents a major constraint to rice production in the country (Food and Agriculture Organization of the United Nations, 2003).

Furthermore, rice production is affected by biotic and abiotic factors (Acharya et al., 2019). Drought, cold, acidity, and salinity are abiotic factors, while pests, weeds, and diseases are biotic factors (Onyango, 2014). Among the biotic factors, fungal diseases alone are estimated to reduce annual rice production by 14% globally (Agrios, 2005), and among the fungal diseases of rice, rice blast caused by *Magnaporthe oryzae* is of significant economic importance and can cause 70%–80% yield losses of rice (Nasruddin & Amin, 2012; Miah et al., 2013).

Pyricularia oryzae Cavara (teleomorph: *Magnaporthe oryzae*) is one of the most important phytopathogenic fungi because it is the causative agent of rice blast diseases, the most destructive and detrimental disease in rice (Gabriel et al., 2022). *Pyricularia oryzae* affects more than 50 species of grasses, such as wheat, barley, oats, and millet (Dean et al., 2012; Langner et al., 2018; Kurrata et al., 2019).

According to Lage (1997), the presence of *Pyricularia* infection, Helminthosporium disease, and weeds (*Echinochloa crus-galli*, *Panicums spp*., *Typha spp*., and *Cyperus spp*.) could slow down rice production (Boulet & Bouhache, 1990). The Food and Agriculture Organization of the United Nations (FAO) stated in 2003 that the most common weed species affecting rice in the Mediterranean region belong to the Poaceae and Cyperaceae. In the Gharb region, the most common weeds are Panicum (*P. repens, Ligustrum obtusifolium Del*.), Typha (*T. latifolia L., T. marsii Bat*.), *Scirpus spp., Cyperus spp*., and *Echinochloa spp*. (Miège, 1951). These species are well adapted to the different agroecosystems where rice is cultivated and can promote the conservation and multiplication of pathogenic species (Pugh & Mulder, 1971; Singh et al., 2008).

Benkirane et al. (2000) observed that Moroccan isolates of *Pyricularia oryzae*, originating from *Stenotaphrum secundatum*, are pathogenic for rice. Likewise, Serghat et al. (2005) found that the fungal pathogen *Pyricularia oryzae*, isolated from *Echinochloa phyllopogon* and *Phragmites australis*, induces leaf lesions and sporulates on the foliage of certain rice varieties.

In Morocco, surveys in the rice-growing area and a study of the mycoflore of rice have revealed several pathogens responsible for the diseases, including foliar diseases. Among these diseases, rice blast (*Pyricularia oryzae*) and rice Helminthosporiose (*Helminthosporium oryzae*) are the most dominant, but their effects on yield are not known in Mediterranean regions such as Morocco (Tajani et al., 2001). In Morocco, most of the cultivated rice varieties are susceptible to several fungal species (Katsura et al., 2007). This shows that in Morocco, rice leaf diseases largely reduce grain weight. The calculated yield losses change depending on the severity of the attack and the efficiency of the fungicide at the treatment stage. Early epidemics were discovered to be more devastating than late epidemics (nearly a 15% yield reduction).To reduce output losses owing to restrictions such as genetic constraints, genetic improvement using biotechnology instruments remains a viable option (Moinina et al., 2018).

The most common approach to combating blast disease is to use resistant strains of rice plants. Initially, the use of resistant strains was effective in controlling blast disease. However, in most cases, host resistance becomes ineffective due to the emergence of recent blast races (Kurrata et al., 2019). High genetic variation, or genomic adaptation, is one of the mechanisms of *P. oryzae* that can overcome host resistance to prevent host

recognition (Longya et al., 2020). In this regard, biological control could be an effective alternative to controlling blast disease. Biocontrol of diseases in plants controls the population of phytopathogens with the aid of living organisms (Heimpel & Mills, 2017; O'Brien, 2017).

In Morocco, pyriculariosis is formerly known, and significant losses have already been reported (Duangporn, 1977; Lakrimi, 1989). During surveys carried out from 1997 to 1999, it was found that this disease causes damage mainly in the Larache region. The absence of fungicides registered for this disease in Morocco does not allow for treatments. As the selection of new rice varieties takes place in the Gharb region, where pyriculariosis pressure is low, pyriculariosis resistance has never been considered in the selection objectives. As a result, the level of resistance of the varieties used by farmers is not known. The major epidemics observed in the Larache region have raised questions about the potential risks of epidemics in the Gharb region and, in particular, about the level of resistance of Moroccan varieties (El Guilli.et al., 2000). The objective of this study was therefore to evaluate the resistance to pyriculariosis of several varieties used or newly selected in Morocco.

Rice in Morocco

In Morocco, the rice sector is socio-economically important. It has performed remarkably well in recent years, thanks to a series of measures taken as part of the Green Morocco Plan, which has helped to organise the sector. The cultivation of rice has experienced a remarkable dynamic, allowing the national production to cover more than 72% of the country's consumption needs The Gharb region contributes to 75% of the national production (MAPM, 2020).

In Morocco, rice consumption is considered to be one of the lowest in the world (1.2 kg of rice per capita), which represents a major constraint for the development of rice production in the country (FAO, 2003). Gharb rice production estimates the average gross yield at 77.7 kg ha^{-1} for a harvested area of 4,999 ha (ORMVAG 2013). In 2004, a study on Gharb showed that the sector had a turnover of 200 million Dh, considered too low compared to the real potential of 600 million Dh. The Plan Maroc Vert (PMV) aims to exploit 9,000 ha by 2020 (The Economist, 2016).

Table 1. Overall indicators for the rice industry by 2020 (Source: ORMVAG, 2013)

Indicators	Initial situation 2010	Horizon 2020
Total surface area (ha)	4,500	9,000
Average yield (t ha^{-1})	7,5	8,0
Production (t)	33,750	72,000
Added value (MDH)	54,00	112,6
Gross margin (DH ha^{-1})	81,400	10,000
Employment (1,000 d.t)	306	585
Number of projects of aggregation	4 projects	5
Number of aggregates	-	100

The Regional Office for the Development of Gharb (ORMVAG, 2013) discovered that some technical, economic, and organisational constraints remain to be overcome in order for this sector to be truly integrated into the Regional Agricultural Development Plan. But since the implementation of the Plan Maroc Vert and given the need to upgrade this important sector at the regional level, the Moroccan government has committed to providing it with the necessary support within the framework of a programme contract linking it to an interprofessional. The overall indicators for the rice industry are shown in Table 1.

Evolution of the rice sector in Morocco

More than 75% of the seeded fields are owned by the agrarian reform cooperatives, which use nearly 8,100 ha of the 12,000 ha designated for rice production in the Gharb. The remaining 25% are owned by collectivists and Melkists (MAPM, 2020).

In Gharb, the total number of rice farmers is 5,500, of which about 1,500 grow rice regularly. The provision of certified seed subsidies, Increased yields at the Gharb level (about 8,108 t ha^{-1}) were made possible by leveling with the complementary, 107 and irrigation control.

In Larache, yields (6.5 t ha^{-1}) are still below target, owing to sparrow attacks and the region's unique environment (cold at the beginning of the season, heat waves during the flowering phase). It's worth noting that the yield achieved in 2018–19 (8.2 t ha^{-1}) was higher than the production culture target of 8 t ha^{-1}. Peaks of more than 11 t ha^{-1} were detected in 5% of the rice growers who were notified. The evolution and production of rice are illustrated in Fig. 1 and Fig. 2.

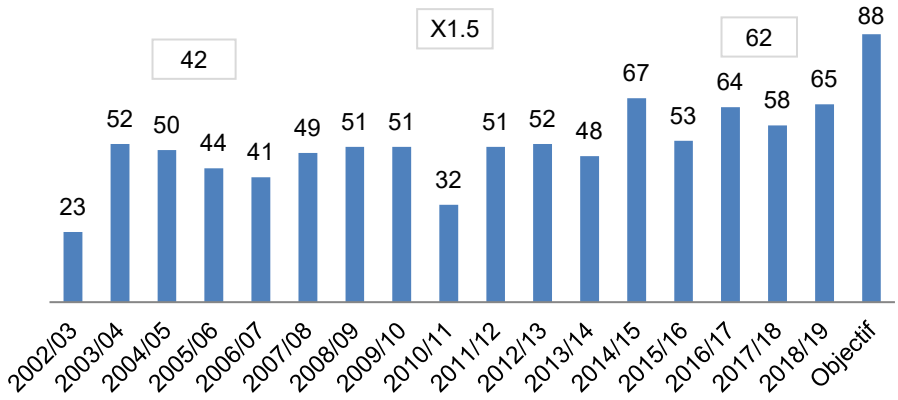

Figure 1. The Evolution of Rice Production (Source: MAPM, 2020).

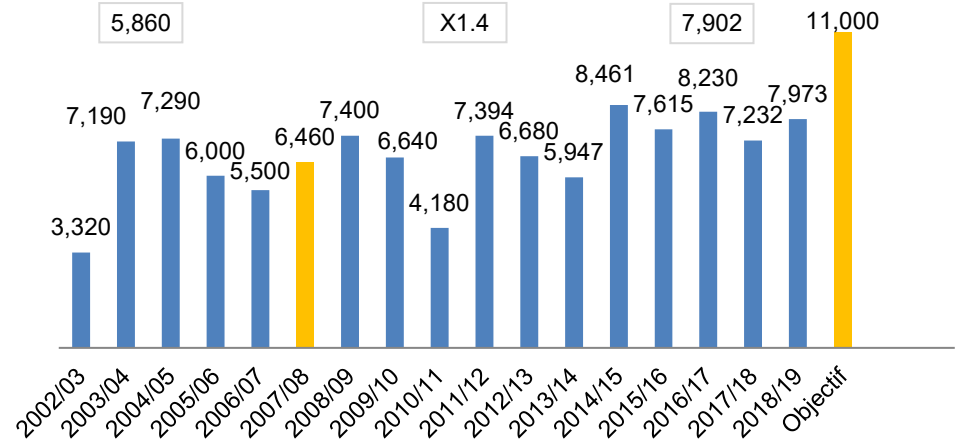

Figure 2. Evolution of the rice area (ha) (Source: MAPM, 2020).

Pedoclimatic characteristics of the Gharb region and their impact on rice cultivation

Western Morocco offers a diverse range of climates, ranging from desert to sub-humid bioclimatic stages. Continentality and latitude are the primary determinants of climate dispersion (Zidane et al., 2010).

Temperature and humidity

Depending on the phenological stage (25 to 31 °C for tillering and 30 to 33 °C for heading), the best temperature for rice development and growth is 25 to 35 °C.

According to Regional Office for the Development of Gharb (ORMVAG, 2013), rice cannot be grown at temperatures below 10 °C or beyond 45 °C. High temperatures in late spring and early summer in Gharb are ideal for this crop.

According to Tajani et al. (1997), the amount of annual rainfall is highly variable. The annual rainfall averages between 450 and 600 mm, with a 90 percent concentration between October and April. In the winter, the average daily temperature is 11 °C, while in the summer it is 27 °C. From mid-April through the end of September, the weather is ideal for rice growing. Rice, on the other hand, is normally planted from June to July due to a scarcity of water before this date.

Soils

In the Gharb, the tirs (vertisols) and merjas soils (vertisols hydromorphic) are well adapted to rice farming, except for those that are too draining (permeable) or too compact (FAO, 2003). Their proportion of total limestone ranges from 0% to 49% (Miège, 1951). Their organic matter concentration ranges between 0.74 and 2.88 percent, and their pH (6.75–8.57) is normally basic, rarely plainly basic, and only rarely mildly acidic (Zidane et al., 2010). Furthermore, surface water NaCl concentrations ranged from 0.2 to 1.7 g L^{-1}, while pore water NaCl concentrations ranged from 0.25 to 3 g L^{-1} (El Bildi et al., 2006). Rice is intensively grown and automated in Morocco. It is grown in enclosures that are built to allow submersion watering. The hydromorphic soils of the Gharb region have benefited from this crop (Lage, 1997).

Rice production techniques in Gharb

Tillage and seeding season

Soil cultivation starts in May and is dependent on the availability of materials as well as weather conditions. The soil must be slightly dry. ORMVAG is in charge of this project, which is sponsored by Plan Maroc Vert (PMV). A rice farmer submits a specification to the ORMVAG before the work begins. The head of the Rice Growers' Cooperative confirms these specifications (ORMVAG, 2013).

The Society of Moroccan Agricultural Works is in charge of soil tillage (STAM). The ploughing depth is 14 to 15 cm, and it is done in one pass with the stubble plough. The cover crop is then passed twice to begin the levelling and planning of the soil. Because the levelling operation is critical for all subsequent processes, this last one is accomplished with a resurfacing or board. Many farmers have dry fields; many farmers plant rice after soaking it in water, on submerged fields, and in submerged water. The soil is ploughed 1 to 2 times. It is planted at 140–200 kg ha^{-1} (Tajani et al., 1997).

Principal varieties

The most prevalent round rice types on the Gharb plain are Elio, Megassa, and Thaiperla, while the most common long rice varieties are Thaibonet, Lido, Arba, and Puntal. The most extensively farmed variety, according to Chataigner (1997), is Elio, which accounts for 80 per cent of all rice-growing acreage. According to FAO (2003), short-grain rice genotypes are the most extensively farmed in Morocco due to their disease resistance compared to long-grain genotypes. The latter are early-maturing and have high production potential, but they require careful watering and soil levelling.

Nutrient requirements

In this area, usual practice is application of fertiliser right after tillage operation. DAP (diammonium phosphate) is administered at a rate of 3 kilogrammes per hectare, either manually (broadcast) or by fertiliser spreader. In terms of cover crop application, urea at a rate of 10 kg ha^{-1} is applied 2 or 3 days after planting, during the growth of the crop (jas). The yield of grains is determined by nitrogen. It is suggested that at least 15 days pass between applications (Tajani et al., 1997). However, excessive nitrogen fertilization often leads to environmental pollution, lodging, and diseases, especially rice diseases, especially rice blast (*Pyricularia oryzae*), so a judicious distribution of this fertilizer is considered necessary.

Constraints related to rice production

In rice production, weeds, pests, and pathogens, especially rice blast (*Pyricularia oryzae*) and rice *Helminthosporium oryzae*, are great economic importance. Rice blast (*Pyricularia oryzae*) and rice helminthosporium (*Helminthosporium oryzae*) are of great economic importance. Oerke (2006) estimated the potential losses from these pests to be 37, 25, and 13%, respectively. Surveys in rice fields in Morocco (Tajani et al., 2001) have identified these dominant fungal diseases, but their effects on yield are not known.

Rice blast disease

The rice blast is distributed in about 85 countries on all continents where rice is grown, both in paddy and upland conditions. In both rice fields and upland conditions, it is one of the most devastating diseases of rice (*Oryza sativa* L.) under favourable conditions Ou, 1985; Miah et al., 2017). In addition to rice, *Pyricularia oryzae* also infects other agronomically important crops, such as barley, wheat, and millet (Valent et al., 1991).

The pathogen of rice blast was first known as *Pyricularia oryzae* Cavara in 1892, but it is indistinguishable from *Pyricularia grisea*, which causes greasy spots on other grasses (Agrios, 2005). The genus Pyricularia, first described in 1880, was named after *Pyricularia grisea* (Cooke) Sacc., the name given to the anamorph of crabgrass isolates. According to Chauhan et al. (2017), *Magnaporthe grise*a (Hebert Barr) is the teleomorph of crabgrass, a flaming ascomycete fungus, and belongs to the family Magnaporthaceae family. The fungus produces several toxins, e.g., *Pyricularin* and *-Picolinic*, which appear to contribute to the development of rice blast (Agrios, 2005).

The rice blast disease cycle

The asexual cycle is the only mode of reproduction observed in nature (Zeigler, 1998). When conditions are favourable, from the mycelium, conidia are produced (Saleh,

2011). There is also a sexual cycle (Fig. 3), which has never been directly observed in nature but is produced in vitro.

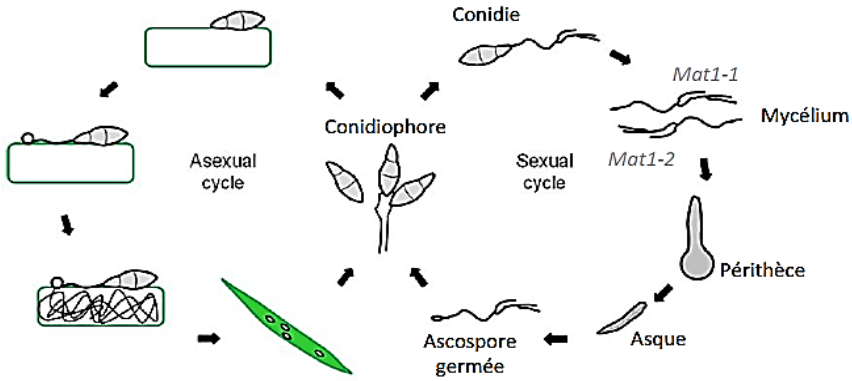

Figure 3. Sexual and asexual reproductive cycles of *Pyricularia oryzae* Source (Saleh, 2011).

The sexual cycle of *Pyricularia oryzae* is suspected to have existed within populations attacking rice in many localised areas of Asia (Zeigler 1998; Tharreau et al., 2009; Saleh et al., 2012; Gladieux et al., 2018; Thierry et al., 2020).

Rice blast is a polycyclic disease that occurs regularly. The infection process of *P. oryzae* can be summarised in five basic steps: (1) conidia generation and dissemination; (2) conidia attachment to a host surface; (3) appressorium creation; (4) penetration of the initial host cell; and (5) invasive hyphae growth (Hamer et al., 1988). During periods of high relative humidity (90 percent or higher), the fungus develops and releases conidia (Kato, 2001; Miah et al., 2017).

When a conidia lands on the surface of a rice leaf, the champignon begins its infection process (Ou, 1985; Wilson & Talbot, 2009). When there is free water, the conidies grow, and the germinating tube becomes an appressorium in which the champignon feeds on the plants (Wilson & Talbot, 2009; Miah et al., 2017). The symptoms begin to appear 4 to 5 days after the infection (Kato, 2001). These sporulent lesions emancipate conidies, which are dispersed by the wind. A single lesion can cause up to 6,000 conidies in one night, and an infected rice spikelet can cause up to 20,000 conidies in one night (Ou, 1981).

The conditions for the development of the disease

Rice blast expression is very variable and is influenced by both environmental and plant-specific variables. Moisture, temperature, fertilizer, and light are the most important elements. Moisture is required for the growth of *Pyricularia oryzae*, particularly for germination and the generation of conidia. Furthermore, elevated nitrogen levels encourage infection. *Pyricularia oryzae* grows best at temperatures between 24 and 28 degrees Celsius. At these temperatures, the fungus can penetrate the rice plant in 6–8 hours if there is enough moisture, although, at 34 °C, it appears impossible (Traoré, 2000).

Symptoms of the disease

The initial symptoms appear as white to grey-green lesions or white to grey-green spots, with dark green borders and green borders. Rice blast can infect most rice organs except the root system. Organs of rice except the root system (Lanoiselet, 2008). Infected seeds are a source of the primary inoculum. Dead infected seeds could serve as the primary inoculum when placed on the field during seedling development (Hubert et al., 2015; Long et al., 2000). If panicle infection occurs early, the grains do not fill and the panicle remains erect. If the panicle is infected later, the seeds become partially filled and, due to the weight of the grain weight of the seeds, the base of the panicle breaks and the panicle (Agrios, 2005).

Pyricularia grisea can infect and develop on different aerial parts of the rice plant. Thus, one distinguishes different symptoms, according to the attacked organ. Foliar blast on the leaf blade (Fig. 4, a), small greyish spots 1 to 2 mm in diameter appear first (Andrianarisoa, 1970). These small spots each correspond to a conidial infection point from which the developing parasite will form spindle-shaped or oval lesions (DPV and GTZ, 1990).

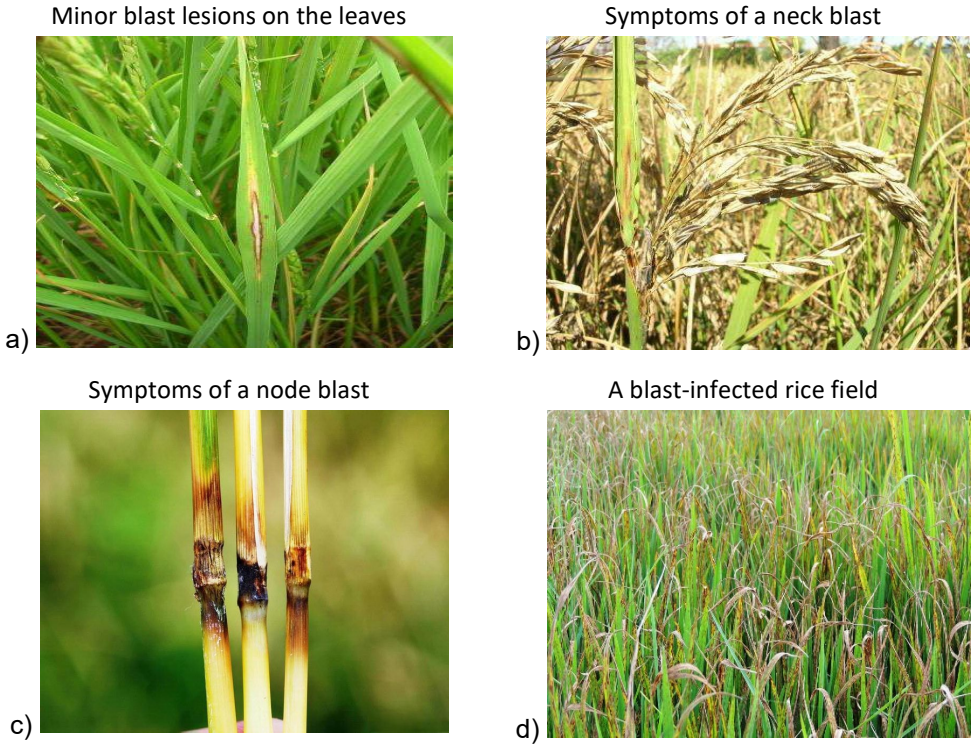

Figure 4. Symptoms of a rice blast (Chauhan et al., 2017): a) minor blast lesions on leaves; b) neck symptoms; c) node blast symptoms; d) blast-infected rice field Spread of blast disease.

At maturity, a typical lesion is characterised by a pale grey or greyish-white central area surrounded by a fairly well-defined brownish area. This is an area of necrosis. At the periphery of this central zone appears a zone of destruction of the chloroplasts. It is light yellow. Following a severe attack on a leaf, the blades can be completely dried,

taking on a burnt aspect. At this stage, the disruption of photosynthetic activity considerably affects the growth of the plant (Andrianarisoa, 1970).

Panicular blast disease

Panicular blast (Fig. 4, b), or neck blast forms the most characteristic symptom of this disease. Brown to black spots is observed on the inflorescence reaches or the spikelet (Hari et al., 1997). A large lesion may form at the base of the panicle which becomes white. In the most extreme cases, the stem eventually breaks. At this stage, the disease prevents grain filling (Sere, 1981). The fungus causes spots on the leaves, nodes, and various parts of panicles and grains, but rarely on the sheath in nature. The spots are elliptical with more or less elongated tips. In general, the centre of the spots is grey or whitish, and the periphery is brown or reddish-brown (Wopereis et al., 2008).

Nodal blast disease

This is an attack of the disease on the nodes of the culms (Fig. 4, c). A brown ring can be seen on these nodes at the beginning of the infection. This colour turns greyish as the cellulose tissue is destroyed. The stem becomes brittle and can easily break at the nodes (Dpv & Gtz, 1990).

Climate factors such as rain and wind play an important role in spore dispersal, while humidity and temperature caused by dew and fog are involved in the development of the fungus and increase its ability to infect. The source of inoculum can be infested rice or crop residues (straw) since the mycelium can survive for up to three years at temperatures between 18 °C and 32 °C and can survive changes in the environment. Conidia can live for one year at a temperature of 8 °C and a relative humidity of 20% (Zeigler et al., 1994).

Global genetic structure of Pyricularia oryzae

Rice *Pyricularia Oryzae* populations have been extensively studied to understand the evolution of the pathogen and to adapt control techniques and breeding programs. These researchers used a variety of molecular markers to characterize global populations of *P. oryza*e, including simple sequence repeats (SSR), sequence-characterised amplified region (SCAR), single nucleotide polymorphism (SNP), and others (Adreit et al., 2007; Tharreau, 2008; Gladieux et al., 2018; Zhong et al., 2018; Thierry, 2019; Thierry et al., 2020). By comparing the number of clonal lineages reported in different investigations, Zeigler (1998) concluded that the genetic diversity of *Pyricularia oryzae* was greater in the area spanning South, East, and Southeast Asia than in other regions of the world. The most in-depth investigations into the genetic organisation of *P. oryzae* populations around the world found three or four distinct groups. This research revealed that Asia will be the core of rice pathogenic population diversity and origin for all world populations (Tharreau et al., 2009; Saleh et al., 2014; Gladieux et al., 2018; Zhong et al., 2018; Thierry et al., 2020).

The control of rice blast disease

Rice blast is controlled using a variety of strategies, some of which are employed in conjunction (Ghazanfar et al., 2009): cultural practises (Biological control), chemical control, and the adoption of resistant varieties (genetic control). Biological control is not used in the field, as far as we know.

Chemical Controls

To control blast pathogen infestations, farmers depend heavily on chemical fungicides because they are readily available and quick-acting. Research conducted in Chitwan, Nepal, found that applying Tricyclazole 22% + Hexaconazole 3% SC three times at weekly intervals from the booting stage resulted in the best disease control (87.03% and 79.62% in leaf and neck blast, respectively), the highest grain yield (4.23 t ha^{-1}), and a 56.09% improvement in yield over the control one (Magar et al., 2015). Experiments performed in Pakistan by Hajano et al. (2012) discovered that using the fungicide mancozeb at 1,000 and 10,000 ppm fully inhibits the mycelial growth of *Magnaporthe grisea*, making it the most effective fungicide. Similarly, experiments conducted in Thailand by Kongcharoen et al. (2020) found that mancozeb exhibited the highest level of fungicidal activity against the blast pathogen *Pyricularia oryzae* with an EC50 value of 0.25 parts per million (ppm). Furthermore, experiments conducted in Nigeria concluded that two systemic fungicides, benomyl and tricyclazole, were found to be effective and significantly increased grain yield over the control one by 18.14% and 42.17%, respectively (Enyinnia, 1996). In an experiment conducted by Padmanabhan et al. (1971), it was found that spraying copper and organic mercury-based fungicides in a schedule covering 5–6 sprays - one spray at the seed bed (on 21-day-old seedlings), two to three sprays at the post-tillering phase at an interval of 10–15 days, and two sprays at ear emergence - one spray before emergence and another 5 days later - were also effective in controlling neck blast infections on local indica varieties. The use of chemicals is non-environmentally friendly (Thapa et al., 2019), and overuse of chemicals for a successive year develops a resistance in the fungus and poses serious threats in the future. Moreover, pesticide exposure leads to acute pesticide poisoning that has adverse health effects on vital body systems such as the digestive, respiratory, and nervous systems, and farmers are the most at risk of pesticide poisoning because of their prolonged exposure during the production season (Pingali & Roger, 2012). The residue of chemicals persists in the grain, straw, and soil, which may cause adverse effects on farm labour (Pingali & Roger, 2012).

Biological control

The indiscriminate use of various plant protection chemicals has resulted in environmental hazards, so finding alternative sources is of immense importance and also preferable (Thapa et al., 2019; Ahamad et al., 2020).

Biological control of plant diseases is typically inexpensive, long-lasting, and safe towards the environment and living organisms, however, biological control can be a slow process, and the search for suitable biocontrol agents requires considerable time and effort (Law et al., 2017).

The first report of a biological agent found effective against *Pyricularia oryzae* was *Chaetomium cochliodes* (Pooja & Katoch, 2014). When the rice seeds were coated with the spore suspension of *C. cochlioides*, the early infection by blast was controlled, and the seedlings were healthy and taller than the control (Pooja & Katoch, 2014). Experiments conducted by Bhusal et al. (2018) showed that seed treatment with *Trichoderma virdi* in 5 mL L^{-1} of water was found to be effective against leaf blast. Furthermore, Hajano et al. (2012) discovered that, of the six bio-control agents tested against *M. oryzae*, *P. lilacinus* inhibited the most, followed by *T. pseudokoningii*, *T. polysporum*, and *T. harzianum*. According to greenhouse studies conducted by Law

et al. (2017), infected rice seedlings treated with Streptomyces resulted in an up to 88.3% reduction in rice blast disease. Furthermore, recent studies on the biocontrol of rice blast showed that *Bacillus subtilis* strain B-332, 1Pe2, 2R37, and 1Re14 were found to be more effective (Changqing et al., 2007; Jin-Hyoung et al., 2008). Rice blast biocontrol experiments revealed that a powder formulation of *Pseudomonas fluorescens* strain Pf1 at 10 g kg^{-1} inhibits rice blast growth (Vidhyasekaran et al., 1997).

In order to achieve successful biological control, the biocontrol agents should be isolated from and applied to locations with similar environmental conditions (Suprapta, 2012).

Genetic control

The use of varieties that are resistant to rice blast disease offers better control strategies.It is less expensive and not as laborious as other methods. Although developing a rice blast disease resistance variety is time-consuming and difficult for plant breeders because the fungus can evolve and mutate to overcome resistance genes (Zhou et al., 2007). Blast-resistant rice genotypes have been developed with the use of marker-assisted backcrossing (Miah et al., 2017).

Cultivation of the host-resistant plants is the most efficient way to manage the disease because it is a convenient, cost-effective, environment-friendly, long-term, reliable, and realistic approach to plant protection for resource-constrained farmers (Ou, 1985; Bonman et al., 1992). Studies show that the degree of resistance increases with an increase in the proportion of silica applied and also with the amount of silicon accumulated in the plant (Pooja et al., 2014).

Generally, horizontal and vertical resistance are used in developing disease resistant cultivars (Rijal & Devkota, 2020). Due to the high genetic variability of the fungus, resistance to infection by *Pyricularia oryzae* can be short-lived (Khemmuk, 2017).

The breakdown of resistance to *Pyricularia oryzae* results from the evolution of genetic variants (races) in the pathogen populations (Liu et al., 2011).

The genetic diversity of rice

The *Oryza sativa* genome comprises more than 150,000 varieties cultivated around the world and about 107,000 accessions in the IRRI gene bank, including 5,000 of which 5,000 are wild species (Courtois, 2007). This diversity comes from natural crosses of *O. sativa* with wild or weedy forms of or weedy forms of *O. rufipogon* or from intra-sativa crosses combined with natural and human selection since domestication (Khush, 2005). To evaluate the genetic diversity of accessions and better exploit its potential, the exploitation of its potential, the use of markers remains indispensable. A good marker should be single-inherited, multi-allelic, and co-dominant. The rice genome has been completely sequenced since 2005, and nearly 400 million DNA 'letters' have been identified and positioned. We used the microsatellite molecular markers of the 'Core Map' of Orjuela et al. (2009). The development of molecular markers during the last decade has offered the possibility of establishing new approaches to improve breeding strategies (Najimi et al., 2003). They have become an essential tool in breeding programmes for new rice varieties (*Oryzaspp.*) for resistance to biotic and abiotic stresses and offer alternatives to the use of traditional phenotypic markers. Molecular genetic markers are of different types (RAPD, RFLP, AFLP, SSR, and SNP). In genetic

diversity studies, microsatellite markers have been the most widely used in rice in recent years (Semon et al., 2005).

The genotyping characterization was conducted using SSR (Simple Sequence Repeats) markers (22 microsatellites), and continued with genetic diversity and polymorphism information content (PIC) analysis (Puspito et al., 2022).

Among the studied set of microsatellite markers, two of the most informative SSR-markers - RM 7481 and PrC3 - showed high efficiency in detecting intraspecific polymorphism of rice varieties. About 400 backcrossed self-pollinated rice lines with introgressed and pyramided resistance genes Pi-1, Pi-2, Pi-33, Pi-ta, Pi-b to *Pyricularia oryzae Cav*. were obtained within the frameworks of program to develop genetic rice sources resistant to blast. The conducted testing for resistance to blast and the assessment by economically valuable traits have allowed to select the prospective rice samples. The plant samples of F2 and BC1F1 generations with combination of resistance to blast genes (Pi) and submergence tolerance gene (Sub1A) in homozygous and heterozygous state that is confirmed be the results of analysis of their DNA have been obtained. The obtained hybrid plants are being tested in breeding nurseries for a complex of economically valuable traits. The best plants will be selected and send to State Variety Testing system. Their involving in rice industry will reduce the use of plant protection chemicals against diseases and weeds, thereby increasing the ecology status of the rice industry (Dubina et al., 2022).

Three SSR markers (introduced by SBS Genetech Co., Ltd., China) linked to rice blast resistance genes; *Pi* genes (Akagi et al., 1996; Temnykh et al., 2001; Hassan et al., 2017) were screened on DNA templates. The details of the used markers and the primer sequences are presented in Table 2.

Table 2. The three used SSR molecular markers, their primers, nucleotide sequences, and essential information

Primer	F/R Primer5′→3′	CL	Linked *Pi* gene	Repet motif	Annealig temperatue	References
RM155	F-GAGATGGCCCCCTCCGTGATGG R-TGCCCTCAATCGGCCACACCTC	12	*Pita-2*	(CTT) 7	68	Akagi et al. (1996);Hassan et al. (2017)
RM512	F-CTGCCTTTCTTACCCCCTTC R-AACCCCTCGCTGGATTCTAG	12	*Pi-12*	(TTTA) 5	60.5	Temnykh et al. (2001);Hassan et al. (2017)
RM541	F-TATAACCGACCTCAGTGCCC R-CCTTACTCCCATGCCATGAG	6	*Pi-9*	(TC) 16	60.5	Temnykh et al. (2001);Hassan et al. (2017)

F/R Primer: forward/reverse primer, CL: chromosomal location.

Currently, approximately 100 genes of resistance (*R*) to rice blast are known; of these, 51% are from *indica* genotypes, 45% from *japonica* genotypes, and 4% from wild species of rice (Sharma et al., 2012) (Table 3). The identified *R* genes have broad nomenclature, and, often, the same resistance gene can have different names (Koide et al., 2009).

Table 3. List of genes manipulated for rice blast resistance

Gene name	Function	Manipulation transgenic	Effects	Reference
OsPi-d2	*R* gene	Overexpression	Resistance to neck blast incidence	Chen et al. (2010)
MoHrip1	Elicitor gene	Overexpression	High resistance against blast	Wang et al. (2017)
OsWRKY53	*R* gene	Overexpression	High resistance against blast	Chujo et al. (2014)
OsGF14b	Induces expression of jasmonic acid (JA)	Overexpression	Resistance to neck blast incidence	Liu et al. (2016)
WRKY45	Induces expression of salicylic acid (SA)	Overexpression	High resistance against blast	Shimono et al. (2007)
CYP71Z18	-	Overexpression	High resistance against blast	Shen et al. (2019)
MoSDT1	Effector protein	Overexpression	High resistance against blast	Wang et al. (2019)
Pi54	*R* gene	Overexpression	High resistance against blast	Singh et al. (2020)
OsCPK4	Calcium-dependent	Overexpression	High resistance against blast	Bundó & Coca (2016)
RACK1A	Receptor for activated C-kinase 1A	Overexpression	High resistance against blast	Nakashima et al. (2008)
OsCDR1	*R* gene	Overexpression	High resistance against blast	Prasad et al. (2009)
OsWRKY13	Regulating defense-related genes in salicylate-and jasmonate-dependent signaling	Overexpression	High resistance against blast	Qiu et al. (2007)
GH3-2	-	Overexpression	High resistance against blast	Fu et al. (2011)
OsGH3.1	Component of the hormonal mechanism regulating	Overexpression	High resistance against blast	Domingo et al. (2009)
OsNAC6	Transcription factor	Overexpression	High resistance against blast	Nakashima et al. (2007)
OsSBP	Homologue of mammalian Selenium-binding proteins	Overexpression	High resistance against blast	Sawada et al. (2004)
OsRacB	Allene oxide synthase gene	Overexpression	High resistance against blast	Jung et al. (2006)
OsAOS2	Allene oxide synthase gene increases the endogenous jasmonic acid level	Overexpression	High resistance against blast	Mei et al. (2006)
OsSERK1	Regulates somatic embryogenesis	Overexpression	High resistance against blast	Hu et al. (2005)
OsOxi1	Regulates basal disease resistance	Overexpression	High resistance against blast	Matsui et al. (2010)
Gns1	Stress-inducible β-glucanase	Overexpression	High resistance against blast	Nishizawa et al. (2003)
Rir1b	Defense-related	Overexpression	High resistance against blast	Schaffrath et al. (2000)
OsWAK1	Wall-associated receptor-like protein kinase gene	Overexpression	High resistance against blast	Li & Li (2009)
OsSYP71	Oxidative stress and rice blast response gene	Overexpression	High resistance against blast	Bao et al. (2012)
BSR1	Putative receptor-like cytoplasmic kinase gene	Overexpression	High resistance against blast	Dubouzet et al. (2011)
OsACS2	Key enzyme of ethylene biosynthesis	Overexpression	High resistance against blast	Helliwell et al. (2013)

The resistance of Moroccan varieties

Several varieties were tested for resistance. The two most widely grown varieties in Morocco are Elio and Thaibonnet. Other varieties include Hayat, Dinar, and Kenz varieties. Nachat, Maghreb, and Bahja are varieties newly registered in the official catalogue by INRA. Farah, INRAM 6, and INRAM 11 are new INRA varieties proposed for the official catalogue. Two other varieties were used as references. Ariete is a French variety whose resistance is acceptable in the Camargue (South of France), and Maratelli is an Italian variety used here as a sensitive control (El Guilli et al., 2000). Sowing seeds in rows was done in trays (45×29×7 cm) containing potting soil that was kept moist after sowing. The trays were then placed in a greenhouse and the seeds were planted (El Guilli et al., 2000).

A collection of Moroccan isolates were collected in 1997 and 1998 from lesions on leaves or panicular stems. The selection of isolates for inoculation was based on the preliminary results of a study of the diversity of the Moroccan population of *M. grisea* using molecular markers and pathogenicity tests (El Guilli.et al., 2000).

Eleven isolates, representative of the different clonal lines and existing breeds in the Moroccan population of *Pyricularia oryzae* were used. For the characterization of the resistance of Farah, 10 additional isolates from different countries (China, Cameroon, Ivory Coast, and Thailand) known for their broad virulence spectrum (13 specific resistance genes mounted on 13 tested) have been inoculated on this variety (El Guilli et al., 2000).

Inoculum preparation and inoculation

Identifying sources of inoculum can help to reduce the disease's occurrence and severity (Raveloson et al., 2011). Residues of infected rice and disease-affected seed are the main sources of primary inoculum for the blast (Long et al., 2001; Guerber & TeBeest, 2006; Raveloson et al., 2013).

The isolated and purified *Pyricularia oryzae* inoculum, stored at 5 °C, was re-cultured in PDA medium (Miura et al., 2005). Procedure was adopted in the preparation of conidial suspension. The inoculated plates were incubated in the dark for 12–14 days at 26 °C. For the inducement of heavy sporulation, the culture was scraped aseptically with a sterile toothbrush, and the plates were exposed to near-ultraviolet light at 25 °C for 10 days. Conidia were dislodged by gently rubbing the incubated plates with a small, sterile toothbrush in sterilized, distilled water. The conidial suspension was well filtered through layers of gauze mesh (aperture 300 lm), and the concentration was adjusted to a final concentration of 1×106 spores per ml using a haemocytometer. Tween 20 was added to the prepared suspension (0.02% Tween 20 in 0.25% gelatin) to enhance the proper adherence of conidia to the rice aerial parts (Jia et al., 2003).

The rice plant leaves were inoculated 20 days after planting by spraying the prepared 1×106 spores per ml of conidial suspension containing 0.02% Tween 20 in 0.25% gelatin per plot using a knapsack sprayer. Spraying was done slowly and carefully to achieve uniformity on the plant's aerial parts until runoff. The inoculum was sprayed around 18:00 hours of the day and ensured that the entire rice plant surface became wet with conidial suspension, and 20 cm was adopted at three stands per hill and later thinned to two stands per hill two weeks after planting (Azgar et al., 2018).

The pathogenicity test was carried out by inoculating *P. oryzae* isolates into the leaves of healthy rice plants. Fungal colonies were harvested using a brush by adding 10 mL of sterile distilled water (dH2O), including 0.02% Tween 20. The *P. oryzae* inoculum was sprayed on rice plants aged 18–21 days after planting (Kurrata et al., 2019).

Disease assessment, data collection, and analysis

Disease scoring of the inoculated rice plants was done 10 days after inoculation (Challagulla et al., 2015). The severity of the disease was estimated and recorded by using the disease rating scale of the Standard Evaluation System of the International Rice Research Institute, Philippines, based on the level of severity of the infection on each entry (International Rice Research Institute [IRRI], 2013). Based on leaf blast scores assessment, the accession was categorized as highly resistant (0), Resistance (1), moderately resistant (2–3), moderately susceptible (4–5), susceptible (6–7), and highly susceptible (8–9) (Standard Evaluation System of IRRI, 2013) as shown in Table 4.

Table 4. Disease rating scale 0–9 by International Rice Research Institute, Phillipines (IRRI, 2013)

Grade	Disease severity	Host response
0	No lesion observed	Highly resistant
1	Small brown specks of pin point size	Resistant moderately
2	Small roundish to slightly elongated, necrotic gray spots, about 1–2 mm in diameter, with adistinct brown margin. Lesions are mostly found on the lower leaves	Resistant moderately
3	Lesion type same as in 2, but significant number of lesions on the upper leaves	Resistant moderately
4	Typical susceptible blast lesions, 3 mm or longer infecting less than 4% of leaf area	Moderately susceptible
5	Typical susceptible blast lesions of 3mm or longer infecting 4–10% of the leaf area	Moderately susceptible
6	Typical susceptible blast lesions of 3 mm or longer infecting 11–25% of the leaf area	Susceptible
7	Typical susceptible blast lesions of 3 mm or longer infecting 26–50% of the leaf area	Susceptible
8	Typical susceptible blast lesions of 3 mm or longer infecting 51–75% of the leaf area many leaves are dead	Highly susceptible
9	Typical susceptible blast lesions of 3 mm or longer infecting more than 75% leaf area affected	Highly susceptible

The severity of leaf blast disease was assessed on three leaves from each of the three plants at 7 days after inoculation and every 7 days until severity stability or leaf senescence using visual quantification based on a diagrammatic scale of 0–9 developed by the International Rice Research Institute, Philippines (IRRI, 2013) (Fig. 5).

The difference between the diseased and control leaf areas (reduction in the number or size of lesions) was used to measure partial resistance (susceptible plants, lesions types 4 to 6). Using partial resistance to compare varieties (El Guilli et al., 2000). Most of the varieties newly registered or proposed for registration (INRAM11, INRAM6, Maghreb, Nachat, and Bahja) are compatible (susceptible) to Moroccan strains, and their partial resistance is lower than that of currently cultivated varieties. It is recommended

that these crops not be planted under conditions favourable to the development of blast disease (for example, too much nitrogen fertilization). In terms of partial resistance, varieties Kenz and Bahja are comparable to varieties Ariete. Under the conditions of rice cultivation in France (Camargue), the level of field resistance of these varieties is acceptable to farmers, and the level of resistance of these varieties would likely be sufficient in the epidemiological context of Morocco. At the foliar level, despite their susceptibility to all or most Moroccan strains, Elio and Thaibonnet varieties have a good level of partial resistance. This characteristic suggests that the varieties should be resistant under normal growing conditions (especially without excessive amounts of nitrogen fertilizer). These varieties should be field-resistant in the Gharb and Larache regions (El Guilli et al., 2000).

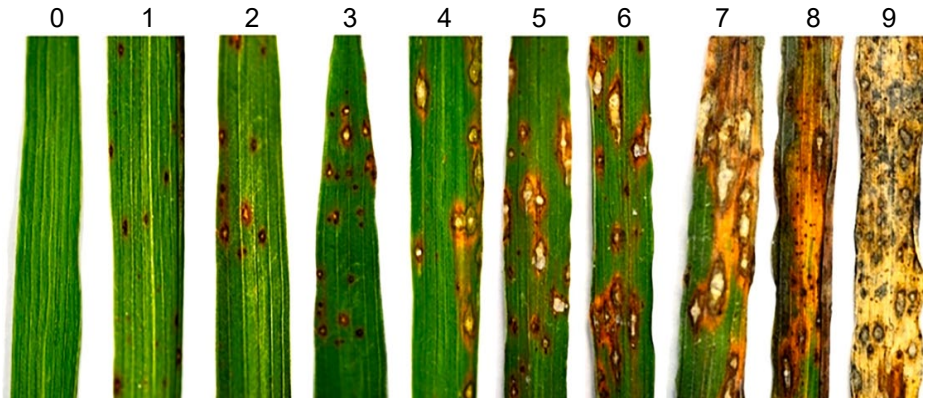

Figure 5. Schematic diagram of the mechanism with the index value for scoring rice blast disease on foliage, from Shrestha et al. (2017).

Trapping experiments with sensitive plants in the Gharb region (results not shown) have shown the presence of *Magnaporthe grisea* strains. The inoculum is therefore present. The absence of a major epidemic in this region could be explained by the cultivation of a variety with a good level of partial resistance (Elio) under conditions not conducive to the development of the disease. On the other hand, epidemics of Thai Bonnet observed at Larache following excessive nitrogen fertilisation appear to show that this partial resistance can be rendered ineffective by inappropriate cultivation practices.

Blast resistance was evaluated at the leaf level in this study. This study allows eliminating the most sensitive varieties at the vegetative stage, but studies of panicle resistance carried out in the field would be a useful complement to this work (El Guilli et al., 2000).

CONCLUSIONS

Rice is a crop of concern since it is a staple food for roughly half of the world's population. Its production is hampered by several biological restrictions. Rice blast (*Pricularia oryzae*), for example, hurts yield. A rice blast is a fungus that attacks rice plants. It is one of the most serious diseases to affect the rice crop, as it can result in

significant yield reductions and possibly crop failure. In the absence of prevention techniques, the annual loss caused by this disease ranges from 10% to 30% of total production, with crop losses reaching 100% in extreme situations for very sensitive types. For this, a characterization of the genetic resources' resistance to pyriculariosis is required, which will allow growers to have resistant varieties at their disposal to alleviate the concerns of harvest loss caused by this disease.

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Red seaweed liquid fertilizer increases growth, chlorophyll and yield of mungbean (*Vigna radiata)*

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Abstract. The demand for chemical fertilizers in Bangladesh is increasing by the day. Seaweed extracts are high in a variety of bioactive substances that can be used as a biostimulant as an alternative to agricultural plants. To assess the impact of foliar spraying of red seaweed (*Gracilaria tenuistipitata* var. *liui*) extracts at 5, 10, 15, 20 and 25% concentrationsin comparison to the control condition (water spray only) and soil application of recommended doses of fertilizer (RDF) as basal on growth, chlorophyll and yield of mungbean variety BU mug5, a pot experiment was conducted at the Department of Agronomy, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, Bangladesh during *Kharif-1* season (March to May 2021). Seven (7) treatments: T_1 – Control (foliar spray using water), T_2 – Recommended doses of fertilizers (RDF) as basal, T_3 – Foliar spray of 5% seaweed extracts, T_4 – Foliar spray of 10% seaweed extracts, T_5 – Foliar spray of 15% seaweed extracts, T_6 – Foliar spray of 20% seaweed extracts and T_7 – Foliar spray of 25% seaweed extracts were imposed following completely randomized design (CRD) with three replications. The results revealed that seaweed liquid fertilizer at 20% concentration increased leaf area, total dry matter and chlorophyll (SPAD value) by 25.00, 40.21 and 9.11% over the control and 15.42, 8.27 and 2.08% compared to RDF, respectively. Seed yield increased by 93.14% when compared to a control with 20% seaweed foliar spray, and by 9.04%, when compared to RDF. Foliar application of 20% seaweed liquid fertilizer performed best among the treatments in terms of mungbean growth and yield, according to findings of the experiment. The results of this study suggest that red seaweed extracts from *Gracilaria tenuistipitata* var. *liui* may be used as a fertilizer to reduce the chemical fertilizer to boost mungbean yield.

Key words: bio-stimulant, mungbean, red algae, yield.

INTRODUCTION

Seaweeds are one of the important marine bio-resources which are now-a-days termed as fantastically promising organic source of nutrient. The major uses of seaweeds are (i) production of phyto-chemicals such as agar-agar, carrageen and alginate (Kaliaperumal & Uthirasivan, 2011) and (ii) as food for human consumption as green vegetable, salad and also in the form of jelly, jam, chocolates and pickles (Chennubhotla et al., 1981) and medicines (Maeda et al., 2007). Seaweed extracts has been found rich in nutrients like nitrogen, phosphorus and higher amount of water soluble potash, other minerals, also rich in vitamins, amino acids, trace elements (Fe, Cu, Co, Ni, Zn and Mn) and plant growth hormones IAA and IBA growth stimulators such as auxin, gibberellins and cytokinin required by plants (Zodape et al., 2001; Zodape et al., 2010). Seaweed extracts are considered biostimulants as opposed to fertilizers because they encourage the plant's defensive and growth responses when applied (Ruso & Berlyn, 1990; Ali et al., 2021a). And nterestingly, seaweed extracts have repeatedly been shown to contribute to plant growth promotion, increased yields (Khan et al., 2013; Patel et al., 2016; Parađiković, 2019). The bio-stimulant present in seaweed extracts increase the vegetative growth, the leaf chlorophyll content, the stomata density, photosynthetic rate, increase water retention capacity in plants (Subramanian et al., 2011) and the fruit production of the plant (Blunden et al., 1996; Spinelli et al., 2009). According to Pascual et al. (2021) the use of seaweed biostimulant dramatically increased the rate of assimilation of rice beans, leading to an increase in height, heavier pods, and more seeds per pod. Foliar application of seaweed extracts has also been reported to enhance the yield of different crops significantly (Zodape et al., 2008; 2009). Seaweed extracts have also delayed of fruit senescence, improved overall plants vigour, improved yield quantity and quality (Featonby-Smith & Van Staden, 1983); improve nutrient uptake by roots (Briceño-Domínguez et al., 2014) resulting in improved water and nutrient use efficiency, thereby enhancing plants growth and vigour (Crouch et al., 1990), and develop tolerance to environmental stress (Zhang & Ervin, 2004). Moreover, manures of seaweeds are also used as a soil amendment in agriculture in many parts of the world (Eyras et al., 1998).

The productivity of the mungbean crop is very low (0.90 ton ha^{-1}) in Bangladesh (BBS, 2021). The low productivity may be attributed to lack of suitable genotypes, adequate nutrient supply (Azadi et al., 2013), improper fertilizer management (Anjum et al., 2006), and so on. Though chemical fertilizers are used in great quantities to compensate nutrient deficiencies and increase yield of mungbean, application of organic fertilizer like seaweed extracts may become a worthy effort to alternate the synthetic fertilizers increasing the yield of mungbean and this may be the most practical means of solving protein malnutrition in Bangladesh in a sustainable way. However, No study has so far been conducted on the effect of *Gracilaria tenuistipitata* var. *liui* (a red seaweed species) on growth, yield and nutritional quality of mungben and its concentration and doses of application have not been standardized. Thus, considering each and every corner of the above discussion, an experiment was conducted on the growth and yield of mungbean aiming to assess the efficacy of foliar application of different doses of seaweed extracts from *G. tenuistipitata* var. *liui*as liquid fertilizer. So, the research work was undertaken with the objectives to study the effects of seaweed extracts on growth, leaf chlorophyll and yield of mungbean crop and to identify suitable doses for mungbean crop.

MATERIALS AND METHODS

Experimental site

The experiment was carried out in pots in a polythene indoor controlled environment at the Department of Agronomy of Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Bangladesh (24° 5′ 23″ N and 90° 15′ 36″ E) in *Kharif-1* season (March to May 2021). The day and night temperatures were 28.5 ± 1.6 and 13.6 ± 1.3 °C, respectively. The plants were grown in plastic pots (0.30 m deep and 0.25 m in diameter), each containing 11 kg of soil. The experimental soil was sandy loam with a field capacity of 28% and a pH of 6.71 (53.12% sand, 33.12% alluvium, and 13.76% clay). The exchanged soil organic carbon, available P, total N, K, cation exchange capacity (CEC), and electrical conductivity (EC) were 0.59%, 0.07 mg per 100 g, 0.06%, 0.76 cmol per kg dry soil, 12.85 cmol per kg dry soil, and 0.03 dS per m, respectively.

Plant materials and seaweed extracts

A high-yielding mungbean variety, BU mug4 developed by Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) was used in this study. Red seaweed species *Gracilaria tenuistipitata* var. *Liui* under family Gracilariaceae is available in Bay of Bengal, Bangladesh was first identified and recorded by Aziz & Alfasane (2020). Fresh seaweed was collected in the morning from the coastal area of the Moheshkhali Channel of the Bay of Bengal (21° 30′ 0″ N and 92° 5′ 0″ E). The seaweed was washed with seawater and tap water, respectively to remove unwanted impurities and transported to the BSMRAU campus. After that, fresh seaweed was dried in the sun and homogenized by a grinder with stainless steel blades at ambient temperature and subsequently utilized for extraction of liquid fertilizer using the method described by Rao & Chatterjee (2014). Hundred (100) g of powdered seaweed suspended in one liter of deionized water was heated to 100 °C in hot water bath for one hour. The extracts was filtered through a muslin cloth and measured and stored in a refrigerator. Considering this as stock solution, seaweed liquid extracts was used to prepare 5%, 10%, 15%, 20% and 25% concentrations by diluting with deionized water. Then the diluted extracts in different concentrations was preserved at room temperature (15 –20 °C) before the spraying. Proximate composition of the seaweed powder from *Gracilaria tenuistipitata* var. *Liui* are presented in Table 1. The crude protein, crude lipid and crude fiber content were determined by the Micro-Kjeldahl method (Guebel et al., 1991), Mehlenbacher (1960) & using the AOAC (2000) method, respectively. To measuring one gram of powdered sample was placed onto the tray of the automatic moisture meter (Model PB-1D2, 544205, Kett Electric laboratory, Made in Japan) for 10–15 minutes while the moisture content was measured. Ash content was determined by following AOAC (1990) method. Following Sarkiyayi & Agar (2010), the percentage of total carbohydrate content was calculated using the formula [Percentage of total carbohydrate content] = 100 -(% moisture + % crude fiber + % crude protein + % crude lipid + % ash). The formula [available energy = (9.3 x fat) + (4.1 x carbohydrates) + (4.1 x protein)] described by Eneche (1991), Chinma & Igyor (2007), and Nwabueze (2007) was used to calculate available energy. The mineral contents (Ca, Mg, Fe, Cu, and P) and heavy metal (Pb) were determined by atomic absorption spectrophotometer (Shimadzu, Model-AA. 610s) following Hitachi, Ltd. (1986). To determine the amount of arsenic in

seaweeds, an improved and verified inductively coupled plasma mass spectrometry (ICP-MS) technique was utilized. A sample of about 0.5 g was put into the digestion vessel, and the sample was washed with 100 mL of distilled water until it reached the bottom of the vessel. After gently stirring the liquid, wait roughly until the vessels are warm to the touch before carefully venting the remaining digestive pressure. The analysis results were then gathered using the NexION 5000 ICP-MS, an inductively coupled plasma mass spectrometry (Wilschefski & Baxter, 2019). According to Nagata et al. (1992), the amount of β-carotene in the fresh sample was quantified and the vitamin C was calculated described by method Pleshkov (1976).

Table 1. Chemical composition of the seaweed extracts from *Gracilaria tenuistipitata* var. *liui*

Constituents	Concentration
Crude protein (%)	24.06 ± 0.03
Crude fiber (%)	5.15 ± 0.09
Crude lipid (%)	0.19 ± 0.06
Carbohydrates (%)	49.50 ± 0.25
Ash (%)	10.02 ± 0.10
Moisture (%)	11.88 ± 0.06
Phosphorus (mg per 100 g dry weight)	579.05 ± 5.36
Calcium (mg per 100 g dry weight)	129.14 ± 1.01
Magnesium (mg per 100 g dry weight)	2.90 ± 0.11
Iron (mg per 100 g dry weight)	75.18 ± 0.15
Copper (mg per 100 g dry weight)	3.29 ± 0.30
Pb (mg per kg dry weight)	0.044
Arsenic (mg per kg dry weight)	5.019
β-carotene (mg per 100 g)	9.21 ± 0.28
Vitamin C (mg per 100 g)	2.12 ± 0.21
Total energy (kcal per 100 g)	303.34 ± 0.89

Treatments and cultural practices

The experiment was consisted of seven (7) treatments: T_1 – Control (0% seaweed extracts, spray with water), T_2 – Recommended doses of fertilizer (RDF) (0% seaweed extracts, spray with water), T_3 – 5.0% seaweed extracts, T_4 – 10.0% seaweed extracts, T_5 – 15.0% seaweed extracts, T_6 – 20.0% seaweed extracts and T_7 – 25.0% seaweed extracts. Ten seeds of mungbean were sown in each pot and well-watered to ascertain uniform germination. Once the plants are fully established, thin them out to keep only six healthy plants in each pot. Different concentrations of seaweed extracts were applied as foliar spray at seedling (15 days after germination) and flowering (30 days after germination) stages. A 50–60 mL seaweed extracts was sprayed on per four plants in a pot. The recommended fertilizers (0.310, 0.421, and 0.365 g urea, triple superphosphate, and muriate of potash, respectively) were mixed uniformly in each pot (T_2) before seed sowing, corresponding to 50–70–60 kg urea, triple superphosphate, and muriate of potash per hectare (FRG, 2018). Required intercultural operations like weeding, insecticides application and irrigation were applied as per requirements.

Experimental design

The experiment was performed using a completely randomized design (CRD) with three replications.

Growth and agronomic measurement

At 15 days after the 2nd time foliar application of seaweed extracts solution three plants were harvested from each pot. Plant growth-related parameters viz. number of leaves per plant, number of branches per plant, individual leaf area, and dry weight of leaf, stem and roots were measured. At the physiological maturity stage, the rest three

plants from each pot were harvested and grain yield and yield contributing traits were recorded.

Measurement of chlorophyll (SPAD values) in leaf

Fully developed three leaves in upper side of one plant in each pot were selected and SPAD values were measured using SPAD meter (SPAD-502Plus KONICA MINOLTA, Japan) at 15 days after the 2nd time foliar application of seaweed extracts.

Statistical analysis

The recorded data underwent an analysis of Completely Randomized Design (CRD) (Gomez & Gomez, 1984) using CropStat statistical software version 7.2. All treatments were compared using Least Significance Difference (*LSD*) test at 5% level of significant.

RESULTS AND DISCUSSION

Effect of seaweed extracts on number of branches per plant, number of leaf per plant and leaf area of mungbean

The foliar application of seaweed extracts significantly influenced the number of branches per mungbean plant. The number of branches per plant had been doubled due to the application of seaweed extracts by compared to the control condition (Table 2). The maximum number of branches was 2.67 per mungbean plant found in plant treated with 20% seaweed extracts. The plants treated with water only provided on an average number of branches (0.83) in mungbean plants. The branches number per plant 2, 1.17, 1.17 and 1.0 were recorded at 15, 25, 10 and 5% seaweed extracts application, respectively. Whereas, the number of branches per plant 2.17 was found in the basal application of chemical fertilizer as recommended this showed 161% more number of branches per plant than the control. The number of branches per plant increased 221, 140, 41, 40 and 20% over the control were when plants were sprayed with 15, 25, 10 and 5% seaweed extracts, respectively. Even the seaweed extracts showed 23.04% higher number of branches per plant in 20% extracts relative to the recommended fertilizer doses.

Table 2. Effect of seaweed extracts on number of branch per plant, number of leaf per plant and leaf area of mungbean

Treatments	Number of branches per plant	Number of leaf per plant	Leaf area (cm^2)
Control	0.83b	10.0c	28.0f
RDF	2.17a	10.83bc	36.26bc
5% Seaweed extracts	1.0b	10.17c	30.16ef
10% Seaweed extracts	1.17b	10.67bc	32.08de
15% Seaweed extracts	2.0a	11.67ab	37.07ab
20% Seaweed extracts	2.67a	12.50a	39.26a
25% Seaweed extracts	1.17b	10.50bc	33.87cd
CV (%)	2.6	7.0	4.8

RDF – Recommended doses of Fertilizers. Means followed by diverse letters in each parameter differ significantly by *LSD* at $p < 0.05$.

The seaweed extracts also influenced the number of leaf per plant remarkably (Table 2). The number of leaf of mungbean plant had been increased by up-to 25% relative to the control condition by 20% seaweed extracts application. The highest number of leaves found 12.5 when the mungbean plant was treated with 20% seaweed extracts and lowest number was 10 found in control condition. The number of leaf per

plant was 10.83 when plants were grown with recommended doses of fertilizers. The number of leaf were found 11.67, 10.67, 10.50 and 10.17 in mungbean plants treated with 15, 10, 25 and 5% seaweed extracts, respectively whichshowed 25, 16.67, 6.67 and 1.67% more leaves compared to control. In relation to recommended fertilizer dose, mungbean showed 8.30% more leaves than the control, at 20% seaweed concentration mungbean produced 15.42% more leaves when mungbean was grown in recommended doses of fertilizers.

Leaf area is an important morphological feature that act as major photosynthetic parts in plant body and influenced the plant growth and development. Maximum plant leaf area were 39.26 cm^2 found in at 20% seaweed extracts application followed by 37.07, 34.20, 32.08, 30.16 and 28.00 cm^2 in mungbean plant treated with 15, 25, 10 and 5% seaweed extracts, respectively. Minimum leaf area 28 cm^2 was recorded in control treatment. In the application of fertilizer as per recommendation, mungbean showed 36.26 cm^2 leaf area which less than that of is found in 20% seaweed extracts treatment and more than that of in 15% seaweed extracts application. In 20, 15, 25, 10 and 5% seaweed extracts application showed 40.21, 32.37, 22.14 14.5 and 7.71% higher leaf area than the control. In comparison between seaweed fertilizer and chemical fertilizer, it was observed that seaweed liquid fertilizer produced 8.27% more leaf area than the chemical fertilizer application.

So, it is clear that the number of branch per plant, number of leaf per plant and leaf area of mungbean were influenced by foliar application of seaweed extracts. Seaweed also showed better growth than the usage of chemical fertilizer. Similar results were also reported by Mohan et al. (1994) in *Cajanus cajan* (L.) *Mill* sp and Sivasankari et al. (2006) in *Vigna sinensis* L. as well as Kamaladhasan & Subramanian (2009) in Red gram. The increased leaf numbers and leaf area along with increased biomass was observed in tomato plants treated with red, brown, and green seaweed extracts reported by Ali et al. (2019) and Ramkissoon et al. (2017). The growth promotion of plants by the application of seaweed extracts have also been reported in several plants viz., marigold (Aldworth & Van Staden, 1987; Russo et al., 1993), strawberry (Battacharyya et al., 2015), maize, and tomato (Alam et al., 2013; Ali et al., 2016; Trivedi et al., 2018a, 2018b), where there was an increase in vegetative growth by the foliar spray of seaweed extracts (Athithan, 2014). *S*eaweed extracts have been shown to positive effect of soybean plant growth at all stages up to harvest (Ali et al., 2019 and 2021b). The maximum number of branches per plant were observed in the plant treated with low concentration of seaweed extracts which also supported by Sridhar & Rengasamy (2010a) found in *Tagetes erecta* (marigold) plant treated with crude extracts from the brown seaweed *Sargassum wightii*. In an another study of *Arachis hypogaea,* it also recorded highest number of branches and leaf area when the plant treated with seaweed liquid extracts than the basal application of recommended fertilizer. Crouch & van Staden (1991) reported that plant leaf area of young tomato plants was improved by seaweed extracts application. The increased growth of the crop may be due to the presence of some growth promoting substances present in the seaweed extracts (Mooney & Van Staden, 1986; Blunden, 1991). In addition, the growth enhancing potential of the seaweed extracts might be attributed to the presence of macro and micronutrients. The growth regulatory substances (seaweed oligosaccharides) induced the biosynthesis of hormones such as phyto-hormones abscisic acid, cytokinin, and auxin in treated plants

(Khan et al., 2009; Aremu et al., 2016; Patel et al., 2018; Ali et al., 2019; Renaut et al., 2019; Mukherjee & Patel (2020) that can promote crop growth.

Effect of seaweed extracts on leaf dry weight, stem dry weight, root dry weight and total dry weight of mungbean

The growth promoting attributes of seaweed extracts on mungbean plants accelerate the dry weight of different plant parts in vegetative stage remarkably. Due to the application of seaweed, the leaf dry weight of mungbean leaves had been increased up-to 94.23% in vegetative stage of plants compared to the control condition (Table 3). At 15 days after the foliar application of seaweed extracts, the highest dry weight of mungbean leaf was estimated as 7.40 g in 20% seaweed extracts application. Whereas, in this similar condition and stage, the plants treated with water only (control) had provided 3.81 g dry weight of leaves in mungbean plants. The second highest 6.03 g of leaf dry weight was observed due to the foliar application of 15% seaweed extracts followed by 4.91, 4.41 and 3.96 g at 25, 10 and 5% seaweed extracts application, respectively. Leaf dry weight (5.85 g) was found in the basal application of chemical fertilizer as recommended which showed 53.54% more dry weight than control. A 58.36, 28.78, 15.75, and 3.94% increment in leaf dry weight over the control was observed with 15, 25, 10, and 5% seaweed application, respectively. Even the seaweed extracts showed 26.5% higher leaf dry weight at 20% extracts compared to the recommended fertilizer.

Table 3. Effect of seaweed extracts on leaf dry weight, stem dry weight, root dry weight and total dry weight of mungbean

Treatments	Leaf dry weight (g)	Stemdry weight (g)	Root dry weight (g)	Total dry weight (g)
Control	3.82e	4.21e	0.47e	8.50d
RDF	5.85bc	6.15ab	0.78b	12.78b
5% Sea weed extracts	3.96de	4.61de	0.60d	9.17d
10% Sea weed extracts	4.41de	4.68de	0.66cd	9.75
15% Sea weed extracts	6.03b	5.16cd	0.73bc	11.92b
20% Sea weed extracts	7.40a	6.86a	0.95a	15.21a
25% Sea weed extracts	4.91cd	5.73bc	0.69bcd	11.33bc
CV (%)	10.8	9.6	8.1	8.6

RDF – Recommended doses of fertilizers, Means followed by diverse letters in each parameter differ significantly by *LSD* at $p < 0.05$.

Dry weight of mungbean stem at vegetative stage had been increased by 62.82% due to the beneficial effect of seaweed extracts compared to the control condition (Table 4). The highest amount of stem dry matter of mungbean was 6.86 g, achieved by the foliar application of 20% seaweed extracts. Conversely, the lowest dry weight of mungbean stem was only 4.21 g found in the untreated plants. Dry weights of mungbean stems, 5.73, 5.16, 4.78, and 4.61 g, were recorded by applying seaweed extracts at concentrations of 25, 15, 10, and 5%, respectively, and 36.11, 22, 39, 13.45, and 9.49% higher than control treatment. When chemical fertilizers were applied in soil, mungbean produced 6.15 g of stem dry weight, which was 45.97% higher than the control, and 20% seaweed extracts produced 11.55% higher stems compared to the recommended fertilizer doses.

The root dry weight of mungbean had been increased up-to 93.75% in vegetative stage of plants compared to the control condition (Table 3). At 15 days after the foliar application of seaweed extracts, the highest root dry weight of mungben was estimated as 0.93 g in 20% seaweed extracts application. In this similar condition and stage, the plants treated with water only provided 0.48 g dry weight of roots in mungbean plants. The second highest 0.71 g of root dry weight was observed due to the foliar application of 15% seaweed extracts followed by 0.69, 0.67 and 0.67 g at 25, 10 and 5% seaweed extracts application, respectively. Root dry weight 0.78g was calculated with the recommended chemical fertilizer application, showing 62.50% more dry weight than the control. At 15, 25, 10, and 5% extracts, root dry weights increased by 47.92%, 43.75%, 39.58%, and 29.17%, respectively, over controls. Even the seaweed extracts showed 19.23% higher root dry weight at 20% extracts compared to the recommended dose.

Total dry weight is one of attributes that indicate the plant growth and development in the vegetative stage. Total dry matter accumulation of mungbean plant was largely influenced by different level of seaweed extracts which is increased up-to 78.94% relative to control (Table 4). The highest amount of total dry weight (15.21 g) of mungbean was measured in 20% seaweed extracts. The second highest total dry weight found in RDF was 12.78 g which was 50.35% higher than that of plant sprayed with water only (control). When the plants were treated with 15, 25, 10, and 5% sea weed extracts solution, the estimated total dry weight of the mungbean plants was 11.92, 11.33, 9.75, and 9.17 g, which was 40.24, 33.29, 14.71, and 7.88% higher than the control. Seaweed demonstrated a 19% higher total dry weight of munbean plants when compared to RDF. Similar findings have demonstrated that adding green and red seaweed extracts, as well as commercial seaweed extracts with compost enhances cucumber vegetative growth, dry and fresh weight, and yield (Ahmed & Shalaby, 2012). Xu & Leskover (2015) reported increased leaf fresh weight and leaf dry weight in spinach plant with the application of seaweed extracts, even if, under the stressed condition. Increment in fresh and dry weight might be due to nitrogen availability and improving soil physical properties as well as improved soil microorganism's activity on the account of seaweed application.

Table 4. Effect of seaweed extracts on number of pods per plant, number of seeds per pod, 100-seed weight and seed yield of mungbean

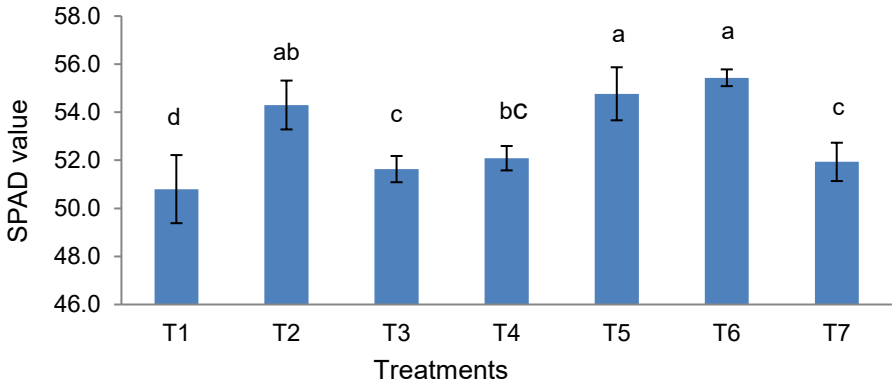
Treatments	Number of pods per plant	Number of seeds per pod	100-seed weight (g)	Seed yield per plant (g)
Control	12.67d	9.87b	4.87f	6.04e
RDF	18.67ab	10.21ab	5.78b	10.69b
5% Sea weed extracts	14.00cd	9.96ab	5.06e	7.05de
10% Sea weed extracts	15.00cd	10.06ab	5.26d	7.9cd
15% Sea weed extracts	16.33bc	10.24a	5.72b	9.63bc
20% Sea weed extracts	20.00a	10.30a	6.21a	11.66a
25% Sea weed extracts	15.67c	10.13ab	5.51c	8.76c
CV (%)	4.9	8.9	5.0	9.7

RDF – Recommended doses of fertilizers, Means followed by diverse letters in each parameter differ significantly by *LSD* at $p < 0.05$.

Seaweed based fertilizers improve plants growth by providing more nitrogen, phosphorus, and potassium, as well as supplying micronutrients and secondary metabolites (Karthick et al., 2013). After using seaweed extracts indicated that fresh and dry weight and leaf area increased probably due to increased nitrogen concentration and improving soil physical conditions through providing more energy for microorganisms helps to improve availability and absorption of mineral nutrients. There have been prior reports of increases in fresh weight, dry weight, root length, stem length, and chlorophyll content due to seaweed extracts (Sridhar & Rengasami, 2011).

Effect of seaweed extracts on chlorophyll (SPAD value) of mungbean

SPAD value which indicates the greenness of plant leaf thereby an indirect idea about the chlorophyll content of the leaves can be obtained it. SPAD value is significantly influenced by the application of seaweed extracts as organic fertilizer (Fig. 1). A SPAD value of 55.43, 9.11% higher than the control, was found at 20% seaweed extracts foliar application, followed by 54.77 at 15%, 7.81% higher than the control. The lowest SPAD value of 50.8 was recorded in the control (water spray only).



T_1 – Control; T_2 – RDF; T_3 – 5% seaweed extracts; T_4 – 10% seaweed extracts; T_5 – 15% seaweed extracts; T_6 – 20% seaweed extracts & T_7 – 25% seaweed extracts. Bars indicates (±*SE*).

Figure 1. Effect of seaweed extracts on SPAD value of mungbean.

The recommended fertilizer dose resulted in a 54.3 SPAD value, which is 6.89% higher than the control, followed by 52.09, 51.93, and 51.63 in the application of 10, 25, and 5% seaweed extracts, which resulted in 2.54, 2.22, and 1.63% more SPAD value than the control, respectively. SPAD values increased from 5% to 20% seaweed extracts application and then decreased at 25% seaweed concentration. Even a 20% concentration of seaweed extracts shows a SPAD 2.09% higher than the recommended fertilizer dose. According to Iswarya et al. (2019), the highest SPAD values were found when mungbean plants were treated with SWE spray (0.25%) to 25 DAS and 35 DAS. Rosa et al. (2021) found that treatment with *Ascophyllum nodusum* on water stressed soybean have higher SPAD value than plants without application and increased pigment concentration was observed using sesweed liquid fertlizer by Arumugam & Anantharaman (2009). Increment of SPAD value in mungbean may be due to the significantly enhanced the growth and nutrient uptake by the foliar applications of seaweed extracts.

Effect of seaweed extracts on yield and yield contributing characters of mungbean

Number of pods per plant

The number of pods per plants is considered as one of most important yield components of mungbean plants. The foliar application of seaweed extracts has a great influence to the number of pods in a mungbean plant. Application of seaweed extracts to mungbean plants increased the number of pods per mungbean plants by 50% compared to control conditions (Table 4). At 15 days after the foliar application of seaweed extracts, the highest number of pods was estimated as 19 in 20% seaweed extracts application. In this similar condition and stage, the plants treated with water only provided on an average 12.67 number of pods in mungbean plants. The number of pods per plant 16.33, 15.67, 15.00 and 13.16 were recorded at 15, 25, 10 and 5% sea weed extracts application, respectively, whereas the number of pods per plant 18.67 was found in the basal application of chemical fertilizer as recommended which showed 47.36% higher than the control. Increases of 28.95, 23.68, 18.42 and 39.47% number of podsper plants over control were observed with seaweed applications of 15, 25, 10 and 5%, respectively. Even the seaweed extracts showed 7.55% higher number of pods per plant in 20% extracts relative to the recommended doses.

Number of seeds per pod

The number of seeds per pod is directly related with the final yield content of mungbean plants. The average numbers of seeds per pod of mungbean were varied due to the inducement of seaweed extracts to the mungbean plants. The application of sea weed extracts to the mungbean plants caused 4.36% higher number of seeds per pod compared to the control condition (Table 4). The highest number of seeds per pod were 10.3 found in at 20% sea weed extracts application followed by 10.24, 10.13, 10.05 and 10.21 in mungbean plant treated with 15, 25, 10 and 5% seaweed extracts,respectively. Minimum number of seeds per podwas recorded in control (9.87) in the application of fertilizer as recommendation, mungbean showed 10.21 numbers of seeds per pod which is 3.44% higher than the control and less than that of found in 20% sea weed application. In 15, 25, 10 and 5% sea weed extracts application showed 3.75, 2.63, 1.82 and 0.91% increased number of seeds per pod over the control, respectively.

100-seed weight

Seed weight is significantly influenced by the application of seaweed extracts as organic fertilizer. The maximum mungbean weight of 100 seed was 6.21 g with 20% seaweed extracts, 27.3% more than the control (4.87 g), followed by 5.72 g in with 15% seaweed extracts which was 17.42% more than with control (Table 4). The lowest weight of 100-seeds was 4.87 g recorded in control (water spray only). 5.78 g 100 kernel weight mungbean found using the recommended dose of fertilizer was 18.47% more than the control.In the application of 25, 10 and 5% seaweed extracts showed 5.51, 5.26 and 5.06 g weight of 100-seed which were 13.3, 8.21 and 3.91% more than the control, respectively.

Seed yield

The yield is one of the most vital components of any crop which defines the productivity of that crop. The foliar application of seaweed extracts has a great influence to the final yield of mungbean plant. The seed yield of mungbean had been increased due to the application of seaweed extracts by 93.14% compared to the control condition (Table 4). The maximum yield was 11.66 g per mungbean plant found in plant treated with 20% sea weed extracts. The plants treated with water only provided on an average 6.04 g seed in mungbean plants. The yield per plant 9.63, 8.76, 7.90 and 7.05 g were recorded at 15, 25, 10 and 5% seaweed extracts application, respectively. Whereas the yield per plant 10.69 g was found in the basal application of chemical fertilizer as recommended which showed 77.13% more yield per plant than the control. About 59.63, 45.17, 30.96 and 16.74% increased seed yield per plant over the control were observed due to 15, 25, 10 and 5% seaweed application, respectively. Even the seaweed extracts showed 9.04% higher yield per plant in 20% extracts relative to the recommended doses. According to Ramamoorthy et al. (2006), the foliar application of aqueous extracts of *Ulva lacuta*, *Turbinariaconoides* and *Sargassum polycystum* gives positive result on the growth and yield of pea and black gram. The foliar application of liquid extracts of *Kappaphycus alvarezii* triggers the yield potency of *Lycopersicon esculentum* (Zodape et al., 2011). Bai et al. (2011) coincide with our findings in the application of liquid extracts of *Pandina pavonia* provides maximum yield of pulses. Kumar & Sahoo (2011) reported higher yield in *Triticum aestivum* upon foliar application of 20% extracts of *Sargassum wightii*. Similar kinds of result are also showed by Xavier et al. (2007); Zodape et al. (2008); Sridhar & Rengasamy (2010b); Thevanathan et al. (2005); Sivasankari et al. (2006) in several crops. Increased in yield may also be related to some nutritional elements, especially iron, zinc and manganese in compost and potassium, calcium, magnesium, sulfur and iron in our used seaweed extracts, which is supported by Zodape et al. (2009) who found increased yield of green gram using *Gracilaria* extracts. These elements can stimulate vegetative growth, chlorophyll biosynthesis and photosynthesis, which in turn affect flowering and fruit production (Ahmed & Shalaby, 2012).

CONCLUSIONS

The foliar application of seaweed extracts derived from *Gracilaria tenuistipitata* var. *liui* in mungbean plants had a positive impacts on number of leaves per plant; number of branches per plant; individual leaf area dry weight of leaf, stem, roots and whole plant; leaf chlorophyll content and yield of mungbean. The application of 20% seaweed extracts increased the grain yield of mungbean plants by 93.14 compared to control (water spray only) conditions and 9.04%, than the recommended fertilizers doses. Among the different concentration of seaweed extracts applied, 20% concentration showed the best performance in the improvement of growth and yield of mungbean plants even when plants were grown as recommended doses of fertilizers. Further study is required, though, to determine more physiological and molecular pathways to boost the development and production of mungbean utilizing the seaweed *Gracilaria tenuistipitata* var. *liui*.

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Agronomic traits determinants of superior varieties and millable cane productivity of sugarcane (*Saccharum officinarum* L.) on dryland, Indonesia

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Abstract. Indonesia is one of the sugar-producing countries in the world, with most of the planting area shifted to dryland, sub-optimal. During the development of production, it is necessary to select varieties that are suitable for dryland. This study aimed to determine the adaptation of superior sugarcane varieties on dryland. This study used a split-plot under repeated Randomized Complete Block Design (RCBD). Six superior sugarcane varieties used in this research were *Saccharum* 'CMG Agribun ', *Saccharum* 'AAS Agribun', *Saccharum* 'ASA Agribun', *Saccharum* 'AMS Agribun', *Saccharum* 'PS864' and *Saccharum* 'Bululawang'. The variables observed were plant height, stem diameter, number of segments, and number of tillers at the age of 13, 15, and 17 WAP (weeks after planting). The results showed that growth parameters, namely plant height at 13 and 15 WAP, number of tillers at 15 and 17 WAP, and stem diameter at 13 and 15 WAP, showed better growth, indicating superior agronomic properties of a sugarcane variety on dryland. *Saccharum* 'AMS Agribun' and *Saccharum* 'Bululawang' varieties, stem diameter, increased with spacing treatment at the early growth of 17 weeks after planting. The *Saccharum* 'PS864' was the best, having the highest average of agronomic values compared to other varieties. The *Saccharum* 'PS864' had the highest plant height and number of internodes. The highest number of tillers was obtained in the *Saccharum* 'AAS Agribun' varieties.

Key words: adaptation, dryland, sugarcane, superior varieties.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a widely grown plant species. The species has been cultivated by millions of farmers in Indonesia to produce sugar as a source of livelihood, which helps the national economy (Indonesian Sugar Cane Statistic, 2020). Besides being used as a processed product, it is also a commodity as raw material for

industrial foods or beverages. Sugarcane is also a potential source of energy from bagasse biomass (Brunerova et al., 2018). The demand for sugar is increasing following the increasing population. However, there is still a gap between the demand and production of domestic sugar.

Southeast Sulawesi is a potential area for planting sugar cane. Southeast Sulawesi can be used as a sugarcane development area by considering various problems or obstacles in the dryland planting area. One of the constraints is most planting areas in Southeast Sulawesi are dominated by Ultisol or Podzolik Red Yellow (PRY). This type of soil with low soil fertility due to high acidity, low organic matter content, macronutrient deficiency, high Al saturation, and very high Fe content (Sujana & Pura, 2015). In addition, the limited water availability on dryland is another important inhibiting factor in using agricultural land to support optimal production.

The success of each planting is dependent on the availability of quality seeds. Unfortunately, most farmers still use poor-quality seeds with genetic (Mulyono, 2011). This is one of the factors causing the low productivity of national sugarcane. The use of quality seeds includes the use of superior varieties. One example of a superior variety of sugarcane is PS864. The best yields of the ridges and furrows planting systems were PS864 compared to Bululawang, PSJT 941, VMC, PS 881, and Kidang Kencana varieties (Rokhman et al., 2014). The superior varieties of sugarcane released by the Center for Plantation Research and Development in 2017 were *Saccharum* 'CMG Agribun ', *Saccharum* 'AAS Agribun', *Saccharum* 'ASA Agribun', *Saccharum* 'AMS Agribun'. These varieties produced millable cane, sucrose content, and sugar in the range of 120–200 tons ha^{-1}, 10–11%, and 12–20 tons ha^{-1}, respectively (Center plantation research and development, 2018).

Superior sugarcane varieties adapted to drought stress, especially on dryland in Southeast Sulawesi. According to Hemaprabha (2014), new sugarcane varieties are released for specific purposes, e.g., sugarcane is rated as a drought-tolerant variety. This is in line with the report of Zhao et al. (2022) that there are variations in agronomic characteristics among sugarcane varieties, such as single-stem weight, height, stem diameter, and millable cane could be under certain climatic conditions. Therefore, the use of high-yielding varieties in this study is expected to provide results on the adaptation of high-yielding sugarcane varieties on dryland in Southeast Sulawesi.

MATERIALS AND METHODS

The research was conducted on the dryland of the Onembute Experimental Garden in Anggondara Village, Palangga District, South Konawe Regency, Southeast Sulawesi. The experiment was located at an altitude of 93 m. According to Koppen's classification, it belongs to a tropical monsoon climate (S: 4°21'07" and E: 122°20'15"). In 2018, the monthly precipitation of January, February, March, April, May, June, July, August, September, October, November, and December were 227.0; 210.0; 231.0; 139.0; 275.5; 386.5; 290.5; 0; 77.0; 2.5; 202.5; 150.09 mm per month. In 2018, the monthly average temperature was 26.69 °C (maximum: 28.0 °C, minimum: 25.2 °C). Monthly sunshine duration (%) in 2018 of January, February, March, April, May, June, July, August, September, October, November, and December were 51, 57, 50, 53, 30, 32, 37, 62, 72, 87, 61, 52.

The experimental design was a split-plot under repeated Randomized Complete Block Design (RCBD). The main plot was the row spacing of planting systems, namely (1) the row spacing was 100 cm (40×100 cm) and (2) the row spacing was 125 cm (40×125 cm). The subplot treatments consisted of 6 varieties, namely (1) *Saccharum* 'CMG Agribun ', (2) *Saccharum* 'AAS Agribun', (3) *Saccharum* 'ASA Agribun', (4) *Saccharum* 'AMS Agribun', (5) *Saccharum* 'PS864' and (6) *Saccharum* 'Bululawang'. The treatment layout was performed by randomizing the subplots (varieties) on the main plot (row spacing). Variables observed were plant height (cm), stem diameter (cm), number of internodes, and number of tillers at 13, 15, and 17 WAP. Plant height was measured from the top of the ground where the sugarcane grew to the tip of the leaf buds/young leaves of the plant. The stem diameter was measured on the second section of the plant from the ground surface using a caliper. The number of internodes was observed by counting the number of internodes that had been formed. The number of tillers was observed by counting the number of tillers that had grown and formed plants.

Statistical analysis

The data were analyzed according to the intervals of observation with analysis of variance (ANOVA) of a split-plot design under RCBD using the SAS 9 program for Windows. If there was an interaction between factors, the interaction effects were compared. Then, the treatment effects were compared based on Duncan's Multiple Range Test at $p \leq 0.05$. A Principal Component Analysis (PCA) Biplot was also carried out on agronomic observation parameters to find out the agronomic traits determinant of superior varieties of sugarcane growth.

Land cultivating

The land used for the experiment was previously planted with corn. Soil tillage was carried out in two stages: primary tillage and secondary tillage. Primary tillage was carried with a rotary plow to uproot the previous crop stubbles and break the soil into clods. Secondary tillage was carried with a harrow to break the soil clods to bring it to a fine tilth and leveling to create ridges and furrows. Ridges and furrows were formed in a field final preparation using a tractor. The depth of the furrows should be around 25 cm. The area of sugarcane planting area was 5 hectares. The physical and chemical properties of soil were texture clay with sand:dust:clay composition 30%:26%:44%, pH 4.5, bulk density 1.33 g cm^{-1}, soil moisture capacity/254 pf = 45.99%, C organic 2.3%, cation exchange capacity (cmol kg^{-1}) 31.65%, available P-content (mg P_2O_5 $100g^{-1}$) 33.77, potential K (mg K_2O 100 g^{-1}) 23,77 (Mulyaningsih et al., 2015).

Planting

Sugarcane seeds used in this experiment was six months old. Sugarcane seeds were planted directly without giving any treatment by stem cutting of sugarcane stalks having two buds. The planting system used was 'single-row planting' with the spacing adapting to the treatment of the main plots, namely (1) row spacing of 125 cm and (2) row spacing of 100 cm.

Plant maintenance

Provision of inorganic fertilizer was in the form of urea 300 kg ha^{-1} and NPK phonska 500 kg ha^{-1} with three application times, at 0, 1.5, and 3 months after planting. Applying organic fertilizer, i.e., cow manure, was done only once, at the beginning of planting during tillage. Soiling, weeding, and fertilizing inorganic fertilizers were carried out simultaneously. Control of plant-disturbing organisms used Nordox 56 WP (containing 56% Copper oxide active ingredient or equivalent to 50% Cu). Watering was done by using a 3-inch hose. During dry season, watering was done every three days; during rainy season, watering was unnecessary. However, when the sugarcane reached 2.5 months old, watering was done less frequently, every five days.

RESULTS AND DISCUSSION

Plant height

The variety significantly affected the height of sugarcane at the ages of 13, 15, and 17 WAP. In addition, the variety interacted with the row spacing and increased the plant height significantly at the age of 15 WAP (Table 1).

Table 1. The result of ANOVA of plant height at the age of 13, 15, and 17 WAP

Source	df	Pr > F Plant height (cm) at different ages		
		13 WAP	15 WAP	17 WAP
Repitation	4	0.9239	0.0709	0.9274
Variety (V)	5	0.0004	<.0001	0.0030
Repitation *V	20	0.7778	0.0231	0.8807
Row spacing (R)	1	0.8964	0.1509	0.9671
V*R	5	0.4778	0.0402	0.2516
CV (%)		13.08	9.25	13.94

Table 2. The effect of varieties on sugarcane plant height

Varieties	Plant height (cm) at different ages	
	13 WAP	17 WAP
CMG Agribun	56.75^{c}	175^{c}
AAS Agribun	63.5^{bc}	205.5^{b}
ASA Agribun	57.3^{c}	187.5^{bc}
AMS Agribun	61.8^{bc}	192.9^{bc}
PS 864	75.15^{a}	232.1^{a}
Bululawang (BL)	67.95^{b}	205.3^{b}

Numbers followed by the same letters in the same columns did not differ significantly at $p < 0.05$ according to Duncan's multiple range test.

Saccharum 'PS864' is a sugarcane variety with the highest plant height compared to other varieties, at ages 13 and 17 WAP (Table 2). The performance of plant height at the age of 13 WAP is shown in Fig. 1.

These findings are in line with Ahmed et al. (2010) that the plant height is influenced by the variety or genotype of each plant. Sugarcane genotypes differ in stem height, whereas the sugarcane stem is the economical part. Sugarcane produces relatively large biomass and high storage of photosynthate in the form of sucrose in the stem (Verma et al., 2013).

At the age of 15 WAP, the *Saccharum* 'PS864' with a row spacing of 40×100 cm produces the highest plant height growth,

Figure 1. Plant performance in the experimental field at the age of 13 WAP.

significantly different than all other treatment combinations, except for the same variety at a denser spacing (40×100 cm) and the *Saccharum* 'Bululawang' at a wider spacing (40×125 cm). Among the varieties, it seems that only the *Saccharum* 'Bululawang' interacts significantly with the row spacing, determining plant height. Saccharum 'Bululawang' has the highest plant height at wide spacing (40×125 cm), which is significantly different from the narrow spacing (40×100 cm) (Table 3).

Plant height is an important parameter that shows the growth process, determined by shoot and root growth parameters (Sulistiono, 2017). Sugarcane plants, with better agronomic characteristics of stem are supported by having better root properties: root length, root surface area, and root diameter at a certain age (Table 4). According to Sulistiono et al. (2018), better root growth properties inoculated by mycorrhizae, i.e., root length, root surface area, and secondary roots, are required to support the growth of the shoots in sugarcane at the early growth.

Table 3. The effect of interaction on sugarcane plant height at the age of 15 WAP

Varieties	Row spacing (cm)	Plant height (cm)
Saccharum 'CMG Agribun'	40×100	157.80 d
Saccharum 'CMG Agribun'	40×125	162.60 d
Saccharum 'AAS Agribun'	40×100	176.80 b–d
Saccharum 'AAS Agribun'	40×125	2,174.60 b–d
Saccharum 'ASA Agribun'	40×100	162.00 d
Saccharum 'ASA Agribun'	40×125	169.40 cd
Saccharum 'AMS Agribun'	40×100	174.60 b–d
Saccharum 'AMS Agribun'	40×125	158.60 d
Saccharum 'PS864'	40×100	201.00ab
Saccharum 'PS864'	40×125	206.20 a
Saccharum 'Bululawang'	40×100	195.60 a–c
Saccharum 'Bululawang'	40×125	159.20 d

Numbers followed by the same letters in the same columns did not differ significantly at $p < 0.05$ according to Duncan's multiple range test.

Table 4. Root properties that promoted increased growth of sugarcane plant height

Root properties	Parameter Estimate	Standard Error	Type II SS	Percentage of influence	*P*–Value
Total root length at the age of 8 WAP	0.342	0.03287	40,693.0	40.33	<.0001
Root surface area at the age of 5 WAP	-0.05	0.00666	20,299.0	20.12	<.0001
Root diameter at the age of 8 WAP	1,377.48	133.872	39,887.0	39.53	<.0001
Sum of Residuals	33.71	First Order Autocorrelation			0.4318
Sum of Squared Residuals	18,522.88	Durbin-Watson D			1.1290
Sum of Squared Residuals - Error SS	-0.000	R^2			0.98

Sulistiono (2017).

In shoot growth, plant height is determined by several growth characteristics. The physiological characteristics of sugarcane shoots that determine plant height are net assimilation rate (NAR), relative growth rate (RGR), and leaf area (LA) at a certain plant age, as shown in Table 5 (Sulistiono, 2017).

The results of this study indicate that at a certain age, the height of sugarcane plants is determined by the interaction between varieties and row spacing. This finding is consistent with the report of Sulistiono (2017) that at a certain age, the interaction effect of variety and row spacing appears to determine sugarcane growth. The leaf area index is significantly determined by the interaction of row spacing and variety at the age of 2 and 9 months after transplanting (Sulistiono, 2017). This is due to plant growth factors among varieties. Varieties with higher plant heights will place leaves to form LA and

leaf area index (LAI), as well as chlorophyll content, which is more optimal in absorbing sunlight. According to Aboagye (2003), optimal LAI is immediately achieved at narrow spacing. Sugarcane plants that have optimal LAI lead to optimal absorption of sunlight by leaf area to be optimal for photosynthesis (Helal & Mengel, 1981). Optimal LAI in sugarcane is achieved at the age of 4 months with a value of 3.08–5.52 (Sulistiono, 2017).

Table 5. Physiological characteristics that promote increased growth of sugarcane plant height

Growth characteristics	Parameter Estimate	Standard Error	Type SS	II Percentage of influence	*P*-Value
NAR at the age of 5 WAP	2,818.508	306.28	33,543.0	44.89	<.0001
RGR at the age of 8 WAP	1,690.822	191.03	31,031.0	41.52	<.0001
LA at the age of 5 WAP	0.076	0.016	81,59.62	10.92	<.0001
LA at the age of 8 WAP	0.0069	0.003	19,93.44	2.67	0.0289
Sum of Residuals	45.482	First Order Autocorrelation			0.453
Sum of Squared Residuals	20,513.28	Durbin-Watson D			1.06
Sum of Squared Residuals-Error SS	-0.000	R^2			0.97

Sulistiono (2017).

Varieties with higher plant heights have an impact on increasing plant fresh biomass weight. Varieties determine the fresh biomass of sugarcane plants (Pereira et al., 2013; Schultz et al., 2017). In addition, differences in varieties also determine the ability to form leaf chlorophyll and adaptive capacity in the planting area, such as saline conditions (Willadino et al., 2011). Knowing the adaptive ability of suitable varieties at the planting location based on the parameters of plant height becomes is important for the selection of varieties.

The optimal sugarcane growth with the best plant height is also influenced by better root properties, such as total root length, root surface area, and root diameter (Table 4). The optimal root properties are important in the growth of sugarcane. Roots are essential for different functions for plant growth, including plant anchorage (Rebouillat et al., 2009), water and mineral nutrient uptake, and synthesis of various essential compounds and plant shoot biomass (Hishi et al., 2015; Nagakura et al., 2015; Sulistiono et al., 2018) and improve the physical conditions of the soil (Cai et al., 2021). Thus, the results of this study show that sugarcane varieties with better plant height agronomic properties indicate having better root properties.

Stem diameter

Variety had a very significant effect on the sugarcane stem diameter at the ages of 13, 15 and 17 WAP. At the age of 17 WAP, there was a significant interaction ($p < 0.05$) between variety and row spacing treatments in affecting the stem diameter (Table 6).

The *Saccharum* 'CMG Agribun', *Saccharum* 'ASA Agribun', and *Saccharum* 'AMS Agribun' provided

Table 6. The result of ANOVA of stem diameter at the age of 13, 15, and 17 WAP

Source	df	Pr > F steam diameter (cm) at different ages		
		13 WAP	15 WAP	17 WAP
Repitation	4	0.4113	0.2998	0.1687
Variety (V)	5	0.0047	0.0080	0.0013
Repitation *V	20	0.8052	0.6297	0.6094
Row spacing (R)	1	0.9964	0.0745	0.0600
V*R	5	0.5838	0.1812	0.0381
CV (%)		10.59	7.66	7.59

larger stem diameters at the age of 13 WAP compared to other varieties. At the age of 15 WAP *Saccharum* 'CMG Agribun', *Saccharum* 'ASA Agribun', *Saccharum* 'AMS Agribun' and *Saccharum* 'PS864' provided the largest stem diameters (Table 7).

Table 7. The effect of varieties on sugarcane stem diameter

Varieties	Stem diameter (cm) at different ages	
	13 WAP	15 WAP
Saccharum 'CMG Agribun'	2.79^{ab}	2.71^{a}
Saccharum 'AAS Agribun'	2.51^{cd}	2.47^{b}
Saccharum 'ASA Agribun'	2.97^{a}	2.74^{a}
Saccharum 'AMS Agribun'	2.79^{ab}	2.68a
Saccharum 'PS864'	2.69^{bc}	2.63^{ab}
Saccharum 'Bululawang'	2.45^{d}	2.44^{b}

Numbers followed by the same letters in the same columns did not differ significantly at $p < 0.05$ according to Duncan's multiple range test.

Table 8. The effect of interaction on stem diameter at the age of 17 WAP

Varieties	Row spacing (cm)	Stem diameter (cm)
Saccharum 'CMG Agribun'	40×100	2.76 b
Saccharum 'CMG Agribun'	40×125	2.90 ab
Saccharum 'AAS Agribun'	40×100	2.43 cd
Saccharum 'AAS Agribun'	40×125	2.65 bc
Saccharum 'ASA Agribun'	40×100	2.75 b
Saccharum 'ASA Agribun'	40×125	2.94ab
Saccharum 'AMS Agribun'	40×100	3.04 a
Saccharum 'AMS Agribun'	40×125	2.75 b
Saccharum 'PS864'	40×100	2.66 bc
Saccharum 'PS864'	40×125	2.69 bc
Saccharum 'Bululawang'	40×100	2.37 d
Saccharum 'Bululawang'	40×125	2.71 bc

Numbers followed by the same letters in the same columns did not differ significantly at $p < 0.05$ according to Duncan's multiple range test.

At the age of 17 WAP, the stem diameter of several varieties increases significantly with differences in row spacing. *Saccharum* 'AMS Agribun' produces the best stem diameter, significantly at denser spacing. Conversely, *Saccharum* 'Bululawang' produces significantly higher stem diameter at looser row spacing (40× 125 cm) (Table 8). This result is in line with the report by Gomathi et al. (2013) and Ahmed et al. (2013) that differences in varieties affect the diameter of the stems produced. In addition, the results are consistent with the report by Sulistiono et al. (2020) that wide inter-rows spacing (75 cm) in the planting material for bud chips results in significantly larger diameters than the dense inter-rows spacing of 45 cm or 30 cm (Sulistiono et al., 2020).

Diameter is an important agronomic characteristic because it determines the weight of sugarcane stalks and the volume of sucrose storage. The weight of millable canes is important for estimating productivity (millable canes). Estimated productivity is known from (1) the number of millable canes per clump, (2) the number of clumps in the planting row, (3) the weight of millable cane per stem, and (4) plant height (Taryono & Sulistiono, 2022). Sugarcane stem weight positively correlates with cane yields (productivity) and determines the productivity of 80.13% (Sulistiono, 2017). According to Jane et al. (2020), an appropriate model should be developed to determine the diameter of the cane stem. It can be used to model the initial growth and production of sugarcane varieties. Varieties with larger diameters will determine the ability of the source steam phloem to accumulate photosynthate (sucrose) in sugarcane stalks in conjunction with the photosynthesis sink process (Chandra et al., 2011; Wang et al., 2013).

Number of internodes

Varieties had a significant effect ($p < 0.05$) on the number of sugarcene internodes at the ages of 13 and 15 WAP, as well as having a very significant effect ($p < 0.01$) at the age of 17 WAP (Table 9).

Table 9. The result of ANOVA of the number of internodes per stalk at the age of 13, 15, and 17 WAP

Source	df	Pr > F number of internodes per stalk at different ages		
		13 WAP	15 WAP	17 WAP
Repitation	4	0.8954	0.6827	0.2593
Variety (V)	5	0.0491	0.0268	0.0082
Repitation *V	20	0.6373	0.5130	0.8183
Row spacing (R)	1	0.7575	0.1179	0.3858
V*R	5	0.4203	0.9180	0.5933
CV (%)		21.19	19.97	18.17

The *Saccharum* 'CMG Agribun', *Saccharum* 'PS864' and *Saccharum* 'Bululawang' provide the highest numbers of internodes that differ significantly from other varieties at the ages of 13 and 15 WAP. Meanwhile, at 17 WAP, the highest number of internodes is obtained at *Saccharum* 'CMG Agribun' and *Saccharum* 'PS864' (Table 10). These results are in line with Santoso et al. (2015) and Ahmed et al. (2013) that different varieties produce differences in the number of internodes per stem. According to Sulistiono et al. (2020), several sugarcane varieties (*Saccharum* 'Kidang Kencana', *Saccharum* 'Bululawang' and *Saccharum* 'PS881') provide a high number of internodes at a wide inter-rows spacing of 60–75×100 cm. On the other hand, *Saccharum* 'PS864' provide a high number of internodes at a narrow inter-rows spacing of 45×100 cm. These results indicate that in certain varieties, the number of internodes can be increased by selecting adaptive variety or row-spacing treatment.

Table 10. The effect of different varieties on the number of internodes per stalk

Varieties	Number of internodes at different ages		
	13 WAP	15 WAP	17 WAP
Saccharum 'CMG	4.3^{a}	5.3^{a}	7.2^{ab}
Saccharum 'AAS	3.7^{ab}	4.2^{b}	6.2^{bc}
Saccharum 'ASA	3.3^{b}	4.2^{b}	6.2^{bc}
Saccharum 'AMS	3.4^{b}	4^{b}	5.8^{c}
Saccharum 'PS864'	$4a^{b}$	4.6^{ab}	7.5^{a}
Saccharum 'Bululawang'	$4a^{b}$	4.7^{ab}	5.7^{c}

Numbers followed by the same letters in the same columns did not differ significantly at $p < 0.05$ according to Duncan's multiple range test.

Sugarcane varieties with more internodes have the potential to increase the organ storing more sugar. According to McCormick et al. (2006), the sucrose content in internodes number 7–12 from the bottom of the stem is about ten times higher than the internodes at the top. Thus, the number of stem internodes becomes an agronomic trait of the superior sugarcane. In general, the results are in line with those reported by Silva et al. (2012), that the number of internodes is one of the determinants of the agronomic superiority of sugarcane.

Number of tillers

Variety had a very significant different ($p < 0.01$) on the number of sugarcane tillers at the ages of 13 and 15 WAP. However, at the age of 17 WAP, both the variety and row spacing treatments do not significantly different on the number of tillers (Table 11).

The *Saccharum* 'AAS Agribun' has the highest number of tillers which is significantly different from other varieties at the age of 13 and 15 WAP (Table 12). This result shows that the number of tillers is a genotype factor and the time of tillering phase is different among varieties. These results align with the reports of Ahmed et al. (2013) that the varieties significantly influence the number of sugarcane tillers.

Saccharum 'AAS Agribun' is a variety that has an ability to produce more tillers, significantly different than other varieties (Table 12). According to Pramuhadi (2010), the emergence of tillers is induced by the success of germination, which is largely determined by the inherent factors, namely varieties (genotype). Furthermore, tillering is determined by the germination process, and the growth of the sprouts itself on the sugarcane stalks underground to become new plants.

Generally, the most important parameter of the number of tillers is the effective tiller. This is because effective tillers will be millable canes for productivity. According to Sulistiono (2017), effective tillers or the number of millable canes per clump are determined by physiological characteristics, namely RGR, LA, and NAR, and specific leaf weight (SLW) at a certain age (Table 13).

Table 11. The result of ANOVA of the number of tillers at the age of 13, 15, and 17 WAP

Source	df	Pr > F number of tillers a different ages		
		13 WAP	15 WAP	17 WAP
Repitation	4	0.6496	0.253	0.6686
Variety (V)	5	0.0020	0.021	0.1366
Repitation *V	20	0.8981	0.6064	0.8206
Row spacing (R)	1	0.4165	0.6594	0.6000
V*R	5	0.7132	0.5286	0.3113
CV (%)		32.06	31.55	32.50

Table 12. The effect of varieties on the number of tillers

Varieties	Number of tillers at different ages	
	13 WAP	15 WAP
Saccharum 'CMG Agribun'	4.1[b]	4.3[b]
Saccharum 'AAS Agribun'	6.7[a]	6.7[a]
Saccharum 'ASA Agribun'	3.8[b]	3.8[b]
Saccharum 'AMS Agribun'	3.9[b]	4[b]
Saccharum 'PS864'	3.5[b]	3.9[b]
Saccharum 'Bululawang'	3.8[b]	4.7[b]

Numbers followed by the same letters in the same columns did not differ significantly at $p < 0.05$ according to Duncan's multiple range test.

Table 13. Physiological characteristics that promoted the amount of millable cane per clump

Physiological characteristics	Parameter Estimate	Standard Error	Type II SS	Percentage of influence	*P*-Value
RGR at the age of 8 WAP	97.563	14.914	46.506	35.80	<.0001
LA at the age of 5 WAP	0.0069	0.0010	43.877	33.78	<.0001
NAR at the age of 5 WAP	80.177	16.891	24.486	18.85	<.0001
SLW at the age of 8 WAP	-84.779	34.781	6.457	4.97	0.0181
LA at the age of 11 WAP	0.00080	0.00038	4.925	3.79	0.037
LA at teh age of 8 WAP	-0.0018	0.00098	3.651	2.81	0.0723
Sum of Residuals	1.508	First Order Autocorrelation			0.148
Sum of Squared Residuals	54.340	Durbin-Watson D			1.659
Sum of Squared Residuals - Error SS	-0.000	R^2			0.96

The *Saccharum* 'PS864' has the highest plant height compared to other varieties, but has the lowest number of tillers. This result complements the report from Rosyady et al. (2017) that the higher the growth of sugarcane stems, the smaller the number of tillers. Sulistiono et al. (2019) reported that the variety and row spacing determine the number of tillers. The wide inter-rows spacing (75 cm) determines the number of tillers significantly different from the dense spacing (60–30 cm) (Sulistiono et al., 2020).

The number of effective tillers is an important agronomic parameter for superior high-yield sugarcane varieties. The number of effective tillers produced by the ability of plant growth is shown in the role of several growth parameters such as RGR, LA, NAR, and SLW at a certain age. The ability of a variety to have a high number of effective tillers indicates its optimal growth ability (Simoes et al., 2018). The number of tillers is the main parameter of superior traits of sugarcane varieties rather than plant height and diameter and to predict cane yield per hectare, which is a superior trait of agro-industrial characters in sugarcane (Grego et al., 2010; Silva et al., 2018).

Tillering depends on climate as a supporting environment factor. According to Samui et al. (2003), climatic elements that affect the tillering phase are minimum temperature, humidity, and high rainfall. The optimal temperature for germination and early growth-formation of tillers is 26–33 °C (Mayer & Clowers, 2011). On the other hand, the critical temperature for sugarcane in non-irrigated land is 19–20 °C (Bacchi, 1977). Meanwhile, the average temperature of the study site was 26.69 °C, with the highest rainfall compared to other months, which was 290.5–365.5 mm per month. This data shows that the climate element is still in the optimal range for the early growth of sugarcane.

Based on the PCA-Biplot, it shows that agronomic traits that show a high contribution to growth are characterized by orange color, namely: Plant Hight at the age of 13 WAP (PH13), Plant Hight at the age of 15 WAP (PH15), Number of tillers at the age of 15 WAP (NT15), Number of tillers at the age of 17 WAP (NT17), Stem Diameter at the age of 13 WAP (SD13) and Stem Diameter at the age of 15 WAP (SD15). On the other hand, the variables showing a medium contribution to growth are shown in yellow, namely: Plant Hight at the age of 17 WAP (PH17), Number of tillers at the age of 13 WAP (NT13), Number of Internodes at the age of 13 WAP (NI13) and Number of Internodes at the age of 17 WAP (NI17), while the lowest contribution is Number of Internodes at the age of 15 WAP (NI15) (Fig. 2).

Fig. 2 shows varieties with agronomic traits, such as higher plant height growth at 13–15 WAP of age, the ability to produce more tillers at 15–17 WAP of age, and higher diameter development at 13–15 WAP of age. These are superior agronomic traits of a variety of sugarcane to be able to grow better on dryland conditions. These growth parameters are superior agronomic traits obtained from the results of this study.

The agronomic properties of the number of tillers, the number of internodes, and the diameter of the stems are not hampered by the climatic conditions on dryland during growth. The agronomic properties that experienced growth pressure are plant height. Sulistiono (2017) reported that plant heights of *Saccharum* 'PS864' and *Saccharum* 'Bululawang' reach 1.95 m and 1.96 m on dryland with clay texture with a ratio of silt:dust:sand of 65.25%: 18.24%: 16.51%, pH 5, 78 and 5.1% organic matter during the rainy season. Meanwhile, the results of this study show that the plant height of *Saccharum* 'PS864' and *Saccharum* 'Bululawang' are 2.32 m and 2.05 m, respectively.

Therefore, the *Saccharum* 'PS864' and *Saccharum* 'Bululawang' do not decrease in plant height.

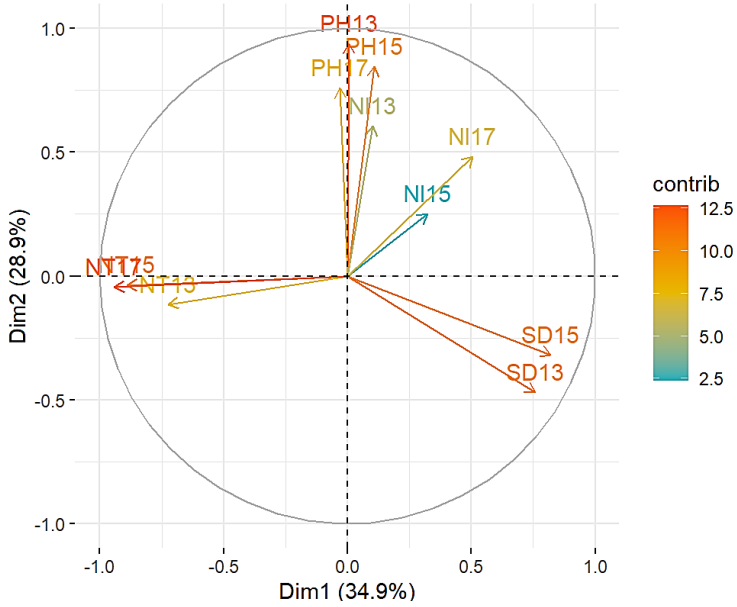

Figure 2. PCA_Biplot: Superior agronomic characteristics determine plant growth.
Caption: High contribution to growth = orange; Medium contribution to growth = yellow; Low contribution to growth = blue.

Saccharum 'CMG Agribun''s height is 14.6% lower than the optimal growth of *Saccharum* 'AAS Agribun'. Zhao et al. (2010) reported that the effect of drought on sugarcane causes a decrease in the growth of stem length, number of internodes, and shoot formation by 19%, 18%, and 45%, respectively. Taryono & Sulistiono (2022) stated that plant height is a grand growth phase susceptible to soil moisture stress. Therefore, low rainfall and the delay of rainy season or insufficient rain for the rapid grand growth phase causes inadequate stem elongation. This impacts the number of effective tillers (millable canes) and lower sugarcane weight (Taryono & Sulistiono, 2022). Rainfall data during the tillering phase is optimal for the 90-day tiller phase. However, the absence of rain in the 3rd month is thought to cause disturbed stem elongation in the CMG Agribun variety.

CONCLUSION

1. The plant height, stem diameter, number of internodes, and number of tillers are strongly influenced by variety. Whereas, on *Saccharum* 'AMS Agribun' and *Saccharum* 'Bululawang', the stem diameter increases affected by the rows spacing treatment at the early growth of 17 WAP. These growth parameters are the determinants of the productivity of millable canes.

2. Growth parameters, namely plant height at 13 and 15 WAP, number of tillers at 15 and 17 WAP, and stem diameter at 13 and 15 WAP, with better growth, indicating superior agronomic properties of a sugarcane variety on dryland.

3. The *Saccharum* 'PS864' has the highest plant height and number of internodes. The highest number of tillers is obtained in the *Saccharum* 'AAS Agribun'.

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Agronomic and physiological response of maize (*Zea mays* L.) hybrids to plant density in the dry and wet Middleveld of Eswatini

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Abstract. One of the factors limiting yield of maize in Eswatini is use of non-optimum plant density for the different maturity group of maize hybrids in different agro-ecologies. Thus, an experiment was conducted at Malkerns (wet Middleveld) and Luve (dry Middleveld) in Eswatini to determine the effects of plant density on growth, yield components and grain yield of maize hybrids. Factorial combinations of three maize hybrids [SC 403 (early maturing), SC 621 (medium maturing), SC 719 (late maturing)] and five plant densities (41,667; 44,444; 47,619; 50,000, and 57,143 plants ha^{-1}) were evaluated in Randomised Complete Block Design in three replications. The results showed that Malkerns had significantly higher crop growth rate (CGR) between V12 and R6 growth stages, relative growth rate (RGR) between V6 and V12 growth stages, mass of thousand kernels (395.60 g), aboveground dry biomass (22.71 t ha^{-1}) and grain yield (7.67 t ha^{-1}). Among maize hybrids, SC 719 produced significantly the highest CGR (18.37 g m^{-2} per day) between V12 and R6, aboveground dry biomass (23.05 t ha^{-1}), number of kernels per m^2 (2074), and grain yield (7.49 ha^{-1}). Moreover, SC 719 grown at Malkerns recorded significantly the highest leaf area index (LAI) at V6, and the highest CGR (31.35 g m^{-2} per day) between V6 and V12 and the tallest plants. The highest density of 57,143 plants ha^{-1} produced the highest LAI, aboveground dry biomass (21.53 t ha^{-1}) and grain yield (7.17 t ha^{-1}). Thus, late maturing maize hybrid SC 719 and plant density of 57,143 plants ha^{-1} (70 cm × 25 cm) can be used to enhance the productivity of maize in the Middleveld of Eswatini.

Key words: crop growth rate, grain yield, leaf area index, relative growth rate, yield components, Swaziland.

INTRODUCTION

Maize (*Zea mays* L.) is the most important crop in sub-Saharan Africa (SSA) and critical to food security with over 300 million Africans depending on it as their main staple food (Shiferaw et al., 2011). In view of its importance, the area of maize production in sub-Saharan Africa has increased by almost 60% from 2007 to 2017 (FAO, 2019). In the southern Africa (excluding South Africa), maize accounts for 19% of the average calorie intake per capita and the demand for the crop as food is increasing as a result of population growth (Shiferaw et al., 2011).

Maize is the main staple crop in Eswatini and constitutes about 95% of the country's cereal production (Dlamini & Masuku, 2011). According to FAO (2019) estimate, maize was grown on an area of 79,130 hectares with an average yield of 1.20 t ha^{-1}. On the other hand, Dlamini & Masuku (2011) reported an average maize grain yield of 4.42 t ha^{-1} on Swazi Nation Land. Thus, the yield obtained in the country is very low and highly variable as compared to the world average grain yield of 5.8 t ha^{-1} and to the yield obtained in southern Africa (4.58 t ha^{-1}) (FAO, 2019). A number of biotic and abiotic constraints contribute to low productivity such as use of inappropriate agronomic practices, drought, declining soil fertility, insufficient technology generation, lack of credit facilities, poor seed quality, diseases, insect pests and weeds (CIMMYT, 2004).

The use of non-optimal plant population per unit area is one of the agronomic practices that can negatively influence maize grain yield (Haarhoff & Swanepoel, 2018). Plant density and arrangement of plants in a unit area greatly determine resource utilisation such as light, nutrients and water; and development of crops particularly that of LAI, plant height, root length and density, yield and yield components, development of diseases and insect pests, and the seed cost (Grassini et al., 2011). Thus, optimisation of plant density is one of the main strategies for increasing yield. High plant density exposes the plant to shading resulting in reductions in leaf development, and leaf photosynthesis per plant thereby reducing the total biomass production and grain yield (Timlin et al., 2014; Yang et al., 2017). On the other hand, if sub-optimal plant density is used, yield will be low due to less number of plants per unit area resulting in less efficient utilization of growth resources. Hence, optimum plant density of maize will lead to effective utilization of soil moisture, nutrients, sunlight and thereby result in higher yields (Liu et al., 2004).

Maize is more affected by variations in plant density than other member of the grass family because of its low tillering ability (Abuzar et al., 2011). Plant density affects grain yield of maize by influencing yield components such as number of ears, number of kernels per ear, and kernel mass (Novacek et al., 2013). However, the optimum plant population of maize depends on several factors such as fertility status of the soil, soil moisture, varieties, and cultural practices (El-Hendawy et al., 2008). When provided with adequate water and nutrient, high plant density can result in an increased number of cobs per unit area, with ultimate increase in grain yield (Bavec & Bavec, 2002). For instance, Ma et al. (2005); Ren et al. (2016); and Haarhoff & Swanepoel (2018) reported that optimum plant population for maize increased with availability of soil moisture through rainfall or irrigation.

A positive association between maize yield and plant population was reported by DeBruin et al. (2017) in modern hybrids, but a contrasting response in older hybrids. The ability of newer hybrids to tolerate increased crowding stress can be attributed to lower lodging frequencies and higher nitrogen use efficiency (Al-Naggar et al., 2011). Optimum plant population density is generally higher for short-season than for long-season maize hybrids since for short-season hybrids, more plants are needed to reach the same amount of cumulative intercepted radiation because of their small leaf area per plant and small leaf area plasticity and a shorter duration of growth (Edwards et al., 2005).

However, in Eswatini, maize population of 44,444 plants ha^{-1} (90 cm × 25 cm) has been recommended without considering the numerous morphological and maturity differences that exist among varieties as well as the existence of soil and climatic differences among the agro-ecologies (Edje & Ossom, 2016). As new maize hybrids with different maturity duration are being developed by seed companies, there is a need to develop appropriate plant population for these varieties for different agro-ecologies of Eswatini. Thus, this study was undertaken to determine the effects of plant density on growth, yield components and grain yield of maize hybrids in the dry and wet Middleveld of Eswatini.

MATERIALS AND METHODS

Description of the experimental sites

A field experiment was conducted under rain-fed conditions in Malkerns Research Station (wet Middleveld) and Luve Experimental Plot (dry Middleveld) from November 2019 to April 2020 growing season. Malkerns Research Station is situated in the upper-Middleveld agro-ecological zone of Eswatini. It is located at an altitude of 740 m, latitude of 26.55°S and longitude of 31.15 °E. Malkerns has an annual rainfall of 800–1,000 mm; and an average annual air temperature of 19.0 °C with the coldest month in June. The annual rainfall of this site is well distributed from October to February with low coefficient of variation (Edje & Ossom, 2016). The soil type is *Ferralsolic* soils or Mdutjane soil series (Murdoch, 1969). The soil at Malkerns is sandy clay, acidic (pH = 5.42) and low in organic carbon (0.82%) (Jones, 2001). Luve Experimental Plot (LEP) is located in the dry Middleveld agro-ecology. The geographical location of LEP is 26.32 °S, 31.47 °E and the elevation is 463 m. The average annual rainfall at LEP is 783 mm with an average annual air temperature of 20.9 °C. The soil at Luve is loamy sand, slightly acidic (pH = 6.08) and low in organic carbon (0.26%) (Jones, 2001).

Table 1. Rainfall and temperature data of the study locations

	Rainfall (mm)		Temperature (°C)	
Location	Malkerns	Luve	Malkerns	Luve
Month				
November 2019	154.4	86.3	22.15	30.13
December 2019	57.8	15.6	21.69	28.42
January 2020	159.2	111.4	23.17	32.91
February 2020	119.8	48.0	22.76	29.70
March 2020	57.8	33.4	20.04	26.50
April 2020	54.6	29.4	19.89	24.30
Total	603.6	324.1	-	-
Mean			21.6	28.66

The rainfall and temperature data of the study locations during the growing period is presented in Table 1. Higher amounts of rainfall at Malkerns and Luve were recorded in the months of November 2019, January and February 2020. Generally, higher seasonal rainfall (603.6 mm) was recorded at Malkerns than at Luve (324.1 mm). Monthly mean temperatures at Malkerns ranged between 19.89 °C and 23.17 °C whilst at Luve it ranged between 24.30 °C and 32.91 °C.

Treatments, experimental design and management of the experiment

The experiment was conducted using factorial combinations of three maize hybrids [(SC 403 (early maturing), SC 621 (medium maturing) and SC 719 (late maturing)] and five plant densities [41,667 (80 cm × 30 cm), 44,444 (90 cm × 25 cm), 47,619 (70 cm × 30 cm), 50,000 (80 cm × 25 cm), 57,143 plant ha^{-1} (70 cm × 25 cm)]. Randomised complete

block design (RCBD) with three replications was used. The hybrids used for the study produce one ear per plant.

Gross plot size was six rows of 4.5 m row length and the net plot size was the central three rows leaving the outer two rows as border and one row for destructive sampling. Spacing of 1 m and 1.5 m was left between plots and blocks, respectively. The gross plot size was 24.30 m^2 (6 rows × 0.9 m × 4.5 m) for 90 cm inter-row spacing, 21.6 m^2 (6 rows × 0.8 m × 4.5 m) for 80 cm inter-row spacing, and 18.90 m^2 (6 rows × 0.7 m × 4.5 m) for 70 cm inter-row spacing. The corresponding net plot sizes were 12.15 m^2 (3 rows × 0.9 m × 4.5 m) for 90 cm inter-row spacing, 10.80 m^2 (3 rows × 0.8 m × 4.5 m) for 80 cm inter-row spacing, and 9.45 m^2 (3 rows × 0.7 m × 4.5 m) for 70 cm inter-row spacing.

Two seeds per hole were sown and later on thinned to one plant per hill when the seedlings developed three leaves. Basal fertilizer [N: P: K, 2: 3:2 (22)] at the rate of 400 kg ha^{-1} containing 25.2 kg N ha^{-1}, 37.6 kg P ha^{-1} and 25.2 kg K ha^{-1} was applied at the time of planting. Five weeks after emergence, the crop was side-dressed with Limestone Ammonium Nitrate (28% N) at the rate of 115 kg ha^{-1} (32.2 kg N ha^{-1}). Six weeks after emergence, the crop was sprayed with Masta 900 (Methomly as an active ingredient) to control the Fall armyworm (*Spodoptera frugiperda*). Weeds were controlled by hand weeding and hoeing from early stage of a crop until to maturity as required.

Crop data collected

Days to silking were recorded by counting the number of days from planting to the date when 50% of plants produced silks in the net plot. The growth parameters of maize were collected at three different growth stages. The first stage was at V6, the second stage was at V12 and the last stage was at physiological maturity (R6) as describe by Ritchie et al. (1993).

For the determination of the LAI, the leaf length and maximum width of all available leaves of five randomly selected plants per net plot were measured and the leaf area was calculated using the method described by McKee (1964) as Leaf area (LA) = Leaf length (cm) × Maximum width of leaf (cm) × 0.73. Then the LAI was calculated as the ratio of total leaf area of five plants (cm^2) to the respective area of land occupied by the plants. The plant height was measured from the ground to the base of the uppermost leaf before tasselling and from the ground to the tip of the tassel after tasselling in centimetre with a measuring tape as an average of five randomly sampled plants per net plot.

In order to determine dry matter, three plants were randomly selected from destructive row of each plot at each sampling and were uprooted for collecting necessary data. The plants were carefully uprooted by pulling just above the root crown and then the remaining roots in the soil were collected by hoeing the ground to a depth of about 70-75 cm. Then the roots were washed with tap water.

Their leaf areas were determined as described above and then the plant parts were separated into root, shoot and leaves. Then, the samples were oven dried at 80 ± 2 °C for 72 hours and the respective dry weights were recorded. Then, the following parameters were calculated as described by Hunt (1978):

a) Crop growth rate (CGR) (g m^{-2} per day) $=\left(\frac{W_2-W_1}{t_2-t_1}\right) \times \frac{1}{A}$

b) Relative growth rate (RGR) (mg g^{-1} per day) = $\left(\frac{(\ln W_2 - \ln W_1)}{t_2 - t_1}\right)$

where, W_1 = dry weight of plant at t_1, W_2 = dry weight of plant at t_2, t_1 and t_2 = time intervals in days, ln = natural logarithm, and A = ground area (cm^2).

Number of kernels per cob was recorded by counting the number of kernels using an electronic seed counter from ten randomly taken cobs from net plot area and converted to per m^2 area. Thousand kernels mass (g) was determined by counting one thousand kernels from a bulk of shelled grains per net plot using electronic seed counter and weighed using sensitive balance and the weight was adjusted to 12.5% moisture content. Aboveground dry biomass was determined by weighing five plants per net plot at harvest after sun drying to a constant weight and the weight was converted to tonnes per hectare. Grain yield was determined by multiplying the number of kernels per m^2 and kernel mass and converted to tonnes per hectare.

Data analysis

Homogeneity of variances was tested using the F-test and since the F-test showed homogeneity of the error variances of the parameters of the two sites, combined analysis of variance was carried out using SAS software version 9.1 (SAS Inistitute, 2003). Mean comparisons were done using Least Significant Difference (*LSD*) test at 5% level of significance where the analysis of variance showed significant differences.

RESULTS AND DISCUSSION

Days to 50% silking

The number of days to 50% silking was significantly ($p < 0.01$) affected by the interaction of location and hybrids (Fig. 1). Maize hybrid SC 719 grown at Malkerns recorded the longest number of days to 50% silking (70.47) which was significantly higher than that of the other hybrids grown at both locations (Fig. 1). In contrast, maize hybrid SC 403 recorded significantly the lowest numbers of days to 50% silking at both locations. The medium maturing hybrid (SC 621) and the early maturing hybrid (SC 403) showed no significance difference in days to 50% silking at the two locations. The difference among the hybrids might be due to the inherent genetic characteristics where hybrid SC 719 requiring longer growing degree days to reach 50% silking than SC 403 and SC 621. Moreover, the relatively low temperature at Malkerns might have delayed days to silking. In agreement with this result, Shafi et al. (2012) reported significant variation in days to 50% silking among four maize varieties where maize variety Jalal-2003 recorded the highest numbers of days to 50% silking (68 days) than the other varieties.

There was significant ($p = 0.0276$) interaction effect of location and plant density in the number of days to 50% silking (Fig. 1). The highest number of days to 50% silking (67.33) was recorded at the highest plant density of 57,143 plants ha^{-1} at Malkerns while, the lowest number of days to 50% silking (64.22) was recorded at the plant density of 47,619 plants ha^{-1} at Luve (Fig. 1). The longest days to silking at the highest plant density at Malkerns might be due to slow growth rate due to intense competition among the plants for growth resources and relatively lower temperature. In line with this result, Imran et al. (2015) reported increasing trend in the numbers of days to silking with increased density of maize.

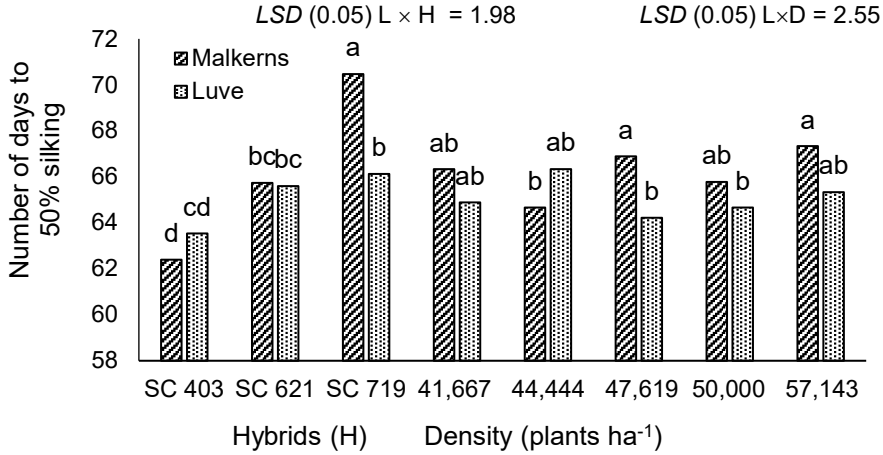

Figure 1. Number of days to 50% silking of maize as affected by the interaction of location by hybrids (H) and location (L) by plant density (D). Means in bars followed by the same letter are not significantly different at 5% level of significance according to Least Significant Difference (*LSD*) test.

Growth parameters

The LAI was significantly ($p < 0.05$) affected by the interaction of hybrids and location at V6 growth stage of maize (Fig. 2). The highest LAI (1.95) was recorded for hybrid SC 719 grown at Malkerns which was only significantly different from maize hybrid SC 403 grown at Malkerns (Fig. 2).

At V12 and R6 growth stages, location had significant ($p < 0.01$) effect on LAI where higher LAI of 4.20 at V12 and 2.70 at R6 were recorded at Malkerns than at Luve (Table 2). The higher LAI at Malkerns could be due to higher rainfall and high clay content of the soils at Malkerns that have retained more moisture and nutrients, making them available to the maize plants.

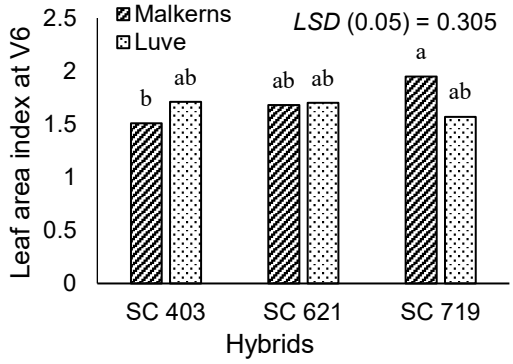

Figure 2. LAI at V6 growth stage of maize as affected by the interaction of location and hybrids. Means on the bars followed by the same letter are not significantly different at 5% level of significance according to Least Significant Difference (*LSD*) test.

Late maturing maize hybrid SC 719 produced significantly highest LAI of 4.30 and 2.67 at V12 and R6, respectively, whilst the early maturing hybrid SC 403 recorded the lowest LAI (Table 2). The highest LAI for SC 719 can be attributed to the extended vegetative growth leading to high number of leaves and rapid leaf expansion. Similar to this result, Jiang et al. (2020) reported greater leaf area and LAI for the long season hybrids than short season hybrids. The higher LAI for long season hybrids results in more light interception during grain filling (Tsimba et al., 2013).

There was significant effect of plant density on LAI at V6, V12 and R6 growth stages and the highest leaf area indexes of 2.02, 4.17 and 2.45, respectively, were recorded at the highest plant density of 57,143 plants ha^{-1} while the lowest leaf area indexes at all the growth stages were from the lowest plant density of 41,667 plants ha^{-1} (Table 2). The LAI showed a decreasing trend at R6 as compared to V12 possibly due to leaf senescence. Moreover, the decrease was highest at the highest plant density which could be due to intense competition for growth resources. In general, as the plant density was increased, the LAI was also increased. Higher LAI recorded at higher plant density can be attributed to higher number of plants per unit area producing more number of leaves which provides maximum interception of solar radiation (Sangoi, 2001). In conformity with this result, Dinh et al. (2015) reported that increasing plant density from 57,000 plants ha^{-1} to 84,000 plants ha^{-1} increased LAI from 3.52 to 4.67 in maize. Likewise,Ndzimandze et al. (2019) found increasing LAI as the density of maize was increased from 44,444 plants ha^{-1} to 57,143 plants ha^{-1}. In general, LAI is influenced by genotype, plant populations, agro-ecological factors and soil fertility (Aliu et al., 2010; Valadabadi & Farahani, 2010).

Table 2. LAI at V12 and R6 growth stages of maize as affected by location, hybrids and plant density. Means in columns followed by the same letter(s) are not significantly different at 5% level of significance according to Least Significance Difference (*LSD*) test; NS = Non-significant

Factor	LAI at V12	LAI at R6
Location		
Malkerns	4.19^{a}	2.70^{a}
Luve	2.81^{b}	1.72^{b}
LSD (0.05)	0.245	0.189
Hybrids		
SC 403	2.81^{c}	1.94^{b}
SC 621	3.40^{b}	2.03^{b}
SC 719	4.30^{a}	2.67^{a}
LSD (0.05)	0.30	0.232
Plant density (plants ha^{-1})		
41,667	2.95^{c}	2.04^{c}
44,444	3.27^{bc}	2.09^{bc}
47,619	3.64^{b}	2.10^{bc}
50.000	3.47^{b}	2.38^{ab}
57,143	4.17^{a}	2.45^{a}
LSD (0.05)	0.387	0.299

Crop growth rate (CGR) was significantly ($p < 0.05$) affected by hybrids and location interaction between V6 and V12 growth stages (Fig. 3). The highest CGR (31.35 g m^{-2} per day) between V6 and V12 growth stages were recorded for maize hybrid SC 719 grown at Malkerns while the lowest CGR (14.77 g m^{-2} per day) was for maize hybrid SC 403 at Luve (Fig. 3).

The lower water availability at Luve might have impaired the late maturing hybrid SC719 from expressing its potential. In line with this result, Ke & Ma (2021) obtained

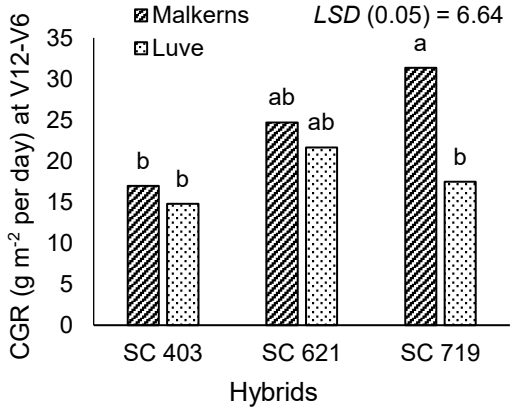

Figure 3. Crop growth rate (CGR) (g m^{-2} per day) of maize at V6-V12 growth stages as affected by the interaction of location and hybrids. Means on the bars followed by the same letter (s) are not significantly different at 5% level of significance according to Least Significant Difference (*LSD*) test.

higher grain yield loss of late maturity hybrid than early maturity hybrid with late planting due to moisture stress.

At growth stages between V12 and R6, there were significant main effects of location and hybrids on CGR (Table 3). Significantly higher CGR (18.16 g m^{-2} per day) was recorded at Malkerns than at Luve. Maize hybrid SC 719 recorded significantly the highest CGR (18.37 g m^{-2} per day) than hybrids SC 403 and SC 621 (Table 3). The higher CGR at Malkerns and for hybrid SC 719 can be attributed to higher LAI recorded resulting in the increased dry matter accumulation per day due to relatively better climatic and soil conditions at Malkerns. CGR depend on the amount of intercepted photosynthetically active radiation, where the leaf area index plays an important role. In line with this result, Adebo & Olaoye (2010) reported positive effect of availability of moisture in the early season on physiological characteristics of maize.

Although the difference was not significant, the highest CGR (17.12 g m^{-2} per day) was recorded at the highest plant density of 57,143 plants ha^{-1} which can be explained to higher accumulation of photosynthates by the maize hybrids due to higher number of plants per unit area. In agreement with this result, Valadabadi & Farahani (2010) obtained higher CGR (34.1 g m^{-2} per day) at higher plant density of 90,000 plants ha^{-1} than at 70,000 plants ha^{-1}.

In general, CGR values from V6 to V12 growth stages were higher than from V12 to R6 growth stages possibly due to rapid growth at the early growth stages owing to less competition among the plants for growth resources. Moreover, the lower CGR from V12 to R6 growth stages might be because of a higher proportion of total plant biomass is represented by non-photosynthetic tissues. Consistent with this result, Hokmalipour & Darbandi (2011) reported that during the early period especially after 30 days after germination, the crop growth rate of maize increased sharply until 90 days after germination then it gradually decreased. Similarly, Valadabadi & Farahani (2010) also reported significant increase in CGR of maize up to 60 days after planting and a sharp decline thereafter.

Table 3. Crop growth rate (CGR) and relative growth rate (RGR) of maize at different growth stages as affected by location, hybrids and plant density. Means in columns followed by the same letter are not significantly different at 5% level of significance according to Least Significance Difference (*LSD*) test; NS = Non-significant

Factor	CGR (g m^{-2} per day)	RGR (mg g^{-1} per day)	RGR (mg g^{-1} per day)
	R6-V12	V12-V6	R6-V12
Location			
Malkerns	18.16[a]	54.80[b]	14.74[a]
Luve	10.29[b]	76.70[a]	13.85[a]
LSD (0.05)	2.5	6.87	NS
Hybrids			
SC 403	13.26[b]	59.20[b]	15.05[a]
SC 621	12.25[b]	68.90[a]	12.77[a]
SC 719	18.37[a]	69.00[a]	15.06[a]
LSD (0.05)	3.07	8.42	NS
Plant density (ha^{-1})			
41,667	13.39[a]	68.40[a]	14.57[a]
44,444	15.59[a]	67.50[a]	15.37[a]
47,619	13.40[a]	67.60[a]	13.12[a]
50,000	13.65[a]	63.70[a]	14.44[a]
57,143	17.12[a]	61.40[a]	13.98[a]
LSD (0.05)	NS	NS	NS

The relative growth rate (RGR) was significantly ($p < 0.05$) affected by location and hybrids between V6 and V12 growth stages (Table 3). Significantly higher RGR (76.70 mg g^{-1} per day) was recorded at Malkerns than at Luve (54.80 mg g^{-1} per day) (Table 3). Among the maize hybrids, late maturing hybrid SC 719 produced the highest RGR (69.0 mg g^{-1} per day) while the early maturing hybrid SC 403 had the lowest RGR (59.2 mg g^{-1} per day) (Table 3). The highest RGR for maize hybrid SC 719 might be attributed to high accumulation of dry matter per day due to the highest LAI. In line with this result, Islam et al. (2019) reported significant difference among eight maize genotypes in RGR that ranged from 82.57 mg g^{-1} per day to 114.1 mg g^{-1} per day. Similarly, Hokmalipour & Darbandi (2011) reported significant differences among three maize cultivars for RGR.

There was no significant difference among the plant densities in RGR, however, the highest RGR (68.4 mg g^{-1} per day) was obtained at the lowest plant densities of 41,667 plants ha^{-1} whereas the lowest RGR (61.4 mg g^{-1} per day) was at the highest plant density of 57,143 plants ha^{-1} (Table 3). Relatively higher RGR at the lower plant density can be attributed to less competition among the plants for growth resources such as sunlight, nutrients and moisture. In agreement with this result, Amanullah et al. (2009) reported that increase in planting density from 4 plants m^{-2} to 10 plants m^{-2} had negative effects on RGR. Similarly, Tajul et al. (2013) found higher RGR (16 mg g^{-1} per day) at lower plant density of 53,000 plants ha^{-1} compared to RGR of 8 mg g^{-1} per day at higher plant density of 80,000 plants ha^{-1}.

The main effects of location, hybrids and density as well as all the interactions were non-significant on RGR between V12 and R6 (Table 3). However, relatively higher RGR (14.74 mg g^{-1} per day) was obtained at Malkerns than at Luve (13.85 mg g^{-1} per day). Maize hybrid SC 719 had the highest RGR (15.06 mg g^{-1} per day) while the lowest RGR (12.77 mg g^{-1} per day) was for hybrid SC 621 (Table 3). Plant density of 44,444 plants ha^{-1} produced the highest RGR (15.37 mg g^{-1} per day) while the lowest RGR (13.12 mg g^{-1} per day) was at a plant density of 47,619 plants ha^{-1}.

Generally, RGR was higher in the vegetative growth stage (V6 to V12) and it decreased sharply at V12 to R6. In conformity with this result, Valadabadi & Farahani (2010) found a sharp decline in RGR as the days after planting were increased from 20 to 80.

The interaction effect of location and hybrids was significant ($p < 0.01$) on plant height of maize at all the growth stages indicating that the hybrids height was not consistent across locations. At V6, the tallest plant (37.77 cm) was recorded for hybrid SC 621 grown at Luve while the shortest plant (29.26 cm) was for hybrid SC 403 grown at Malkerns (Fig. 4). On the other hand, at V12 and R6, the tallest plants were for maize hybrid SC 719 at Malkerns while the shortest plants were for hybrid SC 403 at Luve (Fig. 4). Higher plant height at Luve at the early growth stage (V6) might be due to relatively higher temperature which enhanced early growth of maize (Khaeim et al., 2022). Generally, late maturing hybrid SC 719 had highest plant height at Malkerns at all the growth stages which could be ascribed to higher potential of the hybrid in capturing more sunlight, water and nutrients for photosynthesis than the medium and early maturing hybrids (Sharifi et al., 2009). The hybrids height was not consistent across locations with SC 621 and SC 719 having similar height at Luve. Consistent with this result, Gayosso-Barragán et al. (2020) obtained significant line by location

interaction for plant height of maize. Likewise, Sharifi et al. (2009) reported significant differences in plant height among maize hybrids that ranged from 183.9 cm to 211.59 cm.

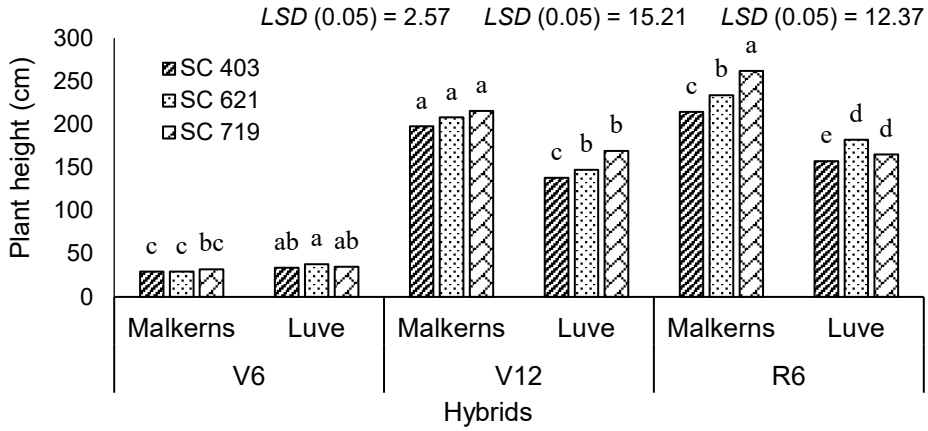

Figure 4. Plant height (cm) of maize at V6, V12 and R6 growth stages as affected by the interaction of location and hybrid. Means on the bars followed by the same letter are not significantly different at 5% level of significance according to Least Significant Difference (*LSD*) test.

Yield components and yield

The main effects of hybrids and plant density were significant on the number of kernels per m^2 whereas the effects of location and the interactions were non-significant (Table 4). Maize hybrid SC 719 produced significantly the highest number of kernels per m^2 (2074) over that of SC 403 (1863) and SC 621 (1804) (Table 4). The highest number of kernels per m^2 for the late maturing hybrid SC 719 could be due to longer vegetative growth period and later silking allowing more LAI to be achieved by the start of the critical period of kernel development. Moreover, the number of kernels depends on traits like ear diameter, ear length and kernel size which are genetically controlled. In agreement with this result, Azam et al. (2007) obtained higher number of kernels per cob for late maturing variety Baber (389 kernels per cob) than early maturing variety Cargill 707 (359 kernels per cob).

Among the plant densities, the highest density of 57,143 plants ha^{-1} produced the maximum number of kernels per m^2 (2031) while the lowest densities of 41,667 and 44,444 plants ha^{-1} produced significantly the lowest number of kernels per m^2 (Table 4). The highest number of kernels per m^2 at the highest plant density might be due to highest number of cobs produced per unit area that compensated the reduction of the kernels numbers per cob (Lashkari et al., 2011). Consistent with this result, Echarte et al. (2000) reported increasing number of kernels per m^2 as the density of maize was increased from 3 to 18 plants m^{-2}.

Mass of thousand kernels is an important yield component, which plays a major role in yield potential of a variety. The main effects of location and hybrids were significant ($p < 0.05$) on the mass of thousand kernels. Significantly higher mass of thousand kernels (395.6 g) was obtained at Malkerns than at Luve (300.4 g) (Table 4) possibly due to better rainfall, temperature and soil conditions at Malkerns. Among the

hybrids, SC 621 and SC 719 recorded significantly the highest mass of thousand kernels of 366.0 g and 361 g, respectively, over that of SC 403 (329.9 g) (Table 4). Higher seed mass for medium and late maturing hybrids might be due to the genetic makeup where longer crop cycle provides more production of dry matter and partitioning to the grain. In accordance with this result, Zamir et al. (2011) obtained higher 1,000-grain weight (241.51 g) for late maturing maize hybrid than for early maturing maize hybrid (234.94 g). Similarly, Esfandiary et al. (2012) reported heavier 1,000-grain weight in late maturing maize variety SC 677 (224 g) as compared to early and medium maturing varieties; SC 500 (190 g), SC 647 (195 g) and SC 633 (209 g).

The highest 1,000 kernels mass (359.30 g) was recorded for the lowest plant density of 41,667 plants ha^{-1}, but it was not significantly different from the other densities (Table 4). Highest kernels mass at the lowest plant density might be due to availability of more photosynthates for grain development because of less interplant competition. In agreement with this result, Abuzar et al. (2011) obtained maximum 1,000 kernels weight (350.0 g) at lower density (80,000 plants ha^{-1}) and the minimum 1,000 kernels weight (166.7 g) at a higher plant population (140,000 plants ha^{-1}). Likewise, Ijaz et al. (2015) also reported decrease in 1,000 grain mass with increased plant density in maize.

Table 4. Yield components and yield of maize as affected by location, hybrids and plant density. Means in columns followed by the same letter are not significantly different at 5% level of significance according to Least Significance Difference (*LSD*) test; NS = Non-significant. Interactions among location, hybrid and density were not significant

Factor	Number of kernels per m^2	1,000 kernels mass (g)	Abovegro und dry biomass (t ha^{-1})	Grain yield (t ha^{-1})
Location				
Malkerns	1939.1[a]	395.60[a]	22.71[a]	7.67[a]
Luve	1888.7[a]	300.40[b]	15.70[b]	5.67[b]
LSD (0.05)	NS	23.709	2.035	1.58
Hybrids				
SC 403	1863[b]	329.90[b]	16.54[b]	6.15[b]
SC 621	1804[b]	366.00[a]	18.03[b]	6.60[b]
SC 719	2074[a]	361.00[ab]	23.05[a]	7.49[a]
LSD (0.05)	178.2	29.038	2.492	0.75
Plant density (plants ha^{-1})				
41,667	1801[b]	359.30[a]	18.07[a]	6.47[a]
44,444	1797[b]	349.40[a]	18.90[a]	6.28[a]
47,619	1938[ab]	343.10[a]	18.59[a]	6.65[a]
50,000	2002[ab]	356.80[a]	18.93[a]	7.14[a]
57,143	2031[a]	352.80[a]	21.53[a]	7.17[a]
LSD (0.05)	230.07	NS	NS	NS

Aboveground dry biomass was significantly ($p < 0.01$) affected by location and hybrids. Significantly higher aboveground dry biomass (22.71 t ha^{-1}) was recorded at Malkerns than at Luve (15.70 t ha^{-1}) (Table 4) possibly due to better growing conditions at Malkerns compared to the higher temperature and lower rainfall observed at Luve (Table 1). The late maturing maize hybrid SC 719 produced significantly the highest aboveground dry biomass (23.05 t ha^{-1}) while the early maturing hybrid SC 403 produced the lowest aboveground dry biomass (16.54 t ha^{-1}) (Table 4). The highest aboveground biomass for late maturing maize hybrid SC 719 could be due to the fact that it took more days to mature and hence had a better chance to utilize more growth resources such as sunlight, soil nutrients and soil moisture to produce higher LAI and plant height resulting in higher photosynthesis and dry matter production. In agreement with this result, Belay (2019) obtained significantly higher aboveground biomass (31.36 t ha^{-1}) for late maturing maize hybrid BH-661 than medium maturing hybrid BH-QPY-545 (20.19 t ha^{-1}). Similarly, Radchenko et al. (2022) reported

significant difference for aboveground biomass among three maize hybrids that ranged from 45.7 t ha^{-1} for hybrid DM Skarb to 55.1 t ha^{-1} for hybrid Forteza.

Maize grown at the highest plant density of 57,143 plants ha^{-1} had the highest aboveground dry biomass (21.53 t ha^{-1}) while the lowest aboveground biomass (18.07 t ha^{-1}) was recorded from the lowest plant density of 41,667 plant ha^{-1} (Table 4). The highest aboveground biomass at the highest plant density could be due to more number of plants per unit area.

Grain yield was significantly affected by location and hybrids (Table 4). Significantly higher grain yield (7.67 t ha^{-1}) was obtained at Malkerns than at Luve (5.67 t ha^{-1}) (Table 4) which can be explained to the optimum rainfall, temperature and soil at Malkerns compared to higher temperature, minimum rainfall and predominantly sandy soil at Luve. The late maturing maize hybrid SC 719 produced significantly the highest grain yield (7.49 t ha^{-1}) than SC 621 (6.60 t ha^{-1}) and SC 403 (6.15 t ha^{-1}) (Table 4). The highest grain yield for the late maturing hybrid SC 719 could be due to the highest growth parameters such as LAI, CGR, plant height and number of kernels as grain yield is positively associated with these parameters. In line with this result, Esfandiary et al. (2012) found the highest grain yield for late maturing maize varieties SC 704 (9,861 kg ha^{-1}) and SC 677 (9,800 kg ha^{-1}) compared to early and medium maturing varieties; SC 500 (9,656 kg ha^{-1}), SC 647 (9,553 kg ha^{-1}) and SC 633 (9,005 kg ha^{-1}). Belay (2019) also reported the maximum grain yield (11.09 t ha^{-1}) for late maturing maize hybrid BH661 than medium maturing maize hybrid BHQPY-545 (9.57 t ha^{-1}).

Though the difference was not significant, the highest grain yield (7.17 t ha^{-1}) was obtained at the highest plant density of 57,143 plants ha^{-1} whereas the lowest grain yield (6.28 t ha^{-1}) was recorded at plant density of 44,444 plants ha^{-1} (Table 4). In general, as the plant density increased, the grain yield showed an increasing trend. Higher grain yield at higher plant density might be due to higher number of plants per unit area which compensated the effects of decrease in other yield components specifically, due to higher grain number per unit area which compensated the decrease in grain weight. Abdul et al. (2007) also found higher grain yield of 5.80 t ha^{-1} at plant density of 90,000 plants ha^{-1} and lower grain yield (4.36 t ha^{-1}) at the lowest density of 30,000 plants ha^{-1}. In contrast to this result, Kadyrov & Kharitonov (2019) reported significant interaction of maize hybrids and seeding rates where the highest yield of early maturing and medium to early maturing hybrids were obtained at the seeding rate of 73,000 seeds ha^{-1} while the medium maturing hybrid produced the highest grain yield (7.21 t ha^{-1}) at the seeding rate of 77,000 seeds ha^{-1}.

CONCLUSIONS

Results of the study showed that maize hybrid SC 719 grown at Malkerns produced the highest LAI, crop growth rate, number of kernels per m^2, aboveground dry biomass and grain yield. As the plant density was increased from 41,667 plants ha^{-1} to 57,143 plants ha^{-1}, the leaf area index, aboveground dry biomass and grain yield showed an increasing trend. Thus, it can be concluded that late maturing maize hybrid SC 719 at the plant density of 57,143 plants ha^{-1} (70 cm × 25 cm) can be used to increase the productivity of maize in the Middleveld of Eswatini. However, further studies are

required to determine the physiological variables influencing biomass accumulation and yield in each location with inclusion of higher plant densities.

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Osmotic stress tolerance in forage oat varieties (*Avena Sativa* L.) based on osmotic potential trials

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Abstract. Forage oats (*Avena sativa* L.) are globally important for milk and meat production, and, to a lesser extent, for the human diet. In Mexico, oats are a strategic crop, occupying the fourth place in cultivated area, only after maize for grain, bean, and sorghum for grain. Droughts are the main problem for oat production in Mexico. This study evaluated the germination and seedling growth of several oat varieties in response to drought stress simulated by PEG-6000 treatments of different osmotic pressure in order to identify drought-resistant genotypes. The Teporaca genotype was the most outstanding in the three levels of OP compared to its control with 0.0 of Osmotic Potential (OP). The Teporaca genotype showed the largest root length and the lowest diminishment of root length under osmotic stress conditions. This genotype also had the largest shoot length in the three osmotic stress levels. Regarding root fresh weight, Babicora stands out with 98.5% and Teporaca with 43% in the most severe level. Teporaca, Menonita, and Babicora showed the outstanding root dry weights of 346.5%, 327.2%, and 251.2%, respectively. These varieties had higher root dry weight than their own controls in water in the most severe level of OP. In conclusion, the Teporaca, Menonita, and Karma genotypes showed the highest osmotic stress tolerance and could be used as sources of favorable alleles to improve oat drought tolerance.

Key words: *Avena sativa*, polyethylene glycol 6000, osmotic pressure.

INTRODUCTION

Forage oats (*Avena sativa* L.) are the sixth most important cereal in the world, right after wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), rice (*Oryza sativa* L.), barley

(*Hordeum vulgare* L.), and sorghum (*Sorghum bicolor (*L.) Moench) (Mariscal-Amaro et al., 2009; Basha, 2020).

Drought is one of the most important problems for worldwide oat production (Farooq et al., 2009; Canales et al., 2021). In Mexico, 670,527 ha were sown with forage oats in 2020. Oats are a strategic crop, occupying the fourth place in terms of cultivated area, only after maize for grain (7,472,356 ha), bean (1,711,962 ha), and sorghum for grain (1,484,126 ha) (SIAP, 2021).

Forage oats are cultivated mainly in rainfed conditions (534,898 ha), representing 79.7% of the total cultivated area in México (SIAP, 2021; Salmerón, 2000; Amado et al., 2000; Osuna-Ceja et al., 2013). In arid and semi-arid regions, drought stress is the main limitation for forage and grain yield, especially for non-irrigated oats (Zhao et al., 2021) and cereals (Batool et al., 2021). To improve drought tolerance, the characteristics of tolerant oat varieties can be genetically selected, such as the leaf water potential and the capacity for osmotic adjustment (González et al., 2008). Moreover, plant precocity, although not necessarily described as a response to osmotic adjustment, is also a physiological characteristic that responds to drought (González et al., 2008).

Under severe water deficit, plants increase their synthesis of abscisic acid (ABA), which reduces the plant cycle and generates a larger concentration of photoassimilates destined for grain production (Maldonado et al., 1997; Coelho et al., 2020). The root is the first organ exposed to the drying soil and the origin of the drought tolerance response (Schachtman & Goodger, 2008). The lack of water in the soil can increase the synthesis of ABA in the roots, which is transported to the bud, causing stomatal closure (Li et al., 2020). In addition, the lack of water can lead to morphological and physiological changes in plants (Canales et al., 2019); in a moderate water deficit, plants tend to reduce the leaf area to minimize water loss and increase root growth (Coelho et al., 2020). To tolerate the osmotic stress of sodic-saline soils, plants also generate proline, which is synthesized in sub-cellular leaf and root compartments (Maldonado et al., 1997; Liu et al., 2020).

Water deficit and nitrogen deficiency (N_2) are the two most important factors limiting physiological activities in crops (Li et al., 2020). In oats, a severe water deficit causes an earlier increase in N_2 absorption, reduces the N_2 daily accumulation rate, and shortens the vegetative cycle (Coelho et al., 2020).

Low molecular hydrophilic substances collectively called osmolytes including mannitol, sugars, and salts modulate osmotic pressure of cell cytoplasm, have been used to control the osmotic pressure, but they have serious disadvantages; for example, they are subject to microbiological decomposition and affect plant metabolism (Kaul, 1966). Hydric stress conditions can be simulated in vitro by using polyethylene glycol (PEG-6000) in controlled environments. The use of PEG-6000 is effective for evaluating genotypes with osmotic stress tolerance at the seedling stage (Michel & Kaufmann, 1973; Basha, 2020). This method has been shown to be efficient in species such as corn, barley and rice (Lu & Neumann, 1998), sorghum (Tsago et al., 2014), wheat (Jatoi et al., 2014), beans (Jimenez-Galindo et al., 2018) and oats (Basha, 2020).

Droughts are currently one of the main problems that prevent crop plants from expressing their genetic potential (Sánchez-Martín et al., 2012). Identifying sources of drought-tolerant germplasms (Sánchez-Martín et al., 2012) and developing cultivars with better drought adaptation is a priority in breeding programs (Canales et al., 2021). Due to the agroclimatic conditions of the Mexican highlands, especially the scarce and irregular distribution of precipitation, it is necessary to identify genotypes of drought-

tolerant oats. Therefore, the purpose of this study was to evaluate osmotic stress tolerance in ten varieties of oats selected by the polyethylene glycol PEG-6000 method in order to identify tolerant parents that may improve commercial oat varieties.

MATERIALS AND METHODS

Plant material

Ten genotypes of *Avena sativa* L. with diverse genetic backgrounds were used (Table 1).

Table 1. Agronomic traits of ten genotypes of oats (*A. sativa*) evaluated for osmotic stress tolerance

Genotype	Source	Drought Response	Characteristics	Grain color	100 seeds weight (g)
Babicora	INIFAP	Tolerant	Precocity	Brown	32.9
Bachiniva	INIFAP	Tolerant	Precocity and high yield	Light brown	36.3
Cuauhtemoc	INIFAP	Unknown	High yield	Light creamy brown	33.0
Cusarare	INIFAP	Unknown	High yield	Pearly	36.9
Karma	INIFAP	Tolerant	Tolerant to rust fungi	Light brown	34.4
Menonita	INIFAP	Moderate tolerant	Resistant to rust fungi	Light yellow	34.2
Papigochic	INIFAP	Unknown	Intermediate cycle	Light brown	28.6
Tamo 386 (Tamo)	TEXAS	Unknown	Long cycle	White	32.9
Teporaca	INIFAP	Tolerant	Resistant to rust fungi	Creamy	33.0
Turquesa	INIFAP	Unknown	High yield	Creamy	32.3

INIFAP (National Institute of Forestry, Agriculture and Livestock Research).

Texas Agricultural Experiment Station, Texas A&M University.

Experimental design

This study was conducted under laboratory conditions, germinating seeds at 28 °C. The study design was completely randomized in a factorial arrangement. Factorial combinations were evaluated in 10 genotypes and four levels of osmotic potential (OP) with three repetitions and two experiments. The experimental unit was a Petri-dish with 10 seeds of each genotype. Levels of osmotic pressure were prepared as 0.0, -0.05, -0.15, and -0.30 MPa using PEG-6000, based on the equation given by Michel & Kaufmann (1973). The bioassays were performed in Petri-dishes of 9.5 cm in diameter with filter paper and 8 mL of a solution containing 0.0, 50.0, 100.0, or 150.0 g of PEG-6000. Seeds were considered germinated when the root or shoot had more than 10 mm in length. After seven days, roots and shoots of the seedlings were measured and weighed. Root and shoot tissues were placed on a stove at 35 °C for seven days and then weighed.

Statistical analysis

An ANOVA was performed using the GLM (General lineal model) procedure (PROC GLM) of the SAS (SAS Institute 2016). The sources of variation were the genotype, experiment, and the interaction genotype × experiment. Genotypes, experiment, and the interaction genotype × experiment were considered fixed effects. Individual ANOVAs were performed by stress levels. The Tukey test was used at $p < 0.05$ to compare means.

Principal Component Analyses (PCA) were carried out using SAS software (SAS Institute 2016). All data were previously standardized with mean = 0 and standard deviation = 1. The first component was used for ordering the genotypes because it explained most of the variability across the OP levels (OP1 = 51.9%, OP2 = 55.4%, and OP3 = 49.5% of the variability explained), and it was considered an osmotic stress tolerance index. Furthermore, 100 seeds for each genotype were weighed (Table 1).

RESULTS

Significant differences were found between varieties in almost all analyzed traits and OP, except for the following: root length at -0.05 and -0.30, shoot length at -0.05, root dry weight at -0.05 and -0.15, shoot fresh weight at -0.15, and shoot dry weight at -0.05 and -0.30.

No significant differences were found between experiments and genotype × experiment interaction at -0.05. No significant differences were found between experiments and genotype × experiment interaction, except for root fresh weight at -0.15.

Significant differences were found between experiments for shoot length, root fresh weight and shoot fresh weight at -0.30. Additionally, there were found significant differences in genotype × experiment interaction in germination and root fresh weight at -0.30.

The Teporaca genotype registered the lowest percentages of germination in all PEG concentration levels (Fig. 1), but the Teporaca germinated plants were more tolerant to osmotic stress than the rest of the varieties (Fig. 2). Moreover, the Teporaca genotype had larger shoot length and root fresh weight compared to the rest of the genotypes in all osmotic stress levels. The Babicora genotype also showed high root fresh weight in the third osmotic stress level (Fig. 1). Teporaca, Menonita, and Babicora had the highest root dry weight in the most severe level of osmotic stress evaluated. Regarding shoot fresh weight, Teporaca stands out in all the OP levels, and Babicora and Cuauhtémoc do so in the last OP level (Fig. 1).

Although there were no significant differences among groups in the osmotic potential of -0.15 and -0.30, the germination of the Teporaca genotype was better in -0.05 OP than in its control. The root and shoot length were much longer in the Teporaca genotype in all levels of osmotic stress. Babicora and Teporaca had the highest root fresh weight in the most severe level of osmotic stress. Teporaca and Menonita had the highest root dry weight in the most severe levels of osmotic stress (Fig. 1).

The results of PCA combining all osmotic pressures confirmed the results observed in the univariate analysis (Figs 1, 2 and 3). By osmotic pressure, the first PC1 explains 51.9% at -0.05 MPa, 55.4% at -0.15 MPa and 49.5% at -0.30 MPa. The genotypes respond positively or negatively to the increase in OP levels. According to the first principal component (which was considered as the tolerance index), the most tolerant genotypes at -0.05 MPa were Teporaca and Karma. The most outstanding genotypes at -0.15 MPa were Teporaca, Turquesa, and Karma. The most tolerant genotypes at -0.30MPa were Teporaca, Babicora, Menonita, and Karma. Tamo was the most susceptible genotype at all OP levels (Fig. 3). The PCA showed a positive response of the Babicora genotype: in the first level, the OP was negative; in the second level, it

began to increase; and in the third level, it increased significantly. The Menonita genotype responded similarly. Conversely, Karma always responded positively (Fig. 3).

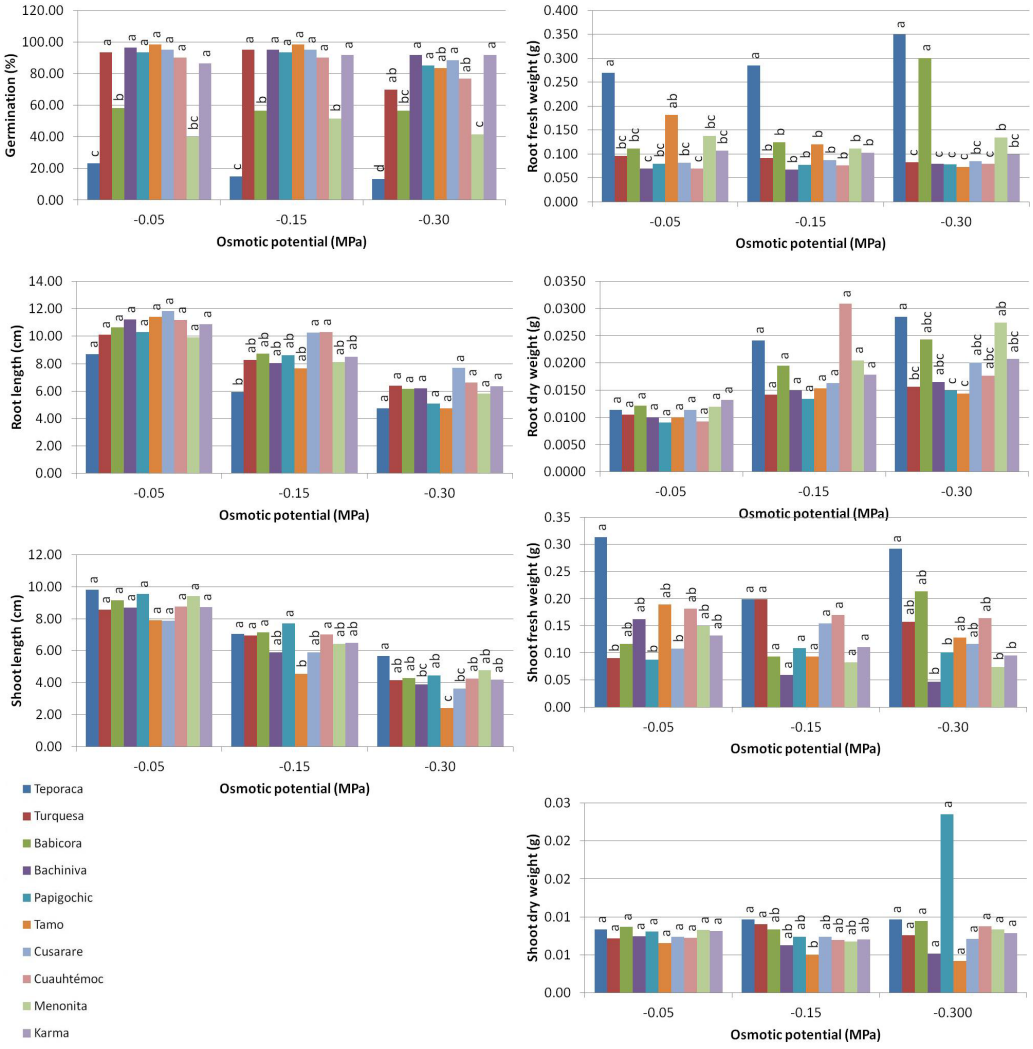

Figure 1. Effect of the different osmotic potentials generated by concentrations of PEG6000 of original data of on different germination traits of the ten oat varieties. The *LSD* for the interaction (genotype × experiment) was calculated with the equation $LSD = Distribution\ T\ (\alpha - DF) * \sqrt{EMS} * \frac{2}{n\ repetitions}$

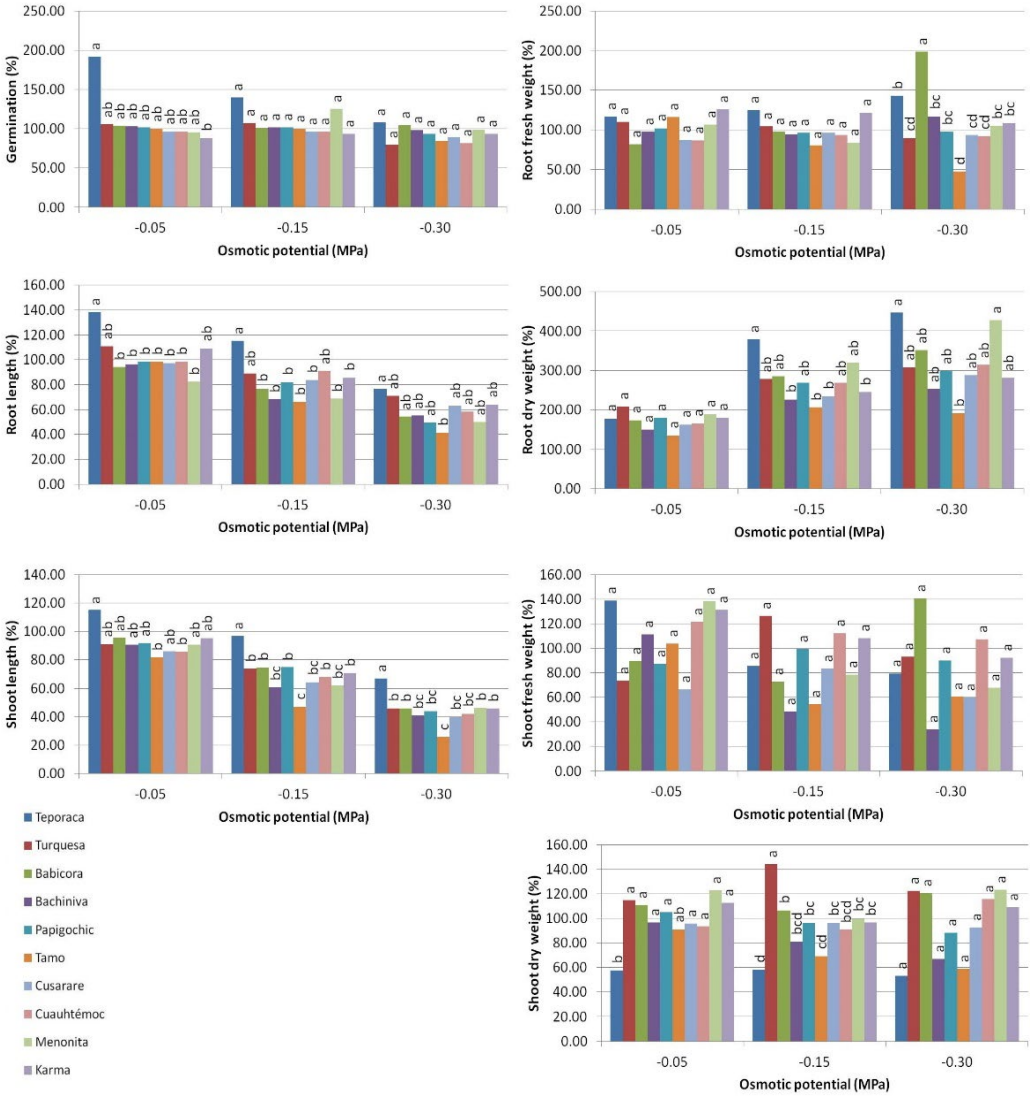

Figure 2. Effects of the osmotic potential on percentage data of ten oats varieties evaluated in vitro under an osmotic potential generated by increasing concentration of PEG6000. The control group is not shown because it is the 100% for each genotype and for seach characteristic. The *LSD* for the interaction (genotype × experiment) was calculated with the equation $LSD = Distribution\ T\ (\alpha - DF) * \sqrt{EMS} * \frac{2}{n\ repetitions}$.

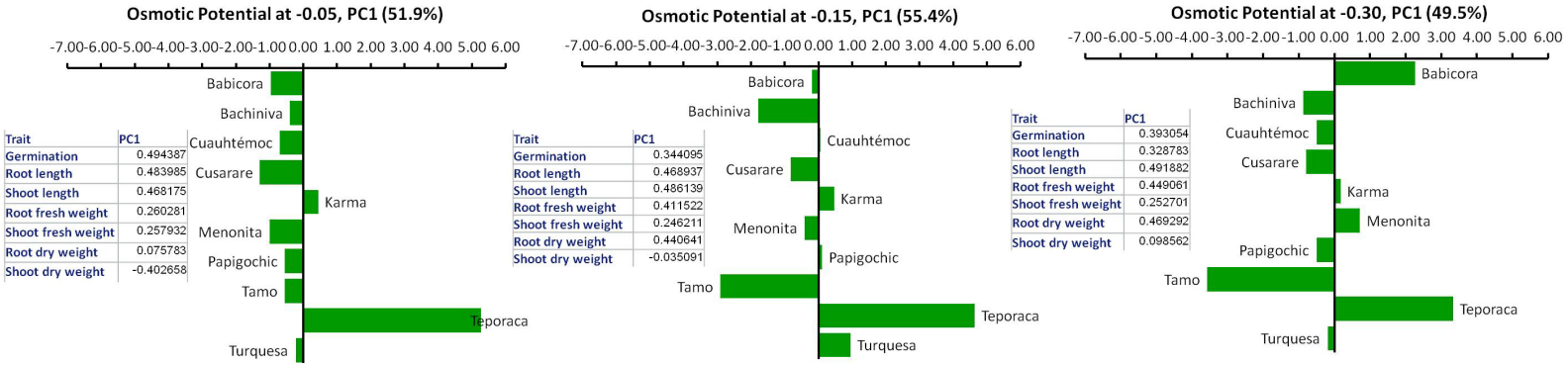

Figure 3. Effect of osmotic potential on ten oat genotypes evaluated in vitro under osmotic potential generated by an increasing concentration of PEG-6000.

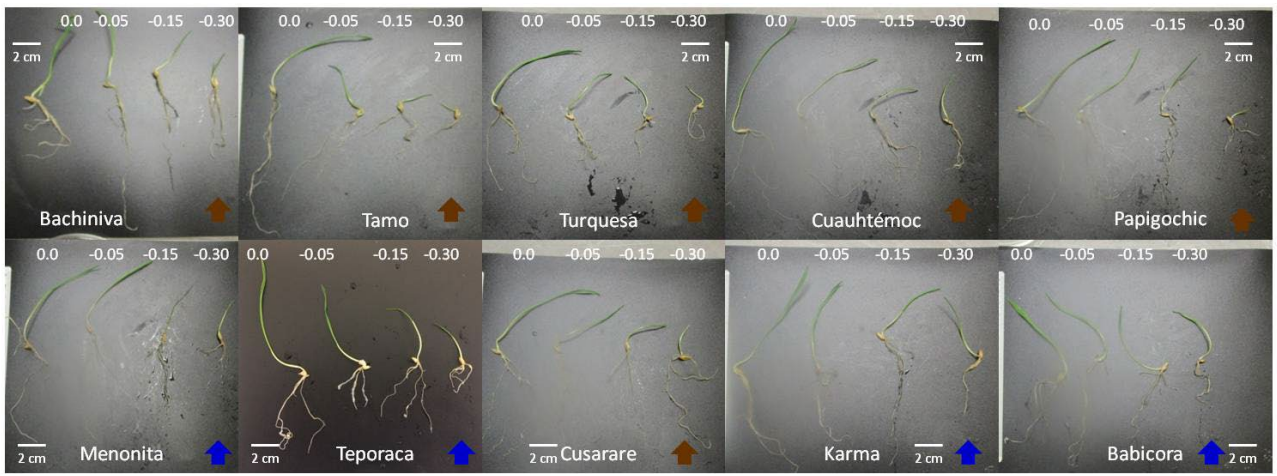

Figure 4. The in vitro effect of osmotic potential on ten oat genotypes evaluated under osmotic potential generated by an increasing concentration of PEG-6000. Blue arrows show longer roots at -0.30MPa, and brown arrows show shorter roots at -0.30 MPa.

The PCA showed outstanding growth and development in the Teporaca genotype in the three osmotic stress levels. In the PCA plot in Fig. 5, Teporaca (genotype no 9) is found in the B rectangle at the top of the Y axis and to the right of all genotypes on the X axis. This can be interpreted as Teporaca being the most tolerant genotype at -0.05. Teporaca is also the most outstanding at -0.15 MPa, as indicated by its appearance in rectangle C (Fig. 5). In rectangle D, Teporaca is also the second most tolerant genotype at -0.30 MPa, only after Babicora. The genotypes with the best response to osmotic stress are Teporaca, Babicora, Menonita, and Karma (Fig. 5). The genotypes with the worst response to osmotic stress are Tamo and Cusarare.

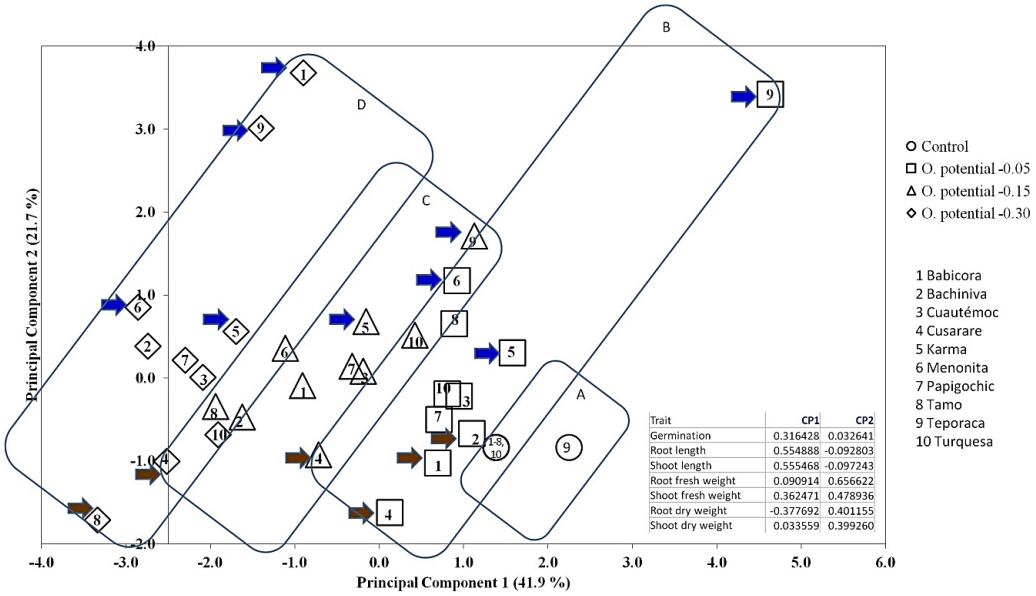

Figure 5. Plot of PC1 vs PC2 for ten oat genotypes and three OP levels. The Blue arrows show genotypes with the best response to OP, and brown arrows show the genotypes with the worst response.

DISCUSSION

The low germination of Teporaca is possibly one of the reasons why farmers did not sow it with the same intensity as other varieties. However, germinated plants from this genotype present a higher tolerance to osmotic stress than the rest. Canales et al. (2021) found that the resistant genotype showed a mild and slow increase in abscisic acid production that allowed maintaining transpiration longer. This response was linked to an increase in root hydraulic conductance by increasing total root length, the length of the thinnest roots, and root conductivity. The study agrees with the present study because the Teporaca genotype, which is the most tolerant, shows the largest roots at all osmotic pressure levels.

Evaluating osmotic stress tolerance in laboratory conditions is backed by Michel & Kaufmann (1973) and Basha, 2020), which measured root length and total root volume. These characteristics indirectly denote the capacity for water exploration and absorption,

which help to explain better the behavior of the genotypes under hydric stress (Jimenez-Galindo et al., 2018; Canales et al., 2019). In addition, the present results agree with Gorny (1995) and Górny & Szolkowska (1996), who found enhanced rooting and improved drought tolerance in progenies of spring barley and oats after a selection for longer juvenile roots. Such characteristics are more complicated to measure in field experiments on irrigation drought (Górny & Szolkowska, 1996). Oat drought resistance is related to many phenological, morphological, and physiological factors (Larsson & Górny, 1988); probably the most important factor is genetic variation in the plant root system (Larsson & Górny, 1988). Using root characters to evaluate drought resistance of breeding materials has been suggested repeatedly (Derera et al., 1969; Taylor & Klepper, 1979).

Tolerance to osmotic stress by Teporaca, Menonita, and Babicora is backed by yields obtained in the field (with precipitations lower than 300 mm), which indicate that these three genotypes outperform the Bachiniva genotype (Salmerón, 2000). Osmotic stress tolerance of the Karma genotype in the present study is supported by field experiments performed by Villaseñor et al. (1998) and Salmerón et al. (2010). In addition, Sánchez-Martín et al. (2012) showed the potential of multivariate analysis as a robust approach to target key mechanisms responsible for drought tolerance in oats. Multivariate analysis can help breeders by accelerating genotype selection in large breeding populations.

The present study contrasts with the drought tolerance reported for the Bachiniva genotype by (Salmerón, 2000; Zamora, 2002) since we found a lower osmotic stress tolerance for this genotype. The contradiction is probably due to previous research assumed Bachiniva's drought tolerance only with field yield data. Relating this genotype with high yield, even in years with little and poor distribution of rain and the present experiment was only carried out under laboratory conditions. The reported tolerance of the Bachiniva genotype is possibly justified by its precocity (Salmerón, 2000), and the apparent contradiction between our study and the previous ones is probably due to the lack of testing of Bachiniva in drought tolerance experiments (Salmerón, 2000; Zamora, 2002).

The Tamo genotype produces a high amount of forage and has a long growing cycle (McDaniel, 1987; Aseeva & Melnichuk, 2018). This last characteristic probably makes it very susceptible to drought, supporting what was observed in the present study and others. Although precocity is linked to osmotic adjustment, several studies indicate that it is a physiological response to drought (González et al., 2008).

In the present study, the most outstanding genotype in terms of osmotic stress tolerance is Teporaca, followed by Babicora, Menonita, and Karma. Searching for agronomic traits and their use for oat breeding is very important (Ociepa, 2019). Evaluating seedlings might ease the phenotyping of plants in the previously mentioned populations Sánchez-Martín et al., 2012; (Canales et al., 2019).

CONCLUSIONS

We identified four commercial varieties of oats with enhanced tolerance to OP: Teporaca, the best option, Babicora, Menonita, and Karma. These varieties are a potential source of favorable alleles for osmotic stress tolerance and should be useful for oat improvement. Tamo and Cusarare were the most susceptible varieties to osmotic

stress. On the contrary to susceptible genotypes, tolerant genotypes have higher germination rate with respect to their control in water, longer roots, and higher root fresh weight, root dry weight, shoot fresh weight, and shoot dry weight. PEG-6000 can be effectively used in genetic improvement studies to select in early stages oat lines with outstanding osmotic stress tolerance. Future research should focus on mapping the genetic regions responsible for osmotic stress tolerance using the PEG method for phenotyping biparental or MAGIC populations.

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Germination characteristics of sorghum (*Sorghum bicolor* L.) affected by temperature variation

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Abstract. Germination was essential in preparation for the subsequent few sorghum growth cycles, but increasing the temperature was a major limiting factor. Temperature change effect, provide information on appropriate sorghum cultivation techniques. Sorghum was the principal cereal grown for food, feed, fodder, starch, fiber, dextrose syrup, biofuels, and bioenergy. This study evaluated the effect of temperature variation on the viability and vigor of sorghum seeds. This research applies Factorial Experiment in Completely Random Design, where the first factor is three genotypes of sorghum Numbu, G1Marapi, and G3Marapi. The second factor was the temperature variation of 18 °C–38 °C. Sorghum seeds were germinated for ten days and placed in the growth chamber. The result showed that every 2 °C temperature increase gives a different response to the germination of sorghum. The optimum temperature for sorghum seed germination was 20 °C–32 °C, with good viability and vigor of > 80%. Root and shoot growth was optimal at 24 °C–28 °C, such as Numbu has a root length of 17.0 cm and shoot length of 18.3 cm at 24 °C, but at 38 °C, no seed germination. However, the morphological mechanisms of sorghum response were a basis for information to get tolerant genotype and maximize utilization of its local germplasm to develop improved cultivars.

Key words: denaturation, germination, high temperature, tolerant, vigor.

INTRODUCTION

Temperature is an abiotic factor that affects plant phenological changes during germination, seed emergence, and vegetative and generative growth. Based on the report of the Intergovernmental Panel on Climate Change (2022), global warming reaching 1.5 °C in the near term (2021–2040) would cause unavoidable increases in multiple climate hazards and risks to ecosystems and humans. High temperature is known to disrupt water for imbibition, ion, and organic solute movement across plant membranes. In addition, the denaturation of enzymes interferes with germination and seedling growth. Temperature is also the primary factor for photosynthesis, but excessive temperatures decline plant leaf photosynthesis and decrease in allocation of dry matter to roots and shoots (Krishnan et al., 2011). However, crop species differ in their

tolerance to temperature and drought stress. Although sorghum is generally considered tolerant, drought stress still significantly hampers its productivity and nutritional quality across its major cultivation areas (Abreha et al., 2022). Pannacci & Bartolini (2018) also report sorghum drought tolerance, low N input, and low water supply, allowing it to maintain high yields.

Sorghum is an important cereal crop in the world that has many benefits. Whole grains of sorghum have essential health benefits, such as free radical scavenging activity, which is associated with antimicrobial properties, reduced oxidative stress, and anti-inflammatory and anti-cancer activity (Rao et al., 2018). These compounds in cereals have exhibited several health benefits, such as anti-diabetic, anti-tumorigenic, and anti-atherosclerogenic effects (Miafo et al., 2019). Sorghum can grow in tropical and subtropical semi-arid regions with deficient rainfall, and high temperatures predominate (Hernández et al., 2020). This crop is a short-day C4 plant (Mullet et al., 2012), and its easy adaptability to hot and dry agroecology makes it a climate change-responsive crop. Sorghum is self-pollinated by nature, outcrossing up to 6% depending on the genotype and growing conditions (Hariprasanna & Patil, 2015).

Rao et al. (2014) reported that the primary production constraints that reduce sorghum productivity are abiotic (nutrient and drought stresses, excess water, temperature extremities), biotic (shoot fly, stem borer, head bugs, grain mold, foliar diseases, charcoal rot). Kange et al. (2014) also state that the abiotic factors include inappropriate temperature, humidity, and rain. Higher temperature (35 °C) has been favorable for all stages of rice weevil, and relative humidity (65%) encourages weevil development (Aslam et al., 2017).

Sorghum is a climate-resilient crop that is naturally tolerant to abiotic stresses and can grow on marginal lands with minimum input. On the other hand, the increasing human population and changing climate have given rise to frequent drought spells, and surging temperatures pose a severe threat to global food security. At the same time, identifying yielding high, temperature-tolerant genotypes remains a professional approach to cope with these challenges. This study evaluated the effects of varying temperatures on the viability and vigor of sorghum.

MATERIALS AND METHODS

The study was carried out at Seed Science Technology Laboratory, Faculty of Agriculture, Andalas University, West Sumatera, Indonesia from August until October 2021. This research applies Factorial Experiment in Completely Random Design, where the first factor was three genotypes of sorghum (Fig. 1), and the second factor was temperature variation 18 °C, 20 °C, 22 °C, 24 °C, 26 °C, 28 °C, 30 °C, 32 °C, 34 °C, 36 °C and 38 °C. Each experimental unit consisted of 50 seeds and was repeated three times (90 units) using the paper test method and placed in the grow room under normal light. The F test analyzed the data in 5% and descriptive qualitative data.

Genotypes G1Marapi and G3Marapi were local sorghum explored in Agam Regency, West Sumatera Province, Indonesia, and Numbu was a superior national variety with high adaptabilities to the environment, such as drought.

Figure 1. Morphology sorghum grain of (a) G1Marapi (b) G3Marapi (c) Numbu.

The sorghum seed quality test observes based on seeds germinating after the radicles had emerged to a length of 2 mm on the following formula.

a. Germination Phenology

Observations were made from 1–10 days after germination (ISTA, 2004) by evaluating and measuring the growth of the sprouts. The data shown only observed the germination steps of the Numbu variety and was not compared to other genotypes because it gives the same results.

b. Germination (%)

Germination Test was calculated from the first observation (I) four days after germination and the second observation (II) ten days after germination (ISTA, 2004),Normal seedlings were collected and calculated with the following formula:

$$G = \frac{\sum Seeds\ that\ germinate\ normally\ \ I+II}{\sum All\ seeds\ germinated} \times 100\% \quad (1)$$

c. First Count Test (%)

First Count Test (FCT) is calculated based on the percentage of regular sprouts on the first count observation (I) four days after they germinated;

$$FCT = \frac{\sum Seed\ germinate\ normally\ count\ I}{\sum All\ seed\ germinated} \times 100\% \quad (2)$$

d. Root and Shoot Growth Test (cm)

The physiological seed quality approach is carried out by observing the growth of sprouts, such as root and shoot growth, ten days after germination. Root and shoot growth describe seed vigor.

RESULTS AND DISCUSSION

Germination Phenology of Sorghum

Germination, or the plant's initial growth, dramatically determines the plant's survival at the next stage. The phenology and growth stages of sorghum start from germination (Fig. 2). The seed germination process begins with the imbibition process, enzyme reactivation, embryo germination initiation, seedcoat cracking, radicle emergence, and plumule emergence (Kamil, 1986).

For a successful germination, seeds should reach an adequate level of hydration during the imbibition phase, to reactivate the seed metabolic processes and stimulate the

growth of embryonic axis. Based on Fig. 2, the emergence of plumula begins two days after germination. On the third day, more than 2 mm of plumule and radicle appeared, meaning germination was successful. The growth and development of sorghum sprouts, such as the appearance of the first leaves, can be seen from the fourth day and fully developed ten days after germination. Plants subjected to severe drought stress require more time to adjust the internal osmotic potential in accordance with the external environment (Abreha et al., 2022).

Figure 2. The germination phenology of sorghum varieties Numbu (0–10 Days After Germinating).

Germination and First Count Test (%)

Viability and vigor was necessary to determine seed quality. Germination was an indicator of the viability index, while the first count test indicates the seed vigor index. The difference in genotype also determines the level of tolerance to high-temperature stress, such as the percentage of germination and the first count test (FCT) in Fig. 3 below.

Based on the percentage of normal germination and first count test, the seeds of sorghum Numbu, G1, and G3 Merapi have good viability and vigor (≥ 80%) only at a temperature of 20–32 °C, at a temperature of 18 °C and 38 °C, none of the seeds can germinate. In contrast, Chadalavada et al. (2021) reported that more than 80% of the sorghum seeds germinate at 15 °C. However, Vanderlip (1993) reported that cool temperatures with high humidity favor the growth of disease organisms; at soil temperatures of 20 °C or more, coleoptiles appear above ground after 3–4 days after germinating and last longer if temperatures decrease.

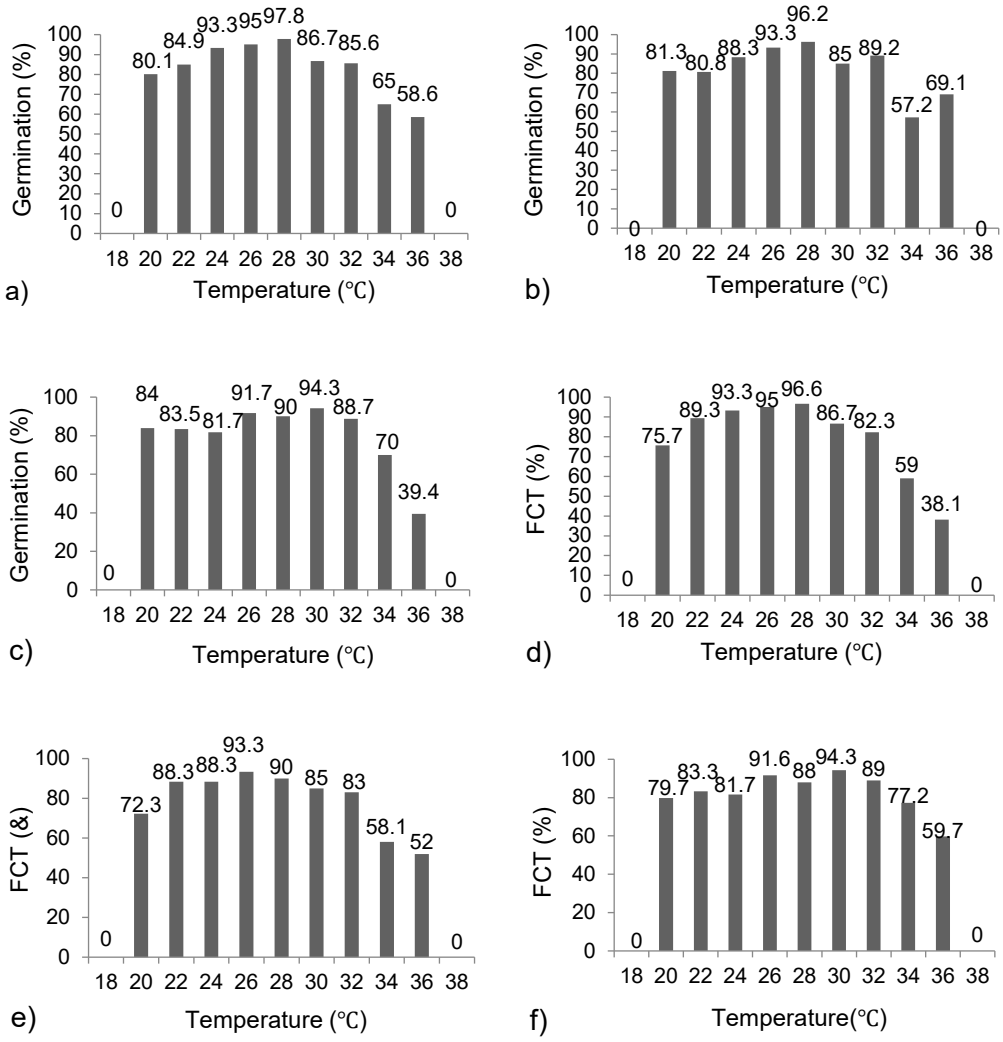

Figure 3. Germination (%) of (a) G1Marapi (b) G3Marapi (c) Numbu and First Count Test (%) of (d) G1Marapi (e) G3Marapi (f) Numbu.

The optimum temperature required for the growth and development of sorghum was 27–30 °C. Growth and yields can be affected beyond 35 °C. It is a short-day plant with a photoperiod requirement of 10–11 h to induce flower formation. Generally, the optimum temperature requirement for sorghum crops was 21–3521–35 °C for germination, 26–34 °C for vegetative growth, and 21–35 °C for reproductive growth (Maiti, 1996). Bartzialis et al. (2020) also explained that the best temperature for sorghum growth is 20–30 °C. However, there is limited information on the availability of resistant sorghum varieties to high temperatures. Sari et al. (2019) reported that the optimum temperature for rice germination was 28–32 °C, and the highest activity of α-amylase was at 40–48 °C but optimum at 48 °C. Mohamed et al. (2009) also reported

that on wheat, the α-amylase activity is above 50% at 40–48 °C with the optimal temperature at 50 °C. In contrast, the temperature optima of *Cucurbita pepo* esterases EIc and EII were 40 °C (Fahmy et al., 2008).

The soil emergence test can be identified when the coleoptile is visible at the soil surface, which takes about four days. Furthermore, sorghum emergence will vary depending on the planting depth, soil moisture, temperate conditions, compaction of soil, and seed vigor. The seedling had three fully expanded leaves, and the collar of 3 leaves is visible, which occurred six days after emergence, and the seedling grew to a height of 20 cm. It can be identified by the appearance of the visible collar in all five leaves, continuous visibility of the first leaf with a round tip, and taking 16 days from emergence. The seedling is thus said to be entered into a 'grand period of growth. The plant grew to a 50 cm height (Rao et al*.,* 2014).

Water stress and extreme temperature are two significant forces impacting germination and plants' reproductive phase. Due to the high temperature, there will be a decrease in seed size and glucose concentration and, at the same time increase in sucrose and raffinose concentrations in grain (Chadalavada et al., 2021).

Root and Shoot Grwoth Test

Based on Fig. 4, genotype G1Marapi had root and shoot growth which relatively increased to 24 °C and began to decrease as the temperature rise. Such as the Numbu variety, the growth of roots and shoots surged to 26 °C and declined from 28 °C to 38 °C. Genotype G3 Merapi fluctuates with the highest root (15.3 cm) and shoots (17.3 cm) at a temperature of 28 °C.

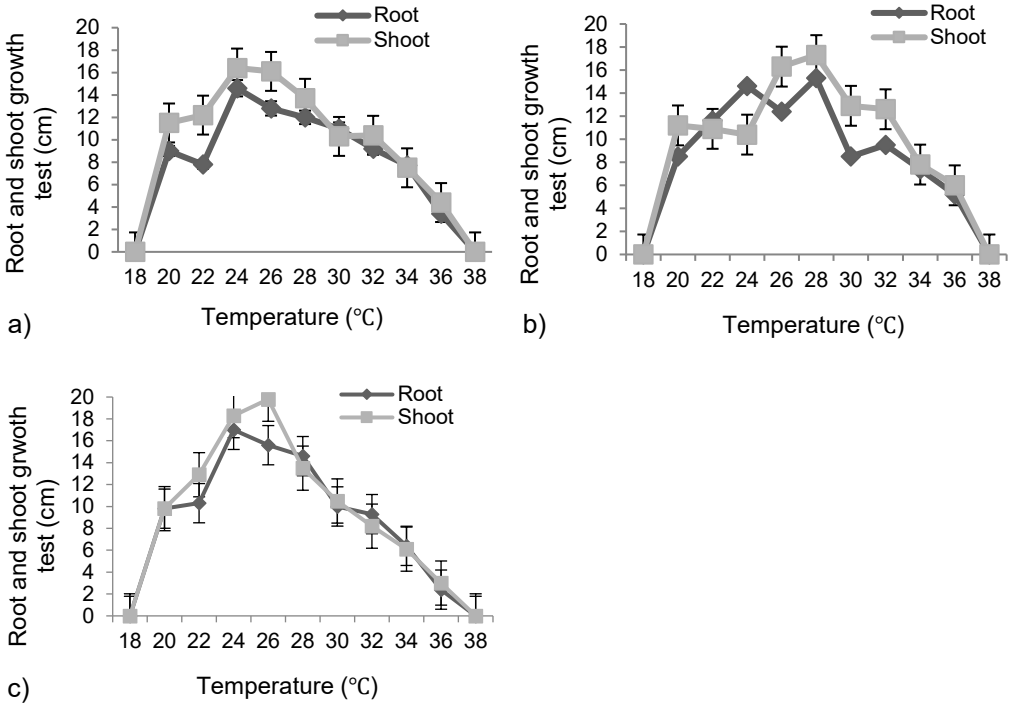

Figure 4. Root and Shooth Growth Test (a) G1Marapi (b) G3Marapi (c) Numbu.

According to Elvira et al. (2015), differences in plant growth are caused by internal influences such as genes and hormones that affect growth through inherited traits. External influences such as nutrients, water, temperature, humidity, and light also affect a plant's characteristics. Taleon et al. (2012) found a strong effect of abiotic stress factors such as light and temperature on the flavonoid content of black sorghum. Heat stress decreases the plant height at maturity, seed set, seed number, and size but does not significantly impact leaf area and dry weight (Chadalavada et al., 2021).

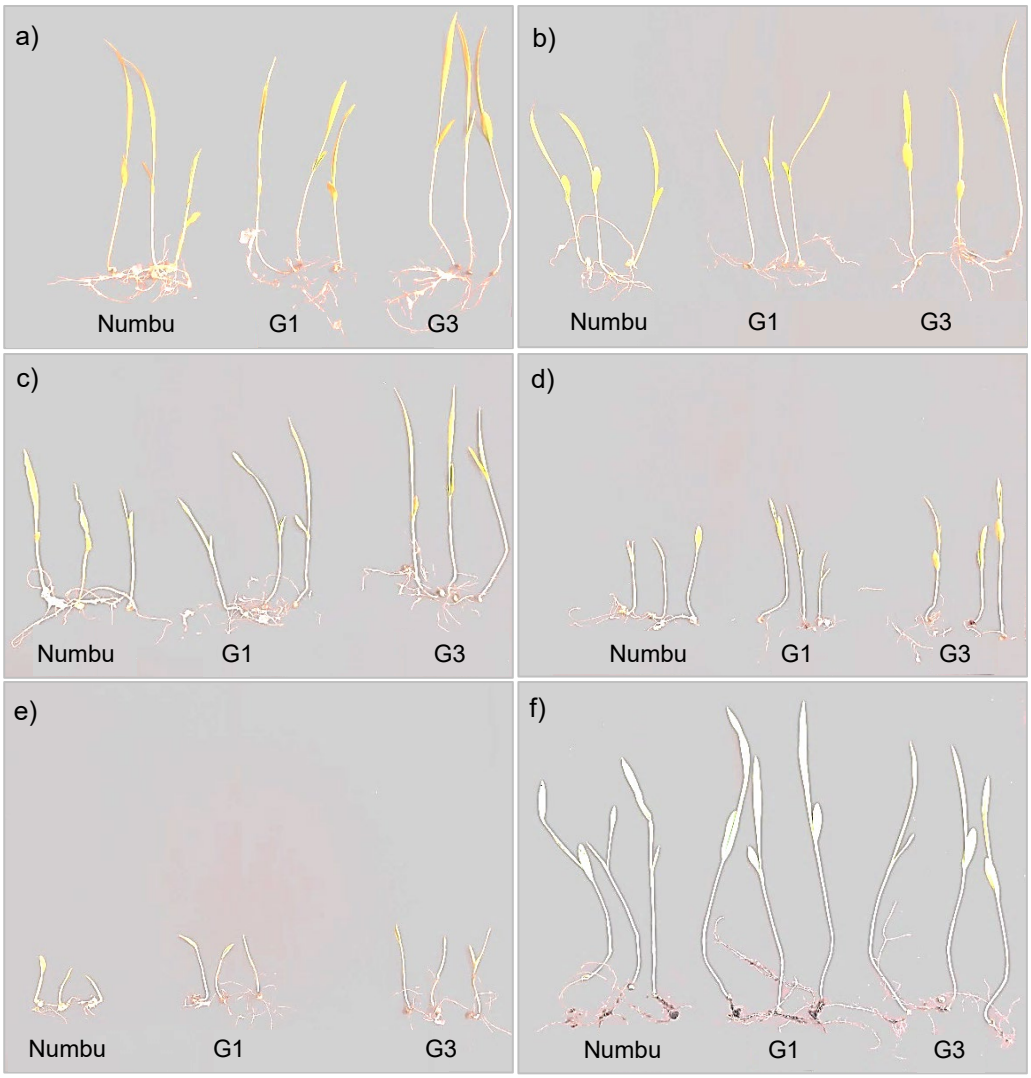

Figure 5. Sprout growth 10 days after seedling (a) 28 °C (b) 30 °C (c) 32 °C (d) 34 °C (e) 36 °C and (f) Soil Emergence Test.

The growth of roots and shoots of sorghum tested was significantly different (Fig. 5). In contrast, the temperature increased beyond the optimum limit, growth decline began to occur, and at 38 °C, no seeds could germinate. If the future temperature increase

above 38, this local sorghum not germinate. Kramer et al. (2021) report that extreme conditions may severely disturb several metabolic processes, resulting in diminished photosynthesis, impeding cell enlargement and division, and finally passing on the cells. However, this problem is feared to be further augmented due to climate change. Global warming manifested through rising temperatures can lead to a severe decline in soil moisture-holding capacity.

Crop establishment is an essential prerequisite for successful crop production. It includes optimum germination and vigor seedling growth. Some yield components in sorghum are determined as early as 30 days after germination (Maman et al., 2004; Wrather, 2009). Hence, in addition to fixing the crop stand and reducing weed competition, timely germination and seedling vigor have important agronomic implications (Weerasooriya et al., 2021).

Genotypes should be tested for their temperature tolerance based on phenology, morphology, physiology, and biochemical behavior at different growth stages from germination to maturity (tillering, jointing, booting, anthesis, grain filling, and physiological maturity stages) due to their variable responses. Assembly of new superior varieties, the inheritance of superior traits must be understood to choose the best parents. Kibalnik et al. (2021) explained that understanding the inheritance of agronomic traits is also necessary to create hybrids with given traits. Hybrid sorghum is characterized by superiority over the parent form for productivity and other critical agronomic properties. In this research, some characteristics of West Sumatra local sorghum genotypes were known for assembling new superior varieties.

CONCLUSIONS

The optimum temperature required for germination of sorghum grain Numbu, G1Marapi, and G3Marapi were 20–32 °C, with good viability and vigor above 80%. High temperatures decrease the germination rate and disturb the growth and development of sorghum, such as the root and shoot of sorghum falling by temperature surge and at 38 °C not germinated.

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Life cycle assessment of shallot farming in Food Estate Hutajulu, North Sumatra, Indonesia

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Abstract. Food Estate is a government program as a solution to meeting food demand. However, in order to meet food needs, environmental impacts must be considered. The study objective was to investigate the impacts of shallot production in Food Estate Hutajulu, Indonesia. The study was conducted with the first stage determining the functional unit, namely an area of 0.2 hectares with a gate-to-gate scope. The second is the inventory data analysis by grouping the categories of nursery, tillage, maintenance, harvesting, and transportation. The third is life cycle impact assessment (LCIA) according to the ISO 14044 standard. Every data obtained from each process was processed using the software OpenLCA 1.11.0; the following is the workflow and use of the software. Processes were made based on the five categories of data (soil processing, planting, maintenance, harvesting and transportation), which had been determined to be connected to flow. The product system was adjusted according to the data in each process and then calculated, and the results of calculation data and graph models appear from each processed data category. Fourth is the interpretation that considers the highest environmental impact, namely acidification in the transportation process of 1.8974 kg SO_2 eq, global warming potential in the transportation process of 415.3188 kg CO_2 eq, eutrophication in the transportation process of 0.4364 kg PO_4 eq, and human toxicity in the maintenance process of 1,409.07377 kg 1,4-DB eq. To minimize the impact on subsequent production, reducing diesel fuel, chemical pesticides and chemical fertilizers are recommended.

Key words: crops cultivation, environmental impact, global warming, LCA, sustainability.

INTRODUCTION

Population growth in Indonesia is increasing. Indonesia's population is expected to continue to increase to 319 million in 2045 (BPS & Bapenas, 2018). As the population grows, food security is expected to be maintained and can meet demand. Existing resources cannot meet current human needs, so advanced agricultural approaches have been developed to meet this urgent need (Mousavi et al., 2022). Various government programs have been implemented to achieve food security. One is the Food Estate program targeted at Central Kalimantan, North Sumatra, South Sumatra, East Nusa Tenggara, and Papua.

Food Estate is a government program that aims to maintain food security during a crisis such as the current Covid-19 pandemic. This program is promoted in specific locations that are considered to have adequate natural resources with different commodities. Based on data from the Ministry of Public Works and Public Housing (PUPR), Indonesia, there are three Food Estate locations in North Sumatra with a total land area of 785 hectares, namely Hutajulu Village, 120.5 hectares, Ria-Ria Village, 411.5 hectares, and Parsingguran Village 253 hectares. In 2021, the government planned to plant shallots (*Allium ascalonicum* L.) and Granola potatoes with an area of 8.8 ha (for shallots) and 7.7 ha (for Granola potatoes), respectively, as a digital farming trial program for Food Estate in Hutajulu Village. Shallots and potatoes, as selected commodities in this Food Estate, generally require specific conditions in the cultivation process. Shallots are one horticultural crop commodity used as a cooking ingredient with high economic value (Prasetyowati et al., 2021). The onion plant (*Allium ascalonicum* L.) is a type of annual plant that belongs to the Liliaceae family. Some shallot varieties in the lowlands have a relatively short lifespan of 55–70 days, depending on the variety and growing season (Baluwo et al., 2021).

The Food Estate program with a digital farming system is expected to achieve maximum total production with maintained commodity quality. This is because all production processes and parameters are controlled in a digital farming system so plants can grow at optimum conditions. Technologies used in intelligent agriculture include liquid fertilizer and pesticide spraying drones, surveillance drones, and soil and weather sensors. Precision Farming does farming practices related to growing crops and raising livestock more accurately and in control. The system can be divided into data collection, data analysis, managerial decisions and variable-rate applications (Cambouris et al., 2014; Balafoutis et al., 2017).

On the other hand, industrialization poses energy unsustainability issues, especially for fossil fuels, and poses severe challenges to food production. Studies have reported the impact of extreme weather events on agricultural production and the application of innovative management strategies to reduce environmental emissions from grain, cash, corn and cotton production in Pakistan (Abbas et al., 2021; Elahi et al., 2022a; Elahi et al., 2022b; Abbas et al., 2022a, 2022b). Improving energy efficiency helps reduce severe environmental impacts, and proper use of energy in agriculture and nurseries leads to sustainable production, cost efficiency and slowing the depletion of fossil fuel sources while preventing air pollution (Karami et al., 2021; Tatli et al., 2021; Elahi & Khalid, 2022; Parhizi et al., 2022). A 5–100% reduction in chemical fertilizers will reduce the environmental impact by 4.38–87.58% and 2.16–43.30%, respectively, on aquatic acidification and global warming in corn production (Abbas et al., 2020; Abbas et al.,

2021). Improved agricultural management practices, production methods, and resource conservation measures through expansion activities are needed to improve energy efficiency. Targeted energy use and replacing diesel with green power can reduce environmental emissions and wasteful use of non-renewable resources.

Farmer education, farming experience, and well-controlled temperature and ventilation systems have significantly improved the energy performance of poultry farms (Elahi et al., 2022c). The agricultural environment structure analysis requires a thorough understanding of energy consumption behaviour and greenhouse gas emissions (GHGs) in crop production. Discharging numerous contaminants with detrimental environmental impacts is one of the drawbacks of considering agriculture inputs (Reichmann & Sala, 2014; Huang et al., 2016; Yan et al., 2019).

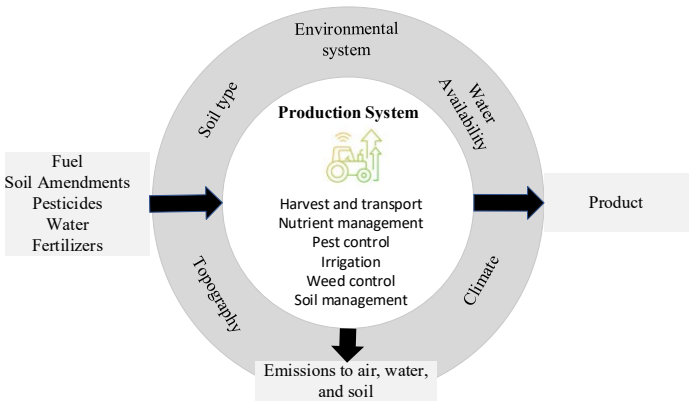

Figure 1. Agricultural life cycle components and flows between environment and production systems.

Life Cycle Assessment (LCA) is a tool used by the International Organization for Standardization (ISO) to analyze a product's potential environmental impact. It examines the entire life cycle of a product, from sourcing and production to product use, packaging, and recycling or final disposal, for components of the agricultural life cycle and the flow between the environment and the production system, as shown in Fig. 1 (Cambouris et al., 2014). In addition, it is intended to evaluate the effects of greenhouse gas emissions on the natural environment. An LCA, also known as a life cycle assessment, is a method of analysis that considers all of the resources related to the inputs and outputs of a production system. This method can also analyze greenhouse gas emissions and other environmental factors, such as acidification, eutrophication, and ecotoxicity. It can analyze and lessen the related environmental problems that result from a particular process or activity. It possesses many tactics that can set it apart from other approaches, such as modelling. The LCA process adheres to the standards produced between 1997 and 2006, beginning with ISO 14040 and going up to ISO 14044. (Hammond & Jones, 2008). The operational stage is comprised of the following four primary steps: defining the goals and scope of the project; doing a Life Cycle Inventory (LCI); performing a Life Cycle Impact Assessment (LCIA); and interpreting the results of the LCIA (Fig. 2) (Rabl & Spadora, 2006; Greenhut et al., 2013; Rahman et al., 2019; Morandini et al., 2020; Sillero et al., 2021).

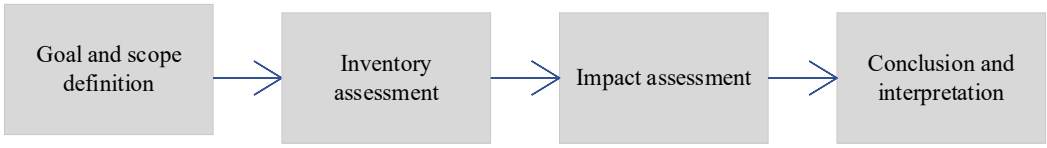

Figure 2. Components of Life Cycle Assessment (The four main components of an LCA are often interdependent, as the outcome of one component will inform how the other components are accomplished).

The agricultural sector is responsible for producing a variety of commodities and services; nevertheless, the industry's expansion has been significantly limited by the paradox of poor land productivity and high population density. Large-scale, high-intensity farming that uses a high input of chemical fertilizers, pesticides, and agricultural film has resulted in negative environmental repercussions, such as damage to natural resources, decreased land productivity, the rapid spread of pests and diseases, and a decline in biodiversity. These effects include harm to natural resources; decreased land productivity; the rapid spread of pests and diseases; and declining biodiversity (Tilman et al., 2001; Tilman et al., 2006; Fan et al., 2022). Operations related to agriculture, forestry, and other land uses contributed 23% of the total net anthropogenic emissions of greenhouse gases (GHGs), including 13% of the world's carbon dioxide (CO_2) emissions, 44% of the world's methane (CH_4) emissions, and 81% of the world's nitrous oxide (N_2O) emissions (IPCC, 2019). It is necessary to undertake a Life Cycle Assessment (LCA) to help farmers and policymakers understand the total environmental impact of agricultural production systems to promote green and low-carbon development of agriculture. This study used this tool opens up new opportunities for 'green marketing' to better use energy, equipment and agrochemical resources to expand land through digital farming systems in Indonesia, especially food estates in North Sumatra. It can even reduce the overall cost of growing shallots in future.

MATERIALS AND METHODS

Life Cycle Assessment (LCA) is a process that evaluates a product's or service's environmental impacts throughout all stages of its life. It is standardized by international regulations ISO 14040:2006 and ISO 14044:2006. The methodology used throughout this study followed the guideline of ISO 14044 (2006). Key features include the use of functional units, the flexibility of methods to implement the process, and the product system, which can be divided into unit processes linked to one another. A specified unit process of a product system defined based on criteria is called a system boundary (Fig. 3).

Materials

The materials used in this study were data and information obtained from the Food Estate Hutajulu for four months, from seedling to harvesting, on aspects of the volume of water use, electrical energy, chemical fertilizer energy, transportation energy, fuel energy, irrigation energy and human labour. The tools used were Personal Computer (PC) with Intel(R) Core(TM) i7-6500U CPU @ 2.50GHz 2.60 GHz with 8.00 GB and 64-bit operating system, x64-based processor as hardware; OpenLCA 1.11.0 as the official life cycle assessment data processing application; elcd_3_2_greendelta_v2_18

and usda_190109_2 as databases; and openlca_lcia_v2_0_5_20200610 as the life cycle impact assessment database.

OpenLCA 1.11.0 is a software used to analyze the stages of research related to life cycle assessment (LCA). There are three application flow types: environmental life to analyze energy in and out of the environment; product flows, whose purpose is to analyze the energy exchanged during the process production takes place; and waste flows. Following are parts of the OpenLCA 1.11.0 software according to GreenDelta (2016): 1) Flows are overall products, materials, or energy as input and output in the product system analysis. There are three types of flows in OpenLCA 1.11.0: a. elementary flows: material or energy from the incoming environment and exit the product process, b. product flows material or energy that undergoes changes or is exchanged during product processing, c. waste flows: material or energy leaving or leaving the product process. 2) Databases use of OpenLCA 1.11.0 requires a database for operation. The database itself is a data assortment matters relating to the production process. 3) Processes are activities that transform inputs into outputs determined based on flow as a quantitative reference.

Methods

Data were collected from the field of 0.2 ha by investigating and measuring included crop type, sowing date, the number of seeds used and variety of cultivation, type and rate of fertilizers used, number of pesticides and fungicides used, fuel consumption and machinery used and amount of physical work for crop period. These energy used were determined according to Sigalingging et al., 2023 and also using the energy coefficient as shown in Table 1.

Table 1. The energy equivalent coefficient on shallot cultivation

		Energy coefficient (MJ per unit)	Unit	Reference
	A. Input			
1	Mechanization			
	Tractor	9–10	kg year^{-1}	Kitani, 1999
2	Fertilizer			
	Nitrogen (N)	78.1	kg	Kitani, 1999
	Phosphate (P_2O_5)	17.4	kg	Kitani, 1999
	Potassium (K_2O)	13.7	kg	Kitani, 1999
3	Pesticides	120	kg	Mohammadi et al., 2008
4	Electricity	12	kWh	Elhami, 2019
5	Manure	0.3	kg	Esengun et al., 2007; Naderi et al., 2019
6	Transports	1.6–4.5	km	Fluck & Baird, 1980
7	Irrigation	0.63	m^3	Yaldiz et al., 1993
	B. Output			
	Shallot	1.85	kg	Kitani, 1999; Allali et al., 2017; Esmaeilzadeh et al., 2020

Fuel consumption was obtained using the Full to Full method to determine how many litres are spent cultivating the land or carrying out other processes with agricultural machines. The Full to Full method was applied before the engine runs, the engine oil tank was filled, and then the engine was run until the processing or process wanted to

run entirely. When finished, the tank was again filled with fuel while measuring how many litres were used up. As long as the agricultural machinery is operating in the field, the operating time is calculated, and the data is then used for the operator's calorie calculation.

Some supporting data are needed to find out the calorie consumption used at work, such as age, weight, height, duration of activity and level of activity performed. The basal metabolism was determined by Eq. 1 (Hutabarat, 2009).

$$Basal\ metabolism = [66.5 + (13.7 \times weight) + (5 \times height) - (6.8 \times age)](\text{kkal}) \quad (1)$$

The conventional way of approaching environmental assessment is represented by a system boundary drawn around a manufacturing process or plant. The materials and energy used in production must be obtained from primary resources and processed before use. At the same time, any product has a further environmental impact on how they are used and ultimately recycled or disposed of. Therefore, the environmental impact of each product or service under Analysis is considered part of the life cycle from start to finish and the boundary of the system to be analyzed (Fig. 3).

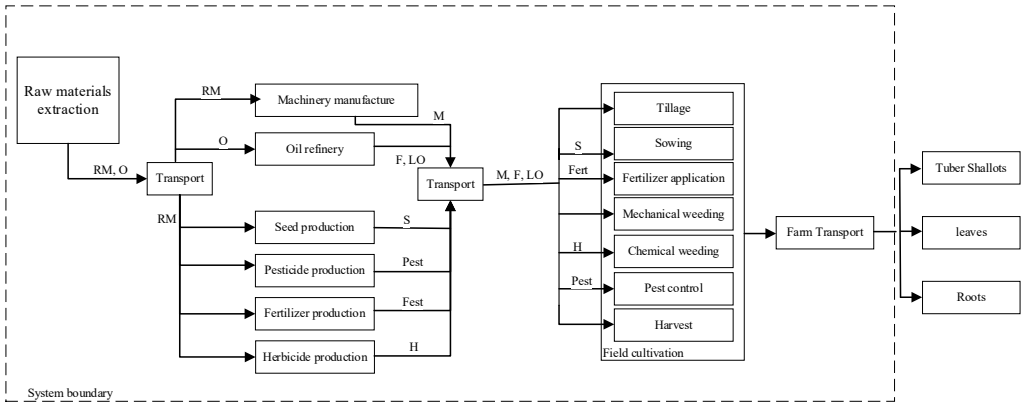

Figure 3. System boundaries for plant production system life cycle assessment by the Crop.LCA tool (RM:Raw Materials; O:Petroleum; F:Fuels; LO:Lubricants; M: Machinery; Fert:Fertilizers; Pests:Insecticides; H:Herbicides; S:Seeds).

Four stages in the life cycle assessment (LCA) are based on ISO 14044:2006 (Fig. 4). 1) Scope/Goal and Scope: The functional unit of this study was the production of shallots in an agricultural area of 0.20 hectares with drone surveillance mapping. The scope used was the Gate-to-Gate Life Cycle Assessment (LCA) method through a review of activities in the shallot production process. 2) Inventory Analysis: Inventory analysis is part of a data set's life cycle assessment process. It flows calculations as input and output data from life cycle assessment stages. Cultivation of shallots has stages including a) nursery with beds; b) tillage using tractors together with trailers; c) planting and maintenance; d) harvesting; and e) transportation. Each stage of the shallot cultivation process uses input from natural resources or energy, with the resulting output in the form of final products and emissions.

The shallot cultivation process starts from the tillage and planting stages. The material used as input is diesel fuel used by the tractor, with each implement having its function for tilling the soil to produce output in the form of fuel combustion emissions.

When spraying pesticides, use a sprayer with a battery input. Pesticides and fertilizers on Food Estates are rumoured to use fertigation techniques to regulate the use of water and fertilizers according to plant needs. The next stage of onion cultivation is harvesting with optimum plant conditions. In the transportation process, diesel is needed to produce output in the form of transportation emissions. 3) LCA analysis: All data were analyzed quantitatively to determine the environmental impact of each stage of the shallot cultivation process. Data was analyzed by calculating input and output at each stage of the production process with a life cycle assessment (LCA) in the energy aspect, including fuel use, electricity use, and gas emission calculations. Every data obtained from each process was processed using the software OpenLCA 1.11.0; the following is the workflow and use of the software. Processes were made based on the five categories of data (soil processing, planting, maintenance,harvesting and transportation), which had been determined to be connected to flow. The product system is adjusted according to the data in each process and then calculated, and the results of calculation data and graph models appear from each processed data category. 4) Life Cycle Impact Assessment (LCIA): The life cycle impact assessment aims to evaluate the impacts generated during the shallot production life cycle, with the primary factor being analyzed as environmental factors. Environmental factors evaluated are acidification, global warming potential, eutrophication, and human toxicity. 5) Interpretation. The data processing results based on inventory analysis and life cycle impact assessment conclude by comparing the values of environmental factors in several studies. The effects are reviewed and evaluated at this last stage to ensure the results are steady with the observed objectives. As proven withinside the diagram, all three different steps are associated with interpretation, indicating that this segment is a significant part of the technique and might constantly result in corrections.

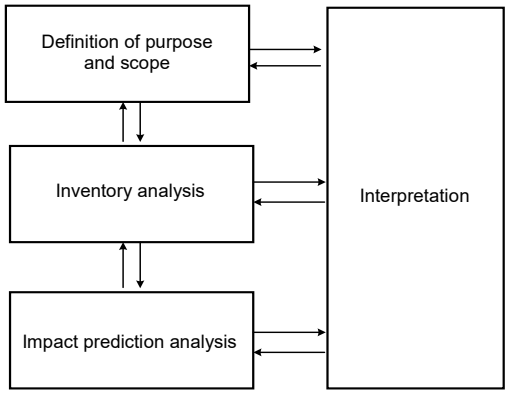

Figure 4. Four stages in the life cycle assessment (LCA) are based on ISO 14044:2006.

RESULTS AND DISCUSSION

Stage 1: Goal and Scope

The goal of LCA in this study is to determine the environmental impacts of acidification, global warming potential, eutrophication, and human toxicity of cultivating shallots in one growing season with an area of 0.2 hectares in Food Estate Hutajulu, Humbang Hasundutan, North Sumatra, Indonesia. The results will be recommended to Food Estate managers for further consideration in cultivating shallots and taking corrective actions for the next growing season to minimise the environmental impact of operations. The scope of this research is the gate-to-gate, which discusses and manages data from soil processing to harvesting shallots in one growing season.

Stage 2: Inventory Data

Inventory data in this study is divided into five categories during the shallot production process: shallot seedling data, soil tillage data, maintenance data, harvesting data and shallot transportation data. Each category analyzed the energy used based on predetermined parameters, namely diesel fuel, fertilizers, pesticides, electricity, human power and irrigation, as shown in Figs 5–8.

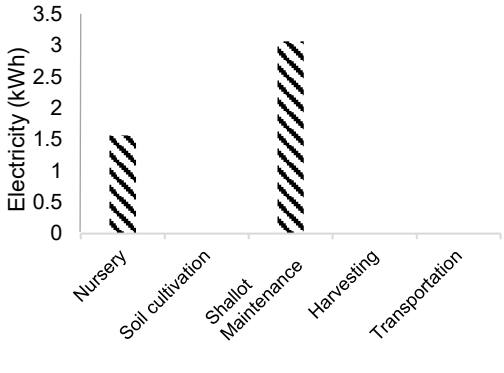

Figure 5. Electric power consumption.

Fig. 5 shows the electricity used starting from the seedling process to harvesting. The use of electricity in shallot cultivation was used for a drone sprayer. A drone sprayer was used to help spray pesticides, insecticides, fertilizers, and watering. The drone sprayer used a volume of 16 litres, the same as an electric sprayer, with a spraying speed of 4 m s^{-1} and a battery capacity of 12,000 mAh. The drone sprayer operates on the surface of the air to spray pesticides and fertilize by remote control using a Wi-Fi connection on the operator's remote control equipped with sensors and a global positioning system (GPS). One season's electricity consumption in the shallot cultivation process was 4.64 kWh (1.568 kWh for the nursery process and 3.072 kWh for shallot maintenance).

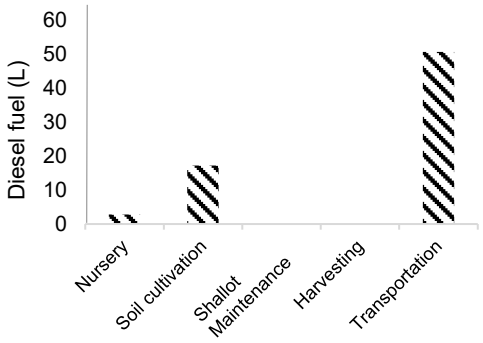

Figure 6. Diesel fuel consumption.

Diesel fuel was used for tractors and transportation, starting from the nursery process until the harvesting process (Fig. 6 and Table 2). In the nursery stage, the initial step for seeding is to cultivate the land where the shallot seeds are sown. The available land area at the Food Estate is 0.04 hectares for later transplanting to an area of 0.2 hectares. This nursery requires several stages so that the plants are suitable for transplanting. The available land in the Food Estate is processed by making beds for sowing shallots with three varieties (Maserati, Lokananta variety, and Sanren). These beds were made by applying organic fertilizer and husk charcoal on the beds before seeding. This stage is done manually. The bed for planting an area of 0.2 hectares is 60.64 meters long and 1 meter wide. Seedling of shallots was made nursery lines on beds with a distance between rows of 15 cm with a density spread of 40 grams per square meter and then covered with husks. Then on the same day, a fungicide was sprayed to prevent the growth of fungi on the beds that had been sown, and then the beds were closed to accelerate seed growth and maintain the air humidity. The hood is made after the seeds are two weeks old or when the shallot seed sprouts have appeared. The hood aims to protect the seeds that have been sown from rainwater and too-hot sunlight. The hood is made of a bamboo

frame and a plastic hood. Maintenance shallots during the nursery stage were carried out manually by watering in the morning or evening to maintain soil and plant moisture, with a total watering of 81.56736 m^3. Then the provision of fungicides, insecticides, and fertilizers regularly keep the plants from pests and diseases, and the plants still have to grow well as needed (Fig. 7 and Table 3).

Table 2. Data on fuel consumption in tillage

Implement	Area (Ha)	Fuel consumption (litre)	Type of fuel	Tractor type
Rotary nursery	0.04	0.540	Diesel	KIOTI DT 4510
Power harrow nursery land	0.04	1.614	Diesel	Farmtrack 120 HP
Nursery field disc bedder	0.04	0.682	Diesel	Kamol 77 HP
Rotary	0.20	2.700	Diesel	KIOTI DT4510
Manure spreader	0.20	3.040	Diesel	Kamol 77 HP
Power harrow	0.20	8.074	Diesel	Farmtrack 120 HP
Disc bedder	0.20	3.410	Diesel	Kamol 77 HP

In soil cultivation stage, the fuel consumption was used for the tractor in soil processing (Fig. 6 and Table 2). Soil processing was carried out in several stages using agricultural mechanization. It started from the rotary stage, levelling, spreading manure, power harrowing, and then making beds. The subsequent tillage was rotary. Rotary on Food Estates used a KIOTI DK4510 tractor with a power of 45 HP, which aimed to chop the soil with a working system; the rotary implement has a knife that moves on an axis driven by a motor so that the knife will chop the soil. On the other hand, land levelling was done by measuring the degree of slope of the land using the Mileseey PF210 600 m Golf Rangefinder. Land with a degree of the slope above 5° was levelled or equalized using a kamol tractor or D31P bulldozer. On valve six land, which is used as a shallot planting area, the degree of slope of the land is 3°, so there is no need for levelling. The shallot planting area at the Food Estate location was spread with manure in cow dung, as much as 7.99 m^3. Manure distribution utilizing mechanization, namely a farm track tractor with a power of 120 HP using a manure spreader implement with a volume of 5.92 m^3 one manure. So the manure spread was carried out twice by the manure spreader. The next stage after spreading the manure is the power harrow. A power harrow is one of the implements whose function is to loosen the soil to a depth of 35 cm, break the soil into smaller sizes, and mix the soil with manure spread over the soil surface. The power harrow uses a rotating blade driven by a rotor of 9 pairs of blades. The power harrow implementation was coupled to a farm track tractor with a power of 120 HP. Making beds on Food Estate land with shallot commodities used a kamol-type tractor with a power of 77 HP in collaboration with a disc bedder implement. The beds' width using a disc bedder was set to 90 cm, with the distance between the beds being 60 cm. On the other hand, diesel fuel was used for transportation. The transportation used in the process of manure is used a Colt Diesel truck with a distance of 207 kilometres and a diesel fuel consumption of 25.875 liters. For shipping the shallot harvest from Hutajulu, Humbang Hasundutan, to towns as far as 198 kilometres using Cold Storage trucks with diesel fuel consumption was 24.75 litres. The highest diesel fuel consumption was used for transportation (Fig. 6) due to the distance of the manure source to the field is too far.

Table 3. OpenLCA input data of fertilizer and pesticide for nursery stage

Active materials	Flow	Total		Information
		(kg)	(kg ha^{-1})	
N	Application of nitrogen fertilizer mix	0.04002	1.0005	Fertilizer
K	Potassium fertilizer, as K	0.00906	0.2265	Fertilizer
P_2O_5	Ammonium nitrate phosphate as P_2O_5	0.02000	0.5000	Fertilizer
Propineb	Propineb	0.14000	3.5000	Pesticide
Propamocarb hydrochloride	Propamocarb HCl	0.30800	7.7000	Pesticide
Mancozeb	Mancozeb	0.16000	4.0000	Pesticide
Dimetomorph	Dimethomorph	0.00788	0.1970	Pesticide
Alkylphenol ethoxylate	Alkylphenol ethoxylate	0.01960	0.4900	Pesticide
Carbosulfan	Carbosulfan	0.00400	0.1000	Pesticide

At the maintenance stage of shallot cultivation, fertilizers, fungicides, and pesticides were applied (Fig. 7 and Table 4), each having a function and role in the plant. NPK Super Folium, a fertilizer formulation containing high doses of NPK and micronutrient elements S, Mg, B, and Zn in the form of white crystals, is 100% soluble in the air without sediment. The function of this fertilizer is to accelerate plant growth, trigger the growth of new shoots, and increase crop yields by increasing fruit/tuber size. Antracol is a fungicide that controls various plant diseases caused by fungi. This fungicide contains the micronutrient Zn, with the active ingredient propineb. Previcur N is a pesticide whose function is to control diseases in shallots with the active ingredient propamocarb hydrochloride. Sidajeb is a pesticide that controls diseases in shallot plants with the active ingredient mancozeb. Acrobat is a pesticide that controls late blight (*Phytophthora infestant*) with the active ingredient dimetomorph. Radix (ZPT) is a growth regulator that stimulates plant roots, growth, flower growth, fertilization, and tubing. Axer is an adhesive for pesticides and foliar fertilizers so that it can survive and penetrate plants. The active ingredients of this product are alkylphenol ethoxylate and sodium succinic sulfonic. Marshall is a pesticide that controls beetles, caterpillars, aphids, seed flies, termites, and armyworms with the active ingredient carbosulfan.

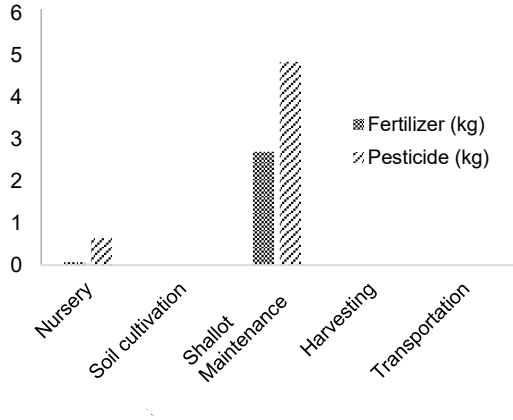

Figure 7. The use of chemical fertilizers and pesticides.

Nebijin is a fungicide that controls club root disease in plants with the active ingredient flusulfamide. Plush is a liquid organic fertilizer to repair soil damage, increase free nitrogen fixation, accelerate plant growth, and increase plant immunity against pests and diseases. Plush contains organic nutrients, N, P_2O_5, K_2O_5, CaO, and MgO. Bazooka 80 WP is a fungicide that controls rot and spot disease on shallot plants in the form of a yellow powder that can be suspended. Bazooka 80 WP has the active ingredient mancozeb 80%. Ultimax 550 EC is one of the clear yellow insecticides in the form of

concentrates to control armyworm pests on onion plants. Ultimax 550 EC produced by PT Agricon has the active ingredients chlorpyrifos and cypermethrin. The cluster is also a product of PT Agricon in the form of a colourless solution. It can be mixed with a solution of insecticide, fungicide, herbicide, and acaricide, which aims to reduce the surface tension of the grains resulting from pesticide spray so that the pesticide sprays evenly on the plants. Captive 200 SC is a fungicide in the form of a white suspension concentrate that controls downy mildew (*Peronosclerospora maydis*), late blight or root disease (*Phytophthora infestans*) on shallots with the active ingredient dimethomorph.

Table 4. Data on fertilizer and pesticide needs in maintenance

Active materials	Flow	Total		Information
		(kg)	(kg ha^{-1})	
N	Application of nitrogen fertilizer mix	1.50300	7.5150	Fertilizer
K	Potassium fertilizer, as K	0.33879	1.6940	Fertilizer
P_2O_5	Ammonium nitrate phosphate as P_2O_5	0.77358	3.8679	Fertilizer
Flusulfamid	Flusulfamid	0.00598	0.0299	Pesticide
Mancozeb	Mancozeb	2.99120	14.956	Pesticide
Chlorpyrifos	Chlorpyrifos	0.18400	0.9200	Pesticide
Cypermethrin	Cypermethrin	0.01840	0.0920	Pesticide
Dimetomorph	Dimetomorph	0.93120	4.6560	Pesticide
Broflanilide	Broflanilide	0.00508	0.0254	Pesticide
Flubendiamide	Flubendiamide	0.02240	0.1120	Pesticide
Monosultap	Monosultap	0.35840	1.7920	Pesticide
Dimehipo	Dimehipo	0.32000	1.6000	Pesticide
Cobalt	Cobalt	0.08000	0.4000	Fertilizer

Fig. 8 summarizes the energy specific of shallot cultivation on the Food Estate Hutajulu. As shown in Fig. 8, transportation, mechanization, and pesticide had the highest energy required, approximately 34%, 21% and 18%, respectively. At the same time, fertilizer (3%), irrigation (11%) and labour/human power (14%) had the lowest energy consumption, contributing less than 15%. In addition, Sigalingging et al. (2023) reported that the energy productivity of shallot production was 33.98 kg kJ^{-1}. The authors also reported a mathematical model to predict energy productivity in different growth phases using a convolutional neural network (CNN).

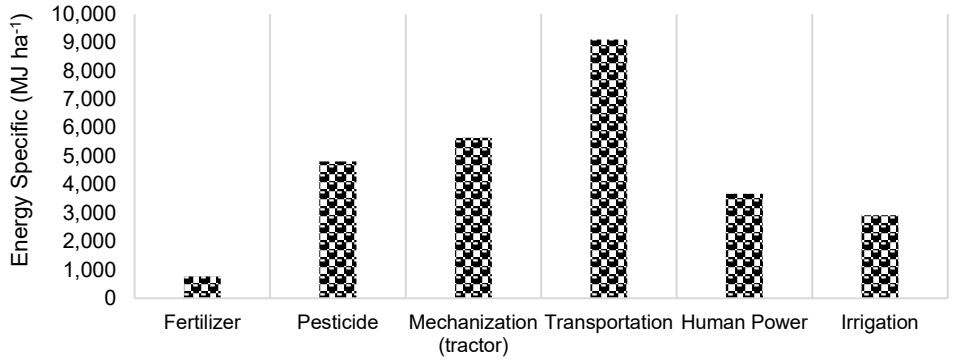

Figure 8. Energy specific of shallot cultivation.

The transplanting of shallots from the Food Estate Hutajulu was done manually with human power. Shallot transplanting was carried out after 51 days of seeding. The land area for transplanting was 0.2 hectares, with a total length of 1,395.5 meters. Similarly, harvesting was done manually with human power. So, to analyze the energy expended during seeding to harvesting, it was calculated using the Hutabarat method (2009) with age, height, weight, duration of work, and a heavy or moderate level of activity. The total human power used for shallot cultivation in Food Estate Hutajulu was 3,682.38 MJ ha^{-1} (Fig. 8). On the other hand, for irrigation, the highest energy specific was during shallot maintenance. The total volume of water used during maintenance was 519.79200 m^3.

Stage 3: Life Cycle Impact Assessment

The field's cultivation process was divided into five categories: shallot seedling, tillage, maintenance, harvesting, and transportation. The five categories of data were processed using the OpenLCA 1.11.0 software. There are four priority contribution impact categories that have an environmental impact (Table 5). Esmaeilzadeh et al., 2020 study on air footprint and the life cycle of edible onion production - a case study in Iran and Abdelkader et al., 2022 on LCA of onion's cultivation processes in Southern Egypt for comparison as shown in Table 6.

Table 5. Results of life cycle impact assessment analysis

Category	Acidification (kg SO_2 eq)	GWP100a (kg CO_2 eq)	Eutrophication (kg PO_4 eq)	Human Toxicity (kg 1,4-DB eq)
Shallot seedling	0.02310	1.27841	0.00545	0.33097
Tillage	0.08750	5.16132	0.01999	1.65311
Shallot maintenance	0.32304	15.98432	0.08017	1,409.07377
Harvesting	0.20647	12.15901	0.04720	3.77075
Transportation	1.89740	415.3188	0.43640	14.51230
Total	2.74541	449.90186	0.58921	1,429.3409

Table 6. Eco invent world average life cycle impact indicators and previous study by others

World average life cycle impact indicators	Esmaeilzadeh et al., 2020	Abdelkader et al., 2022	Unit	Impact
7.45	2.64	1.65	kg SO_2 eq	Acidification
472	324	283	kg CO_2 eq	Global Warming Potential
3.21	1.92	0.66	kg PO_4 eq	Eutrophication
446	446	342	kg 1,4-DB eq	Human Toxicity

Acidification

Acidification produces SO_2 emissions. The acidification value is a degree of air pollution (especially ammonia, sulphur dioxide and nitrogen oxides) in kilograms of sulfur dioxide (SO_2) equivalents (kg SO_2 eq) because of the product existence cycle and contributing to the deposition of acid substances. The resulting 'acid rain' is first-class and regarded as detrimental to forests and lakes. The total impact of acidification in this study was 2.74541 kg SO_2 eq (Table 5), sourced from the transportation process of 1.8974 kg SO_2 eq (Fig. 9). The world average shows that shallot production is equivalent to 7.45 kg SO_2 eq, so the impact of acidity from this study is still low compared to the world average. The study by Esmaeilzadeh et al. (2020) showed that the emissions

produced from the effects of acids on the environment on the production of shallots were 2.64 kg SO_2 eq from agricultural machines, while Abdelkader et al., 2022 study was 1.65 kg SO_2 eq (Table 5). Astuti's study (2019) on the analysis of the potential environmental impact of sugarcane cultivation using a life cycle assessment shows that the LCIA analysis results on the impact of acidification are 1.54 kg SO_2 eq sourced from the harvest and transport stages. The largest source of acidification is in the harvesting process. However, in this study, the value of the acidification impact is still relatively large.

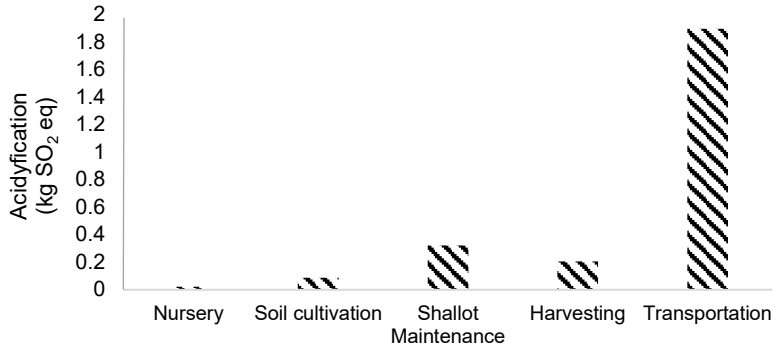

Figure 9. The acidification impact for each category.

The most significant impact of acidification on the environment is the increase in acidity in soil and water systems, with the main pollutants NOx, SOx, NH_3, and HCL being deposited, causing damage to animal and plant populations (Arena, et al., 2003).

Global Warming Potential (GWP 100a)

Global warming is of concern to the world because it can cause various conditions such as rising sea levels, shifting weather, heat waves and other impacts. In this study, the total impact of global warming potential was 449.90186 kg CO_2 eq, with the most significant contributor to GWP being the transportation process of 415.3188 kg CO_2 eq (Table 5 and Fig. 10).

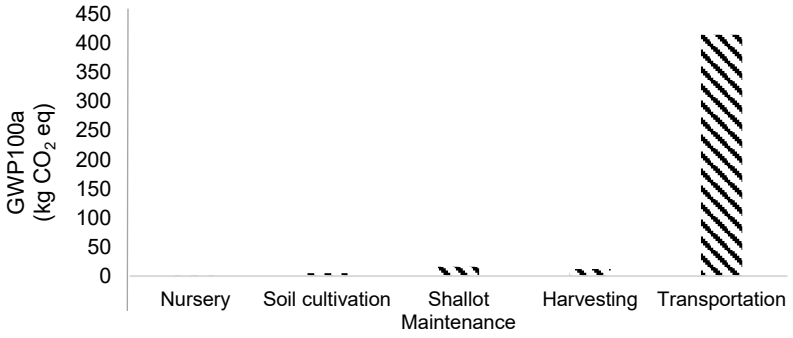

Figure 10. The Global Warming Potential impact for each category.

The global average shows that the impact of global warming was 472 kg CO_2 eq (Table 6), so the results of this study are still below the global average. In the study of Esmaeilzadeh et al. (2020), the impact of global warming on shallot production was 324 kg CO_2 eq. Abdelkader et al. (2022) study on environmental impacts on onion cultivation process in Southern Egypt using the life cycle assessment (LCA) method, the impact value was 3,142.9 kg CO_2 eq.

In Lestari's (2022) study on life cycle assessment (LCA) for the oil palm plantation agro-industry chain, it shows that the plantation process produces an impact of 62 kg CO_2 eq, the distribution process was 1,069 kg CO_2 eq, and the production process was 49 kg CO_2 eq. These results indicate that GWP's impact on shallot production is still lower. Excess doses of fertilizer containing N will produce N_2O emissions, resulting in global warming potential. Through various mechanisms, including an increase in the frequency and severity of heat waves, climate change may impact health. In the near term, health should improve as a consequence of decreasing the consumption of fossil fuels and boosting the utilization of renewable energy sources to combat climate change (Haines et al., 2006). In order to adapt to climate change, public health measures and enhanced surveillance are needed.

Eutrophication

Eutrophication is a process of water pollution caused by phosphate waste, especially in freshwater ecosystems. The results showed the eutrophication was 0.58910 kg PO_4 eq. The most significant contributor to eutrophication was the transport process of 0.4364 kg PO_4 eq (Table 5 and Fig. 11). The global average shows a eutrophication value of 3.21 kg PO_4 eq. This value on the impact of eutrophication on shallot production in Hutajulu was still relatively low. The study by Esmaeilzadeh et al. (2020) showed that the value of the eutrophication impact of shallot production in Iran was 1.92 kg PO_4 eq.

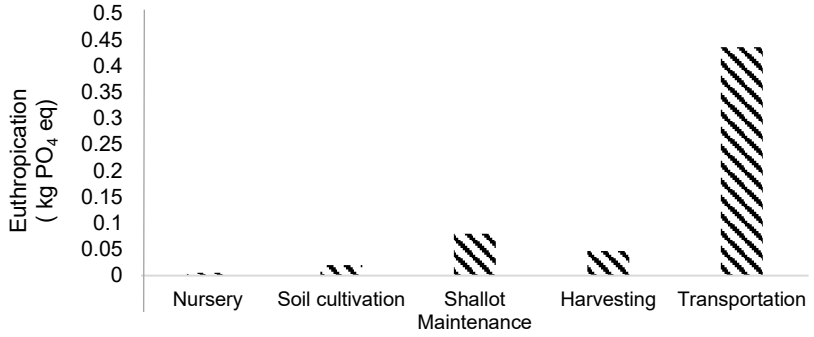

Figure 11. The Eutrophication impact for each category.

In Astuti's research (2019) on the analysis of the potential environmental impact of sugarcane cultivation using a life cycle assessment, it shows that the eutrophication value of 120.24 kg PO_4 eq with the most considerable contribution is the maintenance stage which has an impact on the soil and waters around the land. The most significant contributor to the impact of eutrophication is the maintenance stage. The leading cause of eutrophication is chemical fertilizers which produce nutritional and industrial emissions. Eutrophication has a terrible impact on the aquatic environment because it

can reduce oxygen levels in the water due to the uncontrolled growth rate of aquatic plants so that it covers the waters, and animals will have difficulty getting sunlight. This can be minimized if fertilizers are replaced with organic fertilizers that are safer for the environment.

Human toxicity

Human toxicity is closely related to human health and a process's impact. This indicator is intended to quantify emissions to air, water and soil related to the life cycle of a product that is potentially hazardous to human health. Toxicological factors are calculated using scientific estimates of acceptable or tolerable daily intakes of toxicants. However, they are still in the early stages of development, so absolute values can only be used as a guideline. It can not be used as a rough scale. Units of measure correspond to 1,4-dichlorobenzene (DB), a known carcinogen. In LCA, characterization factors are used to compare hazardous chemical compounds by demonstrating how a particular quantity of pollutant leads to environmental or health impacts. The mid-level comprises mechanisms, whereas the endpoint level focuses on human and environmental harm. Midpoint-level characterization is characterized by better links to environmental flows and reduced uncertainty, whereas endpoints emphasize the environmental significance of the impact but give more significant uncertainty. Characterization variables for human toxicity include destiny in the form of environmental persistence, ingestion and accumulation of chemicals in people, and the ultimate toxicity impact. Such as *chlorpyrifos*, as a pesticide in shallot cultivation in this study, is an organophosphate insecticide proven to cause neurotoxicity and is considered a developmental neurotoxicant. It is mainly used in the agricultural sector but occasionally for household pest control. Exposure pathways include inhalation, ingestion and dermal exposure. Chlorpyrifos exposure has been linked to cognitive dysfunctions, smaller head-circumference new-borns, impaired foetal growth, developmental delay, and behavioural problems in children. *Chlorpyrifos* has been linked to neurodevelopmental effects, reproductive toxicity, and endocrine disruption and is not listed by IARC as a potential carcinogen (Papai et al., 2021).

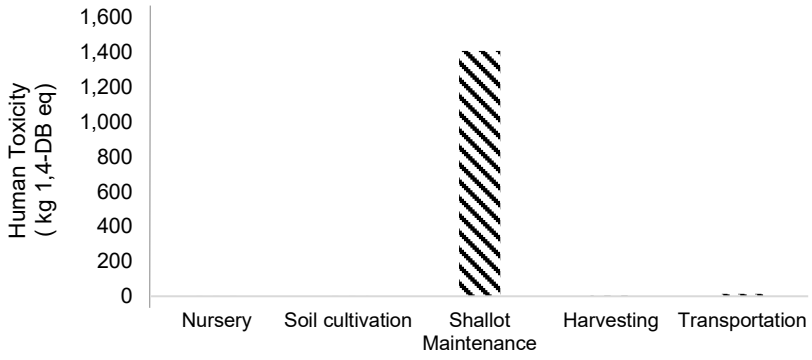

Figure 12. The Human toxicity impact for each category.

The human toxicity obtained was 1,429.34090 kg 1,4-DB eq (Table 5), with the enormous contribution being the maintenance process of 1,409.07377 kg 1,4-DB eq. (Fig. 12). This value is higher than study of Esmaeilzadeh et al. (2020) and Abdelkader et al. (2022). Esmaeilzadeh et al. (2020) reported that the impact value of human toxicity

was 446 kg 1,4-DB eq, with the most considerable contribution from agricultural machinery at 90%, while Abdelkader et al., 2022 reported that the human toxicity on anion cultivation process was 342.5 kg 1,4-DB eq.

Stage 4: Interpretation

The stage of the shallot cultivation process that contributes to the most significant environmental impact is shallot maintenance and transportation process. Both of these processes are dominant due to the application of various chemicals to plants and soil, so the best improvement analysis is to replace inorganic fertilizers with organic fertilizers at the appropriate doses. Chemical fertilizers containing NPK can be replaced with cow dung manure to meet NPK needs. According to Rayne & Aula (2020), manure application to land is a viable option to improve and restore the health of degraded land. Nutrient usage efficiency is key to improving soil biological properties, and improving the absorption from manure may minimize the loss of nutrients and environmental contamination. Organic manure of 3 tonnes ha^{-1} as the base treatment of the study was given to increase soil organic matter, cation exchange capacity, and nutrient and water holding capacity (Sutardi et al., 2022). Organic fertilizers significantly affect the pH, organic C, total K, N and P of the soils. According to Gunawan et al., 2019, applying organic matter can increase the C-organic content of the soil. Additionally, The controlling of diseased pests with the technologies of seeds marinating (40–50 °C), PGPR, yellow and green sticky trap, 15 spots per ha, Pheromone - Exi to trap shallot caterpillar moths, and solar-powered light trap, 20 spots per ha to trap insects (trips, aphids and mites) is effective and reduces pesticide residue. Once every 3–5 days, as opposed to once every 10 to 15 days, pesticides suppress infestations. Followed by the yellow sticky trap, the green sticky trap, the white sticky trap, and pheromone-Exi, solar-powered light traps for plant-disturbing organisms are the most effective (Sutardi & Porwoninsih, 2018). For the Food Estate Hutajulu region, however, further research is required to apply organic manure and biological/new pest control technologies.

CONCLUSIONS

The Food Estate Hutajulu was worked on with the aim of the government wanting to start implementing modern agriculture by implementing complete mechanization, irrigation automation, and utilizing digital cultivation from upstream to downstream. The Food Estate program is one of the government programs with a food development concept carried out in an integrated manner, including agriculture in an area. The background of this program is in line with the soaring demand for Indonesian food, which is proportional to population growth and the availability of sizeable potential land. The most significant contribution in the shallot cultivation on the acidification impact, global warming potential and eutrophication impact was the transport process (1.89740 kg SO_2 eq, 415.31880 kg CO_2 eq, and 0.43640 kg PO_4 eq, respectively). In comparison, the impact of human toxicity was the maintenance process of 1,409.07377 kg 1,4-DB eq. The total energy used was diesel fuel (70.685 litres), active fertilizer ingredients (2.76447 kilograms), active pesticide ingredients (5.47614 kilograms) and electricity (4.64 kWh). The recommended improvement analysis is to replace or reduce the use of chemicals in applying fertilizers, pesticides, and fungicides by using organic fertilizers according to the doses required by plants.

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Resistance of local rice progeny to ferrous iron toxicity between locations, seasons, and salt application in tidal lands

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Abstract. Rice is the main food in Indonesia that grows in various agroecosystems. The challenge is ferrous iron toxicity (FIT), wherein adaptive varieties with high yield potential be required to support increased production. The study objectives were to produce tolerant and widely adapted lines of FIT from local parents, to determine the stability of the lines in various environments and seasons in FIT rice fields, and to determine the response of rice lines to salt application. Two local Fe-tolerant parents that is Cekau and Karya, were used as females to produce lines that were tested for FIT. High-yielding lines and early maturity were selected to represent tolerant, quite tolerant, and moderate to FIT. The research was designed according to a randomized complete block design with 3 replications. Most of the local cultivar descent were resistant to FIT and stable at various locations and seasons. There was an interaction between the lines and the environment in the multilocation test, but in the high-Fe field test, there was no interaction between the lines and the season. Sensitive lines gave higher yields in the dry season than in the rainy season, but the tolerant lines are not affected by the seasons. The long dry season followed by high rainfall caused the accumulation of Fe on the soil surface to increase followed by a decrease in yields of moderate and sensitive lines. The addition of 200 kg ha^{-1} of salt increased the productivity of tolerant, quite tolerant, and moderate lines by improving root quality.

Key words: adaptation, climate, high-yielding variety, production, Indonesia.

INTRODUCTION

Rice (*Oryza sativa* L.) is the main food crop consumed by more than 50% of the world's population (de Oliveira et al., 2020). The area for rice planting is 167 million hectares (m ha); most are conventionally grown in continuously flooded lowlands

(Girsang et al., 2019). Prolonged flooding and poor drainage of iron (Fe)-rich soils create a risk of Fe toxicity (van Oort, 2018). Fe toxicity is one of the most widespread mineral disturbances in lowland rice production (Audebert & Fofana, 2009) due to the formation of excess Fe^{2+} in reduced soil which interferes with plant growth and productivity (dos Santos et al., 2019). Cases of Fe toxicity begin with flooding within a few days or more, which reduces Fe^{3+} to Fe^{2+}, which at high concentrations is toxic to plants (Becker & Asch, 2005) through the mechanism of $FeS_2 + 2Fe^{3+} \rightarrow 3Fe^{2+} + 2S$ (Dent, 1986).

In general, yield losses associated with Fe toxicity typically range from 15% to 30%, but total crop failure can occur in response to severe toxicity in early growth stages (Audebert & Sahrawat, 2000; Becker & Asch, 2005). In West African countries, Fe toxicity can cause rice yield losses of up to 50% (Audebert & Fofana, 2009). The critical level of Fe toxicity in plant tissues varies from 300 to 500 mg Fe kg^{-1} dry mass (Dobermann et al., 2000).

In Riau Province, Indonesia, cases of Fe toxicity often occur in tidal areas, low Fe-rich land, newly opened rice fields, or peatlands. The introduction of new high-yielding varieties (HYV) tolerant of Fe toxicity is not always successful because of the high rate of blast disease, leaf spot, and bacterial leaf blight. Riau Province is located on the equator with high temperature and humidity. In addition, the introduced HYV are not in conformity with the environment and local farmers' preferences.

Initially, the Fe-rich tidal rice fields in Riau-Indonesia planted local rice varieties tolerant to various stresses including Fe toxicity, but the yields are low, thus improvements are required. Crosses of local rice cultivars have been implemented to produce Fe tolerant varieties, broad adapted, high yield, and early maturity.

Although yield reductions can be partially avoided by using the super tolerant rice cultivars to excess Fe, progress in developing high-yielding cultivars with adequate tolerance to Fe toxicity has generally been slow due to the large genotype × environment interaction and high field heterogeneity, which renders rice selection ineffective (Sikirou et al., 2015). Breeding rice varieties with Fe toxicity tolerance is the most effective and economical way to minimize yield losses due to Fe toxicity stress. Tolerance to Fe toxicity in rice is a complex genetic trait, and there is a large genotypic variation in the primary rice gene pool (Liu et al., 2016).

The use of local varieties as one of the crossbreeding parents is strongly recommended, in order to obtain specific superior genes and to expand the genetic background of the superior varieties to be produced (Sitaresmi et al., n.d.), to increase the effectiveness of selection because it has adapted specifically to various stresses including tolerance to Fe toxicity. Two of the local varieties of tidal rice grown in Riau are Cekau and Karya. These two varieties generally grow in Fe-rich tidal soils. For the reason, specialized varieties matching individual environments and varieties adapted to a wide range of Fe toxicity environments should be developed. The environmental conditions, timing and level of Fe stress, the screening system, and other factors play crucial roles in determining genotype responses to Fe toxicity (L.B. Wu et al., 2014). Because the screening technique also affects the genotype's response to Fe toxicity, testing necessity to be performed between environments and seasons.

In 2011, several elite lines were produced from the cross between Cekau × Cisantana, which produced B-53, and Karya × Fatmawati which produced A5 and A15. Due to the interaction between various stresses, the Fe test performed in the laboratory or hydroponics is not always representing the stress in the field, which makes the stress

suffered by plants become more serious. Therefore, field testing is more representative of all interactions that happens. The results of the study will be able to produce stable lines adapted to the tidal lands with the high Fe accumulation depending on the season. New lines produced by crossing local varieties and new high yielding varieties respond to the challenges of facing climate change. The addition of salt is also expected to be a solution to improving the quality of plants which will ultimately increase rice production. Data shows that the tidal area reaches 23% of the land area or 43 million ha in Indonesia (ICALRD, 2015) with local rice productivity only 2–3 t ha^{-1} (Noor, 2004). This is a challenge in getting superior lines, based on Alihamsyah & Ariza (2006) that the use of new high-yielding varieties was able to produce productivity of 4–6 t ha^{-1}. The study objectives were to produce tolerant and broadly adapted lines of Fe toxicity from local parents, to determine the stability of the lines in various environments and seasons in Fe toxicity rice fields, and to determine the response of rice lines to salt application.

MATERIALS AND METHODS

Experimental site

The cross of the Cekau × Cisantana and Karya × Fatmawati varieties was implemented at the Riau Assessment Institute for Agricultural Technology (AIAT), Pekanbaru City in 2009. F1–F3 seeds were planted in Dramaga Village, Bogor City in 2010–2011 (irrigated rice fields). The planting of F4-F6 was conducted in Sungai Solok Village, Kuala Kampar District, Pelalawan Regency (tidal rice fields) in 2012–2013. Selection of the best family from the F8 generation was implemented in Dadahup Village, Kapuas Regency and Mantaren Village, Pulang Pisau Regency, which are tidal rice fields of Fe poisoning in August - November 2013. From the results of family selection, 10 elite lines grew good in Fe poisoning fields. The ten lines were tested in multiple locations at 8 tidal fields of Parit Senang 1 Village (site 1); Parit Senang 2 Village (site 2); and Sungai Selamat Village (site 3) in Sungai Solok Village; Sungai Upih Village (site 4) in Kuala Kampar District, Pelalawan Regency, Riau Province; Rimba Melintang District, Rokan Hilir Regency, Riau Province (site 5); Petak Batuah Village, Kapuas Murung District, Kapuas Regency, Central Kalimantan Province (site 6); Pulau Petak District, Kapuas Regency, Central Kalimantan Province (site 7); and Bunga Raya Village, Bunga Raya District, Siak Regency (site 8) in 2014 (Table 1).

Futhermore, test for Fe poisoning was conducted at Tamanbogo Experimental Station, East Lampung -5.0053205 S, 105.4871335 E, from June to September 2016. The area used for testing was the rice field which have high Fe content (300–400 ppm), 27 m.a.s.l, low fertile sandy clay loam (Ultisol). Research land belonging to the Ministry of Agriculture which is often used for testing rice resistance to Fe.

To determine the effect of growing season on Fe availability and productivity of rice lines were conducted in newly opened rice fields that were poisoned with Fe in Muara Kelantan Village, Siak Regency which contained 334.38 ppm Fe based on soil analyzed in 2017. Previously this land was swamp land then it was opened for rice cultivation for 5 years, but still Fe poisoning because the drainage system is poor. The lines were planted on Fe toxicity land in the dry season and the wet season in 2017–2020. The experiment to determine the effect of salt will be performed at the same location in 2020.

Table 1. Physical and chemical properties of the research area

No	Soil properties and characteristics	Sites						
		Parit Senang	Sungai Selamat	Dadahup	Pulau Petak	Rimba Melintang	Sungai Upih	Siak
1	Sand (%)	30	21.16	0	0	27	23.66	
2	Silt (%)	26	33.50	44	37	38	52.68	
3	Clay (%)	44	45.34	56	63	35	23.66	
4	C organic (%)	1.34	4.88	2.63	3.35	1.37	6.12	2.30
5	N total (%)	0.11	0.30	0.15	0.21	0.13	0.35	0.13
6	C/N	12	16.27	17.68	16.05	11	17.49	17.00
7	pH	5.1	5.20	3.93	4.07	5.5	4.52	4.60
8	Al^{3+} (me 100 g^{-1})	0.28	5.73	8.71	8.02	0.02	0.20	
9	P_{Bray} (ppm)	11.3	16.56	130.5	62.39	24 (Olsen)	59.45	12.47
10	Ca_{dd} (me 100 g^{-1})	5.69	1.80	0.48	0.07	4.26	1.03	0.05
11	Na_{dd} (me 100 g^{-1})	4.48	0.51	0.21	0.27	1.97	0.26	0.03
12	K_{dd} (me 100 g^{-1})	0.75	0.44	0.31	0.22	0.30	0.37	0.57
13	CEC (me 100 g^{-1})	17.17	10.62	8.92	9.07	9.11	19.31	
14	Base saturation (me 100 g^{-1})	14.07	11.57	11.34	05.42	05.08	18.14	
15	P_2O_5 potential (me 100 g^{-1})	34	80.80	45	58	12	170.3	0.22
16	K_2O potential (me 100 g^{-1})	87	90.77	18	13	21	179.3	77.67
17	Fe (ppm)	70	102	148.04	135.10	27.71	265.43	334.38
18	Pyrite content (mg kg^{-1})	71	71	578	-	-	432	0.02
19	Land type	Tidal Swamp	Tidal type C	Tidal type C	Tidal type B	Rainfed	Rainfed	Tidal type C
20	Type of soil	Peaty alluvial	Ultisols	Acid sulfate	Acid sulfate	Alluvial	Alluvial	Alluvial
21	Texture	Clay	Clay	Silty clay	Clay	Clay loam	Silty loam	

Experimental design and management

The research was conducted through five stages of 1). Crossing local varieties with superior varieties. The local cultivars Cekau and Karya are two local rice that is widely grown by farmers in tidal swampland in Pelalawan Regency. The distribution area of this cultivar contains very high Fe, where other varieties usually suffer from Fe toxicity. The Cekau cultivar was crossed with the Cisantana variety and the Karya cultivar was crossed with Fatmawati. Cisantana and Fatmawati varieties intolerance to Fe toxicity. Fifty F1 seeds were planted and had uniform growth. The results of 50 hills of F1 were selected for the 5 best hills to produce F2 seeds, composited, then planted as a whole. From the segregated F2 population, the best 250 hills were selected. Panicles per hills were planted 1 panicle per row. In the F3 generation the best families were selected and from the best families the best hills were selected until the F6 generation according to the pedigree selection method. Seeds from the F6 generation were harvested and composited to be tested for yield ability. The adaptable progeny to Fe-rich soils and high productivity were selected from the F2 to F8 generations. 2). Screening of Fe toxicity-tolerant lines. A total of 10 tidal rice lines were tested by comparison with Mahsuri (tolerant) and IR64 (sensitive) varieties. Rice seeds were sown until they are 21 days old and then transplanted. Each line/variety was planted in 1-row × 5 m, one seed per hill with a spacing of 0.2 m × 0.2 m. The site received 350 kg fertilizer applied two times by broadcasting 75 kg Urea ha^{-1}, 100 kg TSP ha^{-1}, and 100 kg KCl ha^{-1} at crop establishment and 75 kg urea ha^{-1} at 28 days after planting (DAP). Weeding was done before the second fertilization. Pest and diseases protection was done intensively; 3). Multilocation Test. The research was conducted in 8 locations were Dusun Parit Senang 1 (site 1), Parit Senang 2 (site 2), and Dusun Sungai Selamat (site 3) in Sungai Solok Village, Sungai Upih Village (site 4), Kuala Kampar District, Pelalawan Regency, Riau Province; Rimba Melintang District, Rokan Hilir Regency, Riau Province (site 5); Petak Batuah Village, Kapuas Murung District, Kapuas Regency, Central Kalimantan Province (site 6); Pulau Petak District, Kapuas Regency, Central Kalimantan Province (site 7); and Bunga Raya Village, Bunga Raya District, Siak Regency (site 8) (Table 1). The multi-location test in 2013–2014 was conducted with 10 lines from crosses of local varieties were P1F-KK-A1 (A1), P1F-B-A7 (A7), P1F-B-A15 (A15), P1D-KK-A26 (A26), P1D-KK-A45 (A45), P17E-B-A48 (A48), P1D-KK-A48b (A48b), P1D-KK-A67 (A67), P5E-KK-A5 (A5), P253F-B-53 (B-53), with two control varieties were Batang Piaman and Inpara 2. The study used the following technical cultures: (1) seed aged 21 days after sowing; (2) fertilizer at nursery was Urea 50 kg ha^{-1}, TSP 50 kg ha^{-1}, KCl 25 kg ha^{-1}; (3) plant spacing 0.2 m × 0.2 m; (4) two seedlings per hill; (5) basic application was Urea 100 kg ha^{-1}, TSP 150 kg ha^{-1}, KCl 50 kg ha^{-1}, (6) the second application was Urea 50 kg ha^{-1} and KCl 50 kg ha^{-1} at 35 days after transplanting; (7) weeds were controlled with application 2,4-D dimetil amina 865 g L^{-1}, (9) controlling pests and diseases using the Deltametrin 25 g L^{-1} and propinep 70%. 4). Effect of growing season on Fe availability and productivity of rice lines. The Fe-sensitive varieties, Inpara 5 (Nugraha et al., 2016), Fe-tolerant, quite tolerant, and moderate lines were tested using a randomized complete block design with three replicates. Harvest data were analyzed using PBSTAT 1.2 and 3.1. 5). Effect of salt on decreasing the Fe toxicity. The test was implemented in rice fields which always experience Fe toxicity. Three lines and one variety were grown with treatments of 0, 100, and 200 kg NaCl ha^{-1}. The study was designed using a randomized complete block

design with 3 replications. The condition of the soil is mud that is not waterlogged. Seedlings aged 21 days were planted with plant spacing of 0.2 m × 0.2 m. Experimental plots were not irrigated until 10 days. After the soil started to crack, urea, TSP, and KCl were sprinkled with doses of 200 kg ha^{-1}, 100 kg ha^{-1}, and 100 kg ha^{-1}, respectively with water level at 0.3 m and maintained water until seven days after planting. On the next day, the soil is allowed to dry for three days. Then the soil was flooded as high as 0.1 m and sprinkled with salt according to the treatment.

Soil measurements

Multilocation experiment. Soil sampling was conducted at the beginning of the study at a depth of 0–0.3 m from five diagonal points, composited, and analyzed in the laboratory of Kimia Riset Pekanbaru PT. Sarana Inti Pratama. For the experimental effect of the growing season on Fe availability and productivity of rice lines, soil sampling for Fe content analysis was conducted at harvest time in July 2017, February, July, and December 2018, at planting in April and August 2019, and at harvest in January and July 2020. Soil samples were taken from 5 diagonal points at a depth of 0–0.15 m and then composited.

Plant measurements

The grain yield of all experiments was observed from 2.5 m × 2.5 m plots of each replication. The grain is threshed manually and then dried in the sun to a moisture content of 14%. The grain is cleaned of unfilled grains and dirt then weighed and converted to hectares. The variables observed were dry grain yield, adaptability, and stability of lines between locations.

Statistical methods

Observations screening of Fe toxicity-tolerant lines were made on the tolerance of the tested lines with an assessment using the International Rice Research Institute (IRRI) standard (SES, 2014). The experiment of multilocation test was designed according to a randomized complete block design with three replications with an area of each plot of 4 m × 5 m. The data obtained were analyzed by analysis of variance, and the mean difference in treatment was tested by Duncan's statistic at a significant difference level of 5%. Analysis was performed using SAS and PBStat 1.2 and 3.1 software. The adaptability and stability of the lines were tested using stability parameter analysis according to Eberhart & Russell (1966), with a linear model as follows:

$$Y_{ij} = \mu + b_i I_j + \delta_{ij} \qquad I_j = \frac{\Sigma_i Y_{ij}}{t} = \frac{\Sigma_i \Sigma_j Y_{ij}}{ts}$$

Annotation: Y_{ij} = the average yield of the i-cultivar at the j-location; μ = the average of all cultivars at all locations; b_i = regression coefficient of the i-genotype on environmental index, which shows the genotypic response to the environment; I_j = environmental index, i.e. the deviation of the genotypic mean at a season of all averages; t = number of tested genotypes; s = season; δ_{ij} = regression deviation of the *i*-genotype at the *j*-location.

A genotype is considered stable if the regression coefficient (bi) is insignificantly different from one and the standard deviation (Sd) is not different from zero based on

the student's t-test (Eberhart & Russell, n.d.). The value of bi is approximated by $bi = y_{ij}I_{ij} / I^2_j$, while the value of S_d^2 is obtained by:

$$S_d^2 = \frac{\Sigma \delta_{ij}^2}{1-2} - \frac{S_e^2}{r}$$

where S_e^2 = estimated variance galad.

For the second experiment is effect of growing season on Fe availability and productivity of rice lines and effect of salt on decreasing the Fe toxicity were designed using a randomized block design with 3 replications. Harvest data were analyzed using PBSTAT 1.2 and 3.1.

RESULTS AND DISCUSSION

Results

Soil characteristic within the experimental site is shown in Table 1. Soil conditions at the study site were dominated by high clay content except in the Sungai Upih area with a silty loam texture with pH classified as acidic to very acidic for the seven study sites. Soils that contain high fine clay have highly water retention abilities (Girsang et al., 2020) which tend to have poor drainage which has an impact on high soil acidity (Rendana et al., 2021). Furthermore, the content of organic matter is categorized as low to very high. In terms of the macronutrients such as N is in the low to moderate while P and K are in the low to very high category. The exchangeable nutrient such as Ca, K, and K have a great range from very low to very high with the research land types tidal swamp, tidal type B and C, and rainfed. From the overall soil analysis data that the land in the Sungai Upih is more fertile than other locations, this is supported by CEC data, available P, and base saturation with the main limiting factor being Fe toxicity.

Screening of Fe Toxicity Tolerant Lines

There are variations in resistance to Fe toxicity among breeding lines (Table 2). There were many tolerant, quite tolerant, and moderate lines but only three lines with the highest productivity were selected by farmers, namely: B-53, A15, and A5.

Most of the selected lines were tolerant to moderately sensitive to Fe toxicity, have inherited the character of resistance to Fe toxicity from the female parent. The Cekau cultivar which was used as a female parent to produce B-53 and Karya to produce others lines, was a local cultivar that was Fe toxicity tolerant, while the male parent did not have the character. Of the ten elite lines, there were no sensitively lines to Fe toxicity. Differences in tolerance levels between lines from the same parent indicated the presence of several genes

Table 2. Pelalawan swamp rice lines tested for Fe at KP. Tamanbogo, dry season 2016

No	Lines	Score	Category
1.	A45	4.3	Quite tolerant
2.	A26	3.7	Tolerant
3.	A48b	3.7	Tolerant
4.	A67	3.7	Tolerant
5.	A1	5.0	Moderate
6.	A7	4.3	A bit tolerant
7.	A5	5.0	Moderate
8.	A48	5.7	Quite sensitive
9.	A15	4.3	Quite tolerant
10.	B-53	3.7	Tolerant
11.	MAHSURI (control)	1.7	Very Tolerant
12.	IR64 (control)	9.0	Very sensitive

controlling resistance to Fe toxicity. According to Dufey et al. (2015), rice tolerance to Fe toxicity is a quantitative trait controlled by many genes. The expression is strongly influenced by environmental conditions.

In tidal land, double stress often occurs, so plants must face other stresses besides Fe toxicity, such as nutrient deficiency, waterlogging, salinity, acidity, or disease. Local varieties have adapted to these conditions where the symptoms of Fe toxicity are lighter. Some descent of local cultivars inherit this character, they are more resistant to Fe and secondary stress. Therefore, the assembly of Fe-toxicity tolerant varieties should be site-specific, taking into account other stress.

Multilocation Test on Various Fe Levels in Tidal Land

The lines × environment (L × E) interaction was significant in the multilocation test on rice yield. Interaction means the failure of a line to maintain its rank in different environments. The pattern of the influence of the L × E interaction is clearly seen in Fig. 1. There are lines that are stable in various environments, but there are also lines that are specifically adapted to certain environments. It turned out that not all lines that were declared tolerant to Fe toxicity when tested in the laboratory would be strong when tested in the field. On the other hand, there were moderate lines in the laboratory, very good in the field. The A26 and A48b lines were tolerant to Fe toxicity when screened in the laboratory. But in the field, these lines still showed symptoms of Fe toxicity and low yields.

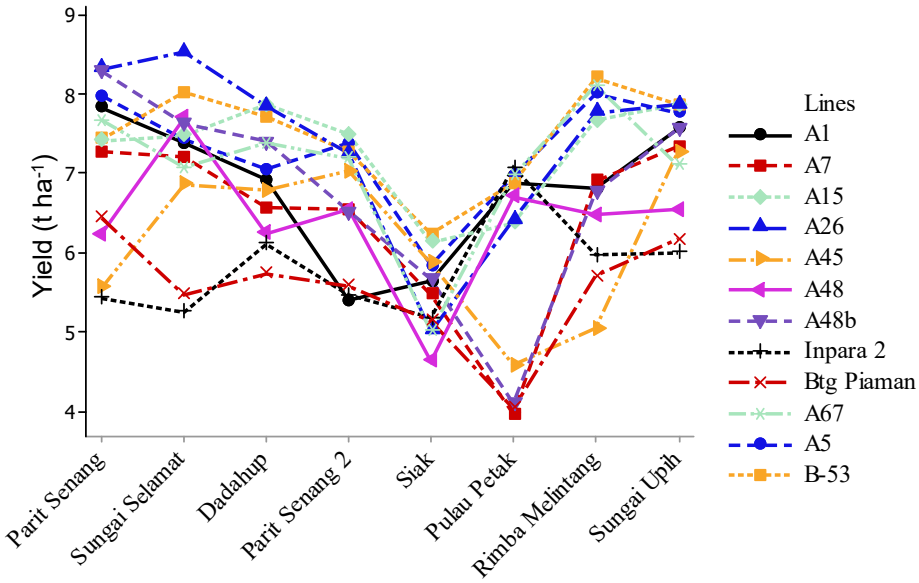

Figure 1. Ranking of lines between locations based on yield.

The A5, A15, and B-53 lines have an average regression coefficient value of the yield on the environmental average (bi) close to 1, their contribution to the genotype × environment interaction (Wi^2) is low, the regression deviation (δi^2) is relatively small (Table 4). This means that these genotypes are stable in eight tidal environments. This stability can also be seen from the AMMI 2 biplot, where the three elite lines are close to the center point (Fig. 2). The three genotypes gave a high average yield.

Despite the higher Fe content, these genotypes produce fairly high yields in more fertile environments such as Sungai Upih, Sungai Selamat, and Parit Senang. There are some lines that are good under pressure or low productivity environments such as Siak and Pulau Petak, namely: A1, A15, A5, and B-53.

The grades of the A15, A5, and B-53 lines did not differ significantly between locations (Table 3, Fig. 1). In this case, it is not clear the effect of moderate, quite tolerant, and tolerant line status on yields in different environments. At the high Fe location in Sungai Upih, the three lines gave no significantly different yields.

The yields of lines A15, A26, A5, and B-53 were all higher than those of control varieties Inpara 2 and Batang Piaman. The average of the four lines across all locations is also quite good. However, especially in Fe-rich regions, such as in Siak, the A5, A15, and B-53 lines produced higher yields than all tested lines/varietals. Up to this point, the yield of existing varieties in the research location is only 3–4 t ha^{-1} dry milled grain (DMG).

Table 4. Parameters of yield stability of elite lines according to Finlay-Wilkinson

Lines/ Varieties	$\bar{y}_{i.}$	b_i	δ_i^2	CV_i	W_i^2
A1	6.80	0.89	2.95	19.70	3.00
A7	6.40	1.52 *	0.68	31.13	3.42
A15	7.28	0.95	0.74	13.14	0.74
A26	7.37	1.73 *	0.37	15.86	0.61
A45	6.12	0.70	4.76	30.45	4.91
A48	6.38	0.84	2.08	15.63	3.28
A48b	6.74	1.74 *	0.79	34.35	5.15
Inpara 2	5.80	0.14	1.17	18.47	6.24
Btg Piaman	5.53	0.84	0.64	23.78	0.97
A67	7.06	1.08	0.88	9.55	1.53
A5	7.30	0.93	0.62	9.65	0.85
B-53	7.45	0.91	0.49	10.45	0.55

Description: * = significantly different from 1; $\bar{y}_{i.}$ = mean genotype yield; b_i = regression coefficient; δ_i^2 = regression deviation; CV_i = coefficient of variance; W_i^2 = contribution to the genotype × environment interaction

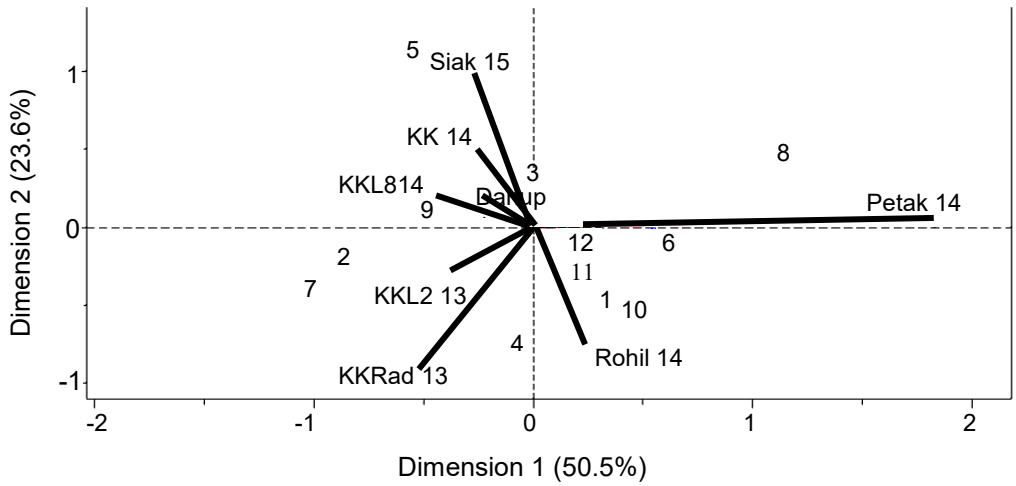

Figure 2. AMMI 2 biplot of grain yields with a conformity level of 50.5 9%.

Note: 1 = A1; 2 = A7; 3 = A15; 4 = A26; 5 = A45; 6 = A48; 7 = A48b; 8 = Inpara 2; 9 = Batang Piaman; 10 = A67; 11 = A5; 12 = B-53; Kkrad13 = Parit Senang; KKL213 = Sungai Selamat; Dahup13 = Dadahup; KK14 = Parit Senang 2; Siak15 = Siak; Petak14 = Pulau Petak; Rohil14 = Rimba Melintang; KKL814 = Sungai Upih.

Table 3. The yield of the dry milled grain of elite lines planted in eight locations

Lines	Locations																Average of lines	
	Parit Senang		Sungai Selamat		Dadahup		Parit Senang 2		Siak		Pulau Petak		R. Melintang		Sungai Upih			
A1	7.82-	ae	7.38	a-g	6.91	a-j	5.39	b-j	5.63	a-j	6.87	a-j	6.80	a-j	7.57	a-g	6.80	ABC
A7	7.27	a-g	7.20	a-g	6.56	a-j	6.53	a-j	5.47	a-j	3.96	j	6.90	a-j	7.33	a-g	6.40	BCD
A15	7.41	a-g	7.48	a-g	7.87	a-e	7.49	a-g	6.13	a-j	6.37	a-j	7.67	a-f	7.87	a-e	7.28	A
A26	8.31	ab	8.52	a	7.84	a-e	7.25	a-g	5.00	e-j	6.40	a-j	7.77	a-e	7.87	a-e	7.37	A
A45	5.55	a-j	6.85	a-j	6.78	a-j	7.02	a-j	5.87	a-j	4.57	g-j	5.03	e-j	7.27	a-g	6.12	CDE
A48	6.23	a-j	7.69	a-f	6.24	a-j	6.53	a-j	4.63	f-j	6.70	a-j	6.47	a-j	6.53	a-j	6.38	BCD
A48b	8.30	ab	7.63	a-g	7.40	a-g	6.51	a-j	5.67	a-j	4.10	hij	6.77	a-j	7.57	a-g	6.74	ABC
Inpara 2	5.43	b-j	5.25	b-j	6.10	a-j	5.46	b-j	5.17	c-j	7.07	a-i	5.97	a-j	6.00	a-j	5.80	DE
Btg Piaman	6.43	a-j	5.47	a-j	5.73	a-j	5.58	a-j	5.13	d-j	4.03	ij	5.70	a-j	6.17	a-j	5.53	E
A67	7.67	a-f	7.07	a-i	7.38	a-g	7.19	a-g	5.00	e-j	6.97	a-j	8.10	a-d	7.10	a-h	7.06	AB
A5	7.97	a-e	7.43	a-g	7.04	a-i	7.38	a-g	5.83	a-j	6.97	a-j	8.00	a-e	7.77	a-e	7.30	A
B-53	7.43	a-g	8.02	a-e	7.71	a-e	7.27	a-g	6.23	a-j	6.87	a-j	8.20	a-c	7.87	a-e	7.45	A
Avg of locations	7.15		7.17		6.96		6.63		5.48		5.91		6.95		7.24			

Note: Numbers followed by the same lowercase or uppercase letters in columns and rows mean that not significantly different according to the Tukey 0.05.

Some tolerant lines still showed symptoms of Fe toxicity of varying severity. But the A5 line, which is moderately to Fe toxic, grows well in Siak and Pulau Petak, although these two sites are more toxic than others. Meanwhile, the Fe-resistant A67 line performed poorly at the Fe-toxic site of Siak.

Although Dadahup had a soil pH of 3.93 and Pulau Petak had a soil pH of 4.07 (very acid), the yields of the three lines were insignificantly different and the average yield was quite good. Soil acidity is not the only factor that suppresses productivity. According to Azura Azman et al. (2014), the optimal pH for rice is 6.

The adverse effects of high Fe concentrations in Pulau Petak, Dadahup, and Siak were greater due to low sodium (Na). The presence of Na in Parit Senang, Sungai Selamat, and Rimba Melintang due to seawater intrusion caused no symptoms of Fe toxicity in the lines seen in Dadahup, Pulau Petak, and Siak. This fact indicates that fluctuation in the toxic effect of Fe in the soil are related to the Na concentration that is important for the Fe balance of plants.

The three lines selected by the farmer were stable between locations with different levels of Fe toxicity, although two of them were only moderately and quite tolerant. This fact shows that the use of parents from the same agroecosystem as the target location for varietal development will produce progeny that adapts to the agroecosystem.

Effect of the Growing Season on Fe Availability and Productivity in Rice Lines

The growing season in Indonesia is divided into two seasons, the dry season (S1) and the rainy season (S2). Generally, S1 occurs in April-September and S2 takes place from October to March. However, in Riau Province which is on the equator, the difference between the S1 and the S2 is often not extreme, which causes the dynamics of Fe in the soil to be similar in the two seasons.

Differences in Fe status in different environments in the multi-site assay did not define the tolerant, slightly tolerant, and moderate characteristics of the three lines. It is suspected that cross-season testing at the location of high Fe can clarify this by testing at S1 and S2. According to X. Wu et al. (2016), environmental conditions affect the level of toxicity.

In a multi-location test, there is an interaction between the line and the environment (L × E). But in the field of high Fe, there is no interaction between lines and seasons. ANOVA of 4 genotypes at 8 planting times showed that both season ($P < 0.000$) and genotype ($P < 0.000$) had very significant effects on rice yield, but their interaction was not significant. The absence of interaction between lines and seasons suggests that performance and genotype ranks are stable between seasons. For example, the B-53 rating has been consistently high from season to season. This is supported by the Finlay-Wilkinson stability analysis (Table 5), where the correlation coefficient values are close to 1. However, the season has a big effect on the concentration of Fe in the tillage layer, Fe is only a small part of the environment that affects plants, and its toxic effects depend on other environmental components, it is site-specific. This also means that the medium and sensitive lines will not outperform the tolerant lines even under favorable growing season conditions, but their yields will be nearly the same in the dry season.

Table 5. Yield stability in the dry season and rainy season in 2017–2020 according to Finlay-Wilkinson Stability Analysis for yield

Genotype	Yield	b_i
A15	4.95	1.10
B-53	5.99	1.13
A5	4.15	0.84
Inpara 5	4.12	0.93

There are variations in Fe content on the soil surface in the S1 and the S2. The Fe content in the tillage layer is very high when the rice fields were flooded after a long S1. The long S1 causes many and deeper soil fractures, leading to extensive oxidation. When rice fields are flooded, Fe^{3+} which is not absorbed by plants turns into Fe^{2+} which is toxic. This can be seen in the high levels of rust deposited on the soil surface when farmers started growing rice at the beginning of S2. At very highconcentrations, Fe on the so il surface can cause damage to seedlings that are just a few days old. At lower Fe concentrations, plants can survive to the reproductive stage but become poisoned during flowering. This fact happened in 2020 when Fe accumulation occurred in the root layer. The leaves of the bottom plants turn orange-yellow in color, bronzing, and the freshness of the leaves is reduced even if the paddy fields are flooded, and the panicles are unfilled and dry.

The difference between S1 and S2 was insignificant in 2017 and 2019 but was significant in 2018 and 2020. However, the ranking of all lines is stable across all seasons and years. In the S2, which was preceded by a long S1, as happened in 2018, yields of sensitive, moderate, and quite tolerant lines fell sharply compared to tolerant lines (Tables 6a, 6b, 6c, 6d, 6e). This continued into 2019 as soil surface Fe levels increased due to heavy rainfall (Fig. 3). The combined analysis of yield data for 2017–2020 shows that the average yield is higher in the S1 and is significantly different from the rice yield in the S2 (Table 6e).

The influence of the S2 and the S1 in 2017 is insignificant due the rainfall is almost flat and is not high every month. The rice fields have not flooded for a long time, there are insignificantof Fe poisoning in the S2. But in 2019, the average rainfall was high and evenly distributed throughout the year, causing waterlogging in S1 and S2, resulting in an increase in Fe on the soil surface, which caused yields to fall. In 2020, heavy rainfall and strong drainage reduced Fe content in the soil surface,

Table 6a. Grain yields of lines/variety in 2017

Lines/ variety	Season 1	Season 2	Average of lines
A15	6.16	5.51	5.84[b]
B-53	7.45	7.29	7.37[a]
A5	5.22	4.46	4.84[c]
Inpara 5	5.51	4.74	5.12[bc]
LSD 5%	-	-	0.90
Avg of seasons	6.08	5.50	

Table 6b. Grain yields of lines/variety in 2018

Lines/ variety	Season 1	Season 2	Average of lines
A15	7.45	3.45	5.45[a]
B-53	7.56	5.13	6.35[a]
A5	5.08	2.76	3.92[b]
Inpara 5	5.38	2.51	3.94[b]
LSD 5%	-	-	1.06
Avg of seasons	6.37[a]	3.46[b]	

Table 6c. Grain yields of lines/variety in 2019

Lines/ variety	Season 1	Season 2	Average of lines
A15	3.54	3.20	3.37[ab]
B-53	4.67	3.33	4.00[a]
A5	2.99	2.64	2.82[b]
Inpara 5	3.15	2.50	2.83[b]
LSD 5%	-	-	0.68
Avg of seasons	3.59	2.92	

Table 6d. Grain yields of lines/variety in 2020

Lines/ variety	Season 1	Season 2	Average of lines
A15	5.14	5.11	5.12[b]
B-53	5.67	6.84	6.26[a]
A5	4.98	5.05	5.02[b]
Inpara 5	4.40	4.80	4.60[b]
LSD 5%	-	-	0.76
Avg of seasons	5.05[b]	5.45[a]	

leading to an increase in crop yields. A significant difference between S1 and S2 was found in 2018 and 2020 (Table 6b, 6d). The lines A5, A1, and B-53 did not show a change in tolerance level in the S2 but all became as if tolerant in the S1 with high yields (Table 6a, 6c, 6d) where the distribution of rainfall is almost flat between S1 and S2.

At specific locations were always Fe poisoned, it was only seen that the tolerant lines were always ranked 1, moderate lines were ranked 2, and the sensitive line was ranked 3 in the S1 and S2. This indicates that the selection of varieties is very important in Fe toxicity areas depending on the growing season and the previous S1. If planting is done in the S2 and water cannot be drained, farmers should plant alternative Fe tolerant varieties with attention to how severe the previous S1 was.

Table 6e. Combined data analysis of grain yields, 2017–2020

Lines/ variety	Season 1	Season 2	Average of lines
A15	5.57	4.32	4.95[b]
B-53	6.34	5.65	5.99[a]
A5	4.57	3.73	4.15[c]
Inpara 5	4.61	3.64	4.12[c]
LSD 5%	-	-	0.80
Avg of seasons	5.27[a]	4.33[b]	

Note: Numbers followed by the same letter in the same column or row insignificantly different based on the 5% level *LSD* test.

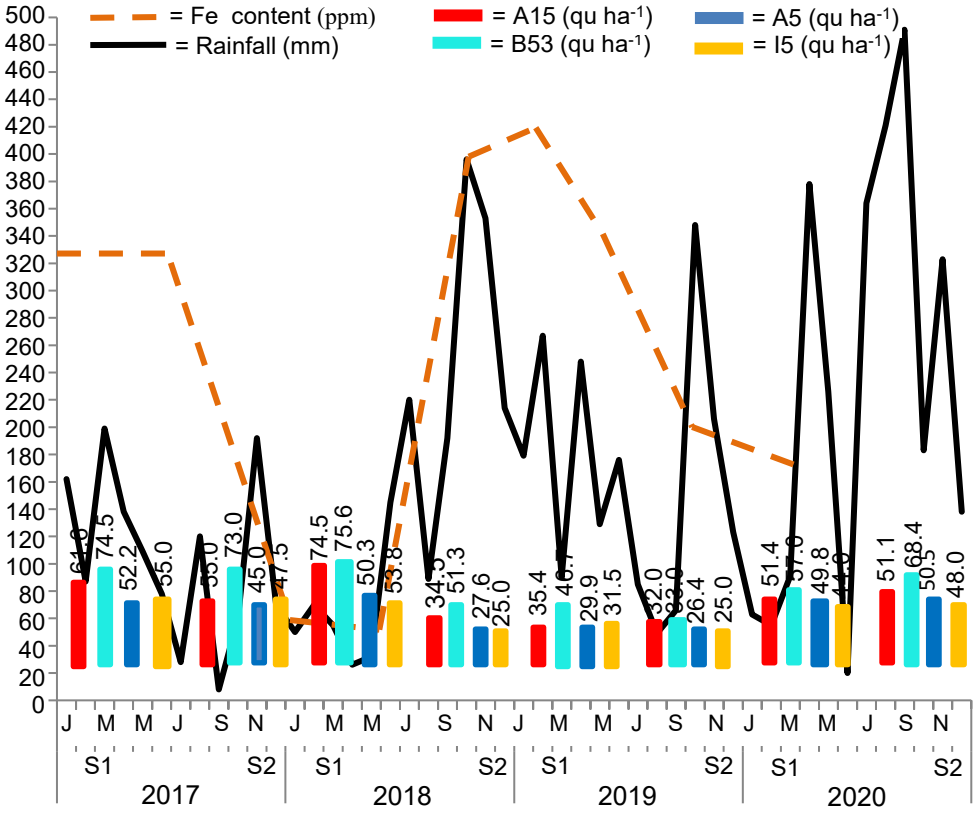

Figure 3. Effect of rainfall on soil surface iron content and rice yields. S1 = dry season, S2 = rainy season.

After a rather long S1, there is a spike in Fe on the soil surface if followed by high rainfall and long-standing water in the rice fields. Along with the increase in Fe, there was a decrease in the yield of non-tolerant lines.

Low rainfall from 2017 to mid-2018 gave high yields for all varieties tolerant, moderate, and sensitive to Fe. In that season, the rice fields are rarely flooded, but the soil is still moist enough for growth even though the soil has cracked. Fe toxicity is minimal because the concentration of Fe on the soil surface is still low, at 70.9 ppm.

A drought that was quite extreme from February to May 2018, did not affect crop yields because rice plants were already in the ripening phase. But the soil fractures are quite numerous, wide, and deep. This caused a spike in Fe on the ground surface during the S2 2018 which caused crop yields to drop drastically and continue into 2019.

In the fourth quarter of 2019 (S2), rainfall was high at transplanting to the vegetative phase, and low before harvest but could not increase yields. The level of toxicity in plants began to decrease because the water was no longer stagnant in the fields due to the disposal of stagnant water through the small canal in the rice field. This significantly reduces soil Fe concentrations and increases crop yields in S1 2020.

Yield changes due to seasonal changes were quite large in A5 and Inpara 5, although their regression coefficient (b_i) is close to 1 (Fig. 2). A15 and B-53 responded to the improvement of the growing environment (bi > 1) although they were tolerant of Fe toxicity, while A5 and Inpara 5 did not respond (bi < 1).

Effect of Salt on Decreasing the Fe Toxicity

The minimum symptom of Fe toxicity in rice plants in coastal areas is the idea in this study. The scarcity of site-specific Fe toxicity tolerant varieties is a limiting factor for farming in Fe-toxicity rice fields during the rainy season. Farmers must cope with environmental improvements wherein Fe toxicity is not too severe. The material that can be used is sea salt (NaCl).

The salt treatment and variety had a significant effect ($P = 0.00$) on the yield, but the salt × variety interaction had no significant effect ($P = 0.2434$) on the yield. Fe-tolerant lines and Fe-sensitive lines react differently to salt treatment. The B-53 line gave a positive response to a NaCl dose of 200 kg ha^{-1} with an increase in yield of 1.41 t ha^{-1} DMG compared to 0 kg ha^{-1} NaCl (Table 7). Fe-sensitive varieties A5 and Inpara 5, were still depressed due to Fe even though they were given salt as a suppress or of the effect of Fe. It is suspected that salt only slightly reduces the effect of Fe.

Table 7. The yield of tolerant and sensitive varieties to Fe toxicity in the salt treatment

Varieties	Salt dose (kg ha^{-1})			Varieties avg.
	0	100	200	
A15	4.20	4.64	5.01	4.62^{b}
A5	3.25	3.75	3.95	3.65^{c}
B-53	5.26	5.47	6.67	5.80^{a}
Inpara 5	3.70	3.56	3.55	3.60^{c}
LSD 5%	-	-	-	0.51
Salt average	4.10^{b}	4.36^{b}	4.80^{a}	

Note: Numbers followed by the same letter in the same column or row insignificantly different based on the 5% level *LSD* test.

The effect of varieties to control the toxic effects of Fe is very important. In the existing conditions, the tolerant line B-53 can produce 5.26 t ha^{-1} DMG, 1–2 t higher than other lines. Its tolerance to salinity causes yields to increase with the addition of salt and on the other hand, salt suppresses the toxic effects of Fe. The increase in yield due to improved environmental conditions showed that the character of tolerance to Fe in the

B-53 line seemed to be inductive. B-53 can adapt to their stressed natural environment, as well as be able to develop their genetic characteristics in a better environment.

Salt treatment reduced the symptoms of Fe toxicity in all varieties. Moderate and sensitive lines to Fe toxicity showed bronzing symptoms in the vegetative to generative phase with mild-moderate intensity. The results of this study indicate the importance of adding salt to areas where Fe toxicity is very low in Na content. Low-dose salt can be implemented when Fe levels increase, usually after a long dry season followed by a rainy season.

Fe plaque that covered the roots was very easy to find in Fe-rich and Na-low locations but was not found in the roots of plants grown in Na-rich locations even though Fe was abundant. Under conditions of high Na concentration in the soil, the rice roots are white but the penetration is shallow and the roots are rather brittle. This causes the plants to be easily removed.

The roots are rarely covered by Fe plaque and have the ability to regenerate the roots, where they can still absorb water and nutrients well are characteristics of Fe toxicity tolerant varieties. This can be seen from the number of roots of plants that tolerate Fe toxicity compared with sensitive plants. In dry conditions, sensitive lines also grow new roots on the soil surface, but cannot keep up with the activity of tolerant varieties.

The Fe plaque covering the roots is believed to be the reason for the low output of farmers. Fe plaque inhibits the absorption of nutrients and water.

The roots of Fe-sensitive lines were brownish-black from the base to the tip of the roots (4a), while the tolerant lines still had plaque-free roots about 1/3 of the root length from the tips in high Fe areas (4b). The root color of the sensitive (4c) and tolerant (4d) lines became white with the addition of 200 kg ha^{-1} of salt.

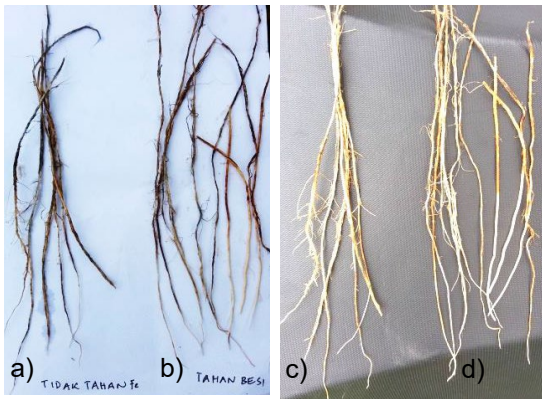

Figure 4. Performance of rice roots sensitive (a) and tolerant (b) to Fe toxicity without NaCl, sensitive (c) and tolerant (d) to Fe toxicity in NaCl treatment.

Discussion

Fe plays an important role in various metabolic processes of organisms (Grillet & Schmidt, 2019), biosynthesis of chlorophyll, carotenoids, and many proteins (Adamski et al., 2012). However important Fe is in life, Fe is toxic to most plants and animals at higher concentrations (White & Brown, 2010). Various levels of plant response to Fe indicate the diversity of controlling genes. Understanding the tolerance level of varieties to Fe toxicity is very important to choose the right variety in the dry season and the rainy season preceded by a long dry season

Breeding rice varieties tolerant to Fe toxicity is more effective and economical to reduce yield losses. Tolerances to Fe toxicities in rice are genetically complex traits; there is large genotypic variation in the primary rice gene pool (Liu et al., 2016), and they are inherited quantitatively. The existence of resistance categories such as sensitive,

moderate, and tolerant to Fe toxicity due to differences in the genes that control it. Therefore, the assembly of high yielding varieties tolerant to Fe toxicity should use parents from the target location because they have adapted to the environment.

The number of genes involved in resistance to Fe toxicity causes different resistance categories to be very tolerant, tolerant, moderately tolerant, moderate, and sensitive to Fe toxicity. Furthermore, according to L.B. Wu et al. (2014), environmental conditions, timing and levels of Fe stress, screening systems, and other factors play important roles in determining genotype responses to Fe toxicity. Noor et al. (2022) reported that the yield of medium tolerant and fully tolerant genotypes treated with organic matter will increase due to a decrease in Fe toxicity.

When the above resistance categories are combined with environmental conditions and soil Fe content, interactions will be more complicated and more important to screen for site-specific genotypes. According to Becker & Asch, (2005), toxicity levels depend on the region, the soil type, the cropping season, and the severity and duration of Fe-toxicity occurrence; genotypes strongly differ in their response patterns and their ability to cope with excess amounts of Fe^{2+}. The finding of QTL in Fe stress conditions and without Fe-stress conditions (Zhang et al. (2017), indicated that the expression of most of the genes was constitutional and inductive. The constitutional expression causes the yield of the varieties not to increase even though they are grown under conditions without Fe stress because some of the energy is used to form secondary metabolites.

There are quite a number of genes controlling the resistance of rice plants to Fe toxicity with varying strengths, so it is necessary to use QTL pyramiding to develop Fe resistant lines in rice (Rasheed et al., 2020). Varietal assembly or gene pyramiding can be done by selecting genes that are only expressed during Fe-stress. Zhang et al. (2017) reported that 29 QTL were identified in the Fe stress experiment, including four detected only in a control condition, 12 detected only under Fe stress condition, and 13 commonly detected under both control and Fe stress conditions

However, the introgression of tolerance traits into high-yielding germplasm has been slow-owing to the complexity of tolerance mechanisms and large genotype-by-environment effects (Kirk et al., 2022). There were wide variations in Fe-toxicity tolerance responses on rice lines, which depended on stress duration, strength, and plant development stage. Some genotypes might show contrast performances depending on how and the location of experiments were accomplished. The exposure of Fe^{2+} excess affected roots and continued to severe reduction of chlorophyll concentration in the leaf, as will be shown as the bronzing spot. Nevertheless, there was no reduction in the tolerant one (Stein et al., 2019). The use of local parents in this study has minimized the interaction of genotype $^{\times}$ environment with a large number of lines that adapt and tolerate Fe poisoning.

The continuously flooded may be able to break the defense system of the rice genotype which has only a few genes controlling toxicity to Fe. Fe toxicity in rice plants can reduce plant height, dry weight, number of productive tillers, number of panicles, number of filled grains, delaying flowering and ripening (Audebert & Fofana, 2009), decrease plant production (Amnal, 2009). One of the weaknesses of traditional swampland is no water system where the land is flooded during the rainy season.

A significant negative correlation between grain yield and leaf bronzing score (LBS) at 60 DAS was found in Fe treatments (Sikirou et al., 2016). On average, in Fe-toxic soils, one unit increase in LBS was related to a yield decrease of about 20%. In

the field situations, it is reported that an increase in LBS score by one unit reduced yield by 390 kg ha^{-1} (Audebert & Fofana, 2009).

Fe^{2+} concentration up to 3.2 mM did not damage rice roots while induced IP formation obviously (Fu et al., 2018). Under excess Fe were detected (i) nutritional deficiencies, especially of calcium and magnesium in leaves; (ii) negligible changes in grain nutritional composition, independently of Si application; (iii) decreases in net photosynthetic rates, stomatal conductance, and electron transport rate, in parallel to decreased grain yield components, especially in the Fe-sensitive cultivar (dos Santos et al., 2020). Fe^{2+} concentrations of 300 to 400 ppm were highly toxic to rice and resulted in low plant nutrient availability (Ikehashi & Ponnamperuma, 1978).

Fe toxicity can also occur in normal soils at low soil pH when harmful organic acids and hydrogen sulfide accumulate (Liu et al., 2016. Excess Fe can cause direct poisoning and nutritional imbalances, during the vegetative and reproductive stages (Sahrawat, 2004; Müller et al., 2015), and P, K, Ca, Mg, and Mn deficiency (Olaleye et al., 2001; Audebert & Fofana, 2009; Stein et al., 2009). Genotypes with enhanced Fe storage in roots and stems may be better suited to such conditions (Kirk et al., 2022). The application of fertilizers significantly decreased average shoot Fe concentrations partly due to Fe exclusion favored by enhanced root plaque formation (Rakotoson et al., 2019). Excessive S supply (60 and 120 mg kg^{-1}) significantly decreased IP on the root surface during flooded conditions (Yang et al., 2016).

In the vegetative phase, the toxic effect of Fe on rice plants has been associated with a decrease in the net CO_2 (A) assimilation rate due to the limitations of stomata and non-stomata photosynthesis (Pereira et al., 2013). At the reproductive stage, excess Fe causes a significant decrease in the number of tillers and grain fertility, which in turn reduces grain yield. This loss may be substantial, depending on the cultivar, time of Fe poisoning, stress intensity, and management strategy (eg, mineral fertilization) (Olaleye et al., 2001; Audebert & Fofana, 2009; Stein et al., 2009).

In cultivation techniques, there are ways to eliminate the effects of Fe toxicity, for example choosing rice varieties tolerant to Fe toxicity (Becker & Asch, 2005), water management, amelioration, application of fertilization, and use of high yielding varieties of Fe toxicity tolerant (Khairullah et al., 2021), the addition of salt, application of Si (dos Santos et al., 2020), or liming (Suriyagoda et al., 2017). However, none of those options is universally applicable or efficient under the diverse environmental conditions where Fe toxicity is expressed (Becker & Asch, 2005).

Fe bioavailability to plants is reduced in saline soils, but salting should not be excessive because plants will be faced with two major challenges for poor crop productivity: high salinity and Fe deficiency (Sultana et al., 2021). The concentration of plant-available Fe in saline-alkaline soils is often low due to immobilization (Li et al., 2016). In alkaline soils, Fe occurs mainly in the form of insoluble hydroxides and oxides, limiting its bioavailability for plants (Li et al., 2016).

The use of Si can be recommended as an effective management strategy to reduce the negative impact of Fe toxicity on the photosynthetic performance of rice and crop yields with no effects on the grains (dos Santos et al., 2020). Application of dolomite to lowland rice fields affected by Fe^{2+} toxicity can increase grain yield while reducing the negative impacts of Fe^{2+} toxicity. The magnitude of these positive responses would vary depending on variety, season, and soil conditions (Suriyagoda et al., 2017).

The intermittent water system showed dominance in increasing soil pH at planting 0, 7, and 14 days after application of the water system. The planting time of 14 days showed the lowest soil pH. The intermittent water system and planting time of 14 days and 21 days after application of the water system showed the lowest soil Fe content. Intermittent water management increased rice growth and yield higher than flooded continuously by 13.6% and/or flushing system by 13.2%. An intermittent system after one week was accompanied by a delay in planting time of 14 days to 21 days after being flooded (Khairullah et al., 2021).

CONCLUSIONS

1. The use of local varieties from Fe toxicity areas as parents for crosses resulted in offspring that were tolerant to Fe toxicity and were stable between locations and seasons.
2. The change from the dry season to the wet season caused the yield of sensitive varieties to decrease more than the moderate and tolerant lines. Sensitive varieties and moderate lines gave higher yields in the drough season than in the wet season.
3. The long dry season followed by high rainfall caused the accumulation of Fe on the soil surface followed by a decrease in yields of moderate and sensitive lines.
4. The addition of 200 kg ha^{-1} of salt increased the productivity of tolerant, quite tolerant, and moderate lines by improving root quality.
5. The stability in the multi-location test can be used as a reference for stability in inter-seasonal fluctuations of soil Fe content.

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DATA AVAILABILITY STATEMENT. The data presented in this study are available upon request from the corresponding author. The data are not publicly available yet but will be in due course.

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Response of regulation of resistance genes, reactive oxygen species, and antioxidant enzymes to salicylic acid treatments in drought tolerant rice

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Abstract. Drought is one of the most important issues in crop production which may disrupt physiological processes and biochemical metabolism in rice plants, including the emergence of plant resistance gene expression such as OsAB13 and OsLEA, the formation of ROS (Reactive Oxygen Species), namely hydrogen peroxide (H_2O_2), as well as the emergence of gene expression related to antioxidant enzyme activity such as OsAPX1, OsCATA, Mn-SOD, Cu/Zn-SOD, and APX Cytosolic. Besides the emergence of plant resistance gene expression, ROS, and changes in gene expression related to antioxidant enzymes, rice plants also produce salicylic acid which acts as an endogenous signal to activate plant resistance gene responses and can encourage plant resistance responses such as antioxidant enzyme activity. The treatments in this study included: control, 15% PEG 6000, 15% PEG 6000 + SA 1 mM, and SA 1 mM. The results showed that the interaction between treatments and rice plant varieties significantly affected plant height, root length, total chlorophyll, and H_2O_2 content. The expression of the OsAPX1, OsCATA, Mn-SOD, and Cu/Zn-SOD genes increased in the 15% PEG 6000 treatment compared to the control. In the 15% PEG 6000 + SA 1 mM treatment, there was an increase in gene expression of APX Cytosolic, Cu/Zn-SOD, and OsCATA compared to the 15% PEG 6000 treatment. The OsLEA is expressed in Siakraya and Sertani 1 as an indicator of resistance to drought stress, and the OsAB13 is expressed in Indragiri, Sertani 1, and Siakraya varieties as an indicator of resistance to drought stress.

Key words: drought stress, antioxidant enzymes, resistance genes, reactive oxygen species, and rice plants.

INTRODUCTION

Drought is one of the most critical constraints in crop production, including rice, which may reduce the chlorophyll content (Niu et al., 2022) as well as the absorption of

water and nutrients by plant roots. That conditions will disrupt the physiological and biochemical processes of the chemical metabolism of rice plants. Physiological and biochemical processes that occur due to drought stress conditions is a part of plant survival mechanism (Ubaidillah et al., 2019).

Rice plants under drought stress produce ROS as a result of the accumulation of oxygen (O_2) in the cells (Wang et al., 2005; (Ubaidillah et al., 2013). ROS consists of free radical groups that can trigger cell damage. The freest radicals formed in plant tissues are superoxide, which will later be converted into H_2O_2 and converted into hydroxyl radicals (OH-) which cause lipid peroxidation in cell membranes (Nahar et al., 2016). Excessive ROS production causes plants to make efforts to maintain their survival by producing enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT) (Farssi et al., 2022), ascorbate peroxidase (APX), glutathione peroxidase (GPX), and peroxiredoxin (PrxR) (Miller et al., 2010).

Rice plants also produce salicylic acid, a compound that acts as an endogenous signal to activate the response of genes related to plant resistance, including drought stress (Roumani et al., 2019), and is able to induce plant resistance responses such as the activation of antioxidant enzymes (Liu et al., 2015). Based on previous studies, salicylic acid plays a role in plant resistance.

Salicylic acid (SA) is a phenolic endogenous growth regulator and also a signaling molecule that participates in the regulation of physiological processes in plants such as growth, photosynthesis and other metabolic processes. Salicylic acid can modulate the antioxidant defense system thereby reducing oxidative stress. Salicylic acid regulates plant-water relations, photosynthetic rate, nitrogen metabolism, and proline metabolism when under abiotic stress conditions (Jayakannan et al., 2015).

This research was conducted to carried out the response of antioxidant genes and other genes that correlated with the resistance factor of Indonesian local tolerant rice. It should be noted that the IR64 variety was a moderately resistant control used as a comparison in this study. This research was expected to be able to provide information about physiological and biochemical changes in several varieties of rice plants that experience drought.

91 rice germplasm have been characterized and evaluated for resistance to drought stress based on the Standard Evaluation System for Rice IRRI (2013), and 8 candidates for rice varieties that are resistant to drought stress have been obtained, of which 3 rice were used in this study including Siak Raya, Sertani 1 and Indragiri. This study aims to evaluate the tolerance level of rice varieties to drought stress and to determine the growth response and expression of antioxidant genes in drought tolerant rice under drought stress conditions and what is the role of SA in increasing the resistance response.

MATERIALS AND METHODS

Plant material and Treatment

The three varieties used in this study are Siakraya, Indragiri, Sertani 1 and IR64 used as moderate resistance control. Rice seeds were soaked with fungicide for 1 week before ready to be planted in the pot tray for 14 days. Healthy rice plants were selected, with characteristic of leaves are green and free from disease, the number of leaves is 4 to 5, and the stems are upright. Plant treatment was carried out by placing each experimental unit portray into a tub containing the treatment solution and keeping it from

changing in concentration. The treatment solutions were in the form of control, 15% PEG 6000, 15% PEG 6000 + SA 1 mM, and SA 1 mM. Plants that have been treated are adjusted with water every day up to the initial volume limit when the treatment is applied. Plants were maintained by controlling their environmental conditions from pests and ensuring the water and nutrients are maintained and the room is controlled. Each plant then being observed for its height, root length, total chlorophyll content, H_2O_2 content, as well as the resistance gene expression.

Morphological characterization

The plant height dan root length was determined after 8 days treatment. Plant height was measured from the root at ground level to the tip of the tallest shoot. Root length was measured from the base of the root to the tip of the root.

Chlorophyll and hydrogen peroxide content analysis

The total leaf chlorophyll content was calculated using leaf samples that were taken 8 days after treatment with the spectrophotometric method using 80% acetone and measuring the absorbance of chlorophyll using a spectrophotometer at a wavelength of 645 nm and 663 nm (Ahmad et al., 2019). The formula for calculating chlorophyll is as follows:

$$\text{Total chlorophyll (mg g}^{-1} = \frac{20.2\ (\text{A } 645)\ +\ 8.02\ (\text{A663})\ \times\ \text{V}}{1{,}000} \times \text{W} \qquad (1)$$

The content of hydrogen peroxide was measured according to the method (Christou et al., 2014). A total of 0.1 g of leaf sample was homogenized into 1 mL of 0.1% Trichloroacetoacid (TCA), then centrifuged at 10,000× g for 15 m. A total of 0.5 mL of the supernatant was taken and adjusted to 0.5 mL of 10 M phosphate buffer with pH 7.0 and 1 mL of 1 M potassium iodide. The solution was then incubated at room temperature for 30 m, then the absorbance was measured at a wavelength of 390 nm. The calibration standard curve was used as a standard in determining the content of H_2O_2.

Gene Expression analysis

The callus samples were taken on the 14th and 28th days of medium regeneration and frozen immediately in liquid nitrogen. Then the isolated total RNA with some modifications to the manufacturer's Gene All Ribospin Plant RNA Mini Kit (GeneAll Biotech, Korea). The 260 nm/280 nm measurement at a level between 1.8 and 2.2 for cDNA synthesis and RT-PCR was obtained using nanodrop (TECAN® Infinite M200 Multi-Detection Microplate Reader Part).

After verifying RNA quality, The RNA sample is converted into cDNA by reverse transcription using ReverTra Ace® RT Master Mix with gDNA Remover (Toyobo, Osaka, Japan) treats 0.5 μg of total RNA. This process eliminates and replaces genomic DNA with a single-stranded cDNA. Total RNA was incubated at 37 °C for the DNase reaction for 5 m and reverse transcription reaction. Finally, gene-specific primers for the expression of OsAB13, OsAPX1, OsCATA, Mn-SOD, Cu/Zn-SOD and APX Cytosolic genes (Table 1) have been used in real-time PCR applications.

Table 1. Primer Sequences for Gene Expression Analysis

Gene	Primer	Source
OsACTIN	Forward: 5' TCCATCTTGGCATCTCTCAG 3' Reverse: 5' GTACCCGCATCAGGCATCTG 3'	Kim et al. (2007)
OsAPX1	Forward: 5' CCAAGGGTTCTGACCACCTA 3' Reverse: 5' CAAGGTCCCTCAAAACCAGA 3'	Kim et al. (2007)
OsCATA	Forward: 5' CGGATAGACAGGAGAGGTTCA 3' Reverse: 5' AATCTTCACCCCCAACGACT 3'	Kim et al (2007)
Mn-SOD	Forward: 5' GGAAACAACTGCTAACCAGGAC 3' Reverse: 5' GCAATGTACACAAGGTCCAGAA 3'	Kim et al. (2007)
Cu/Zn-SOD	Forward: 5' CAATGCTGAAGGTGTAGCTGAG 3' Reverse: 5' GCGAAATCCATGTGATACAAGA 3'	Kim et al. (2007)
APX Cytosolic	Forward: 5' AGTACATTGCCCGTGGTACTCT 3' Reverse: 5' CGCATTTCATACCAACACATCT 3'	Kim et al. (2007)
OsAB13	Forward: 5' CCC AAC AAC AAA AGC AGG AT 3' Reverse: 5' CCT TTG TAT TGG ACG AGA CG 3'	Zhou et al. (2020)
OsLEA	Forward: 5' CCC AAG CTT AAA ATG GCG TCG AGG CAG GAC A 3' Reverse: 5' TGC TCT AGA TCA TGG CAA GAC TGC TGA TGT ATG g 3'	Zhou et al. (2020)

PCR analysis is performed in a total volume of 10 µL containing 5 µL of 2 × GoTaq® Green Master Mix, 1 µL cDNA templates, 2 µL Nuclease-free water and 1 µL Forward (F) and Reverse (R) primer to detect the presence of a specific nucleic acid sequence using the GoTaq® Green Master Mix kit (Promega). The PCR amplification profile consisted of an initial denaturation of 95 °C for 2 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 53 °C for 30 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 5 min. An electrophoresed 2% agarose gel in 1 X TAE buffer stained with GreenStar™ was used for PCR analysis. In addition, the UV transilluminator was used to visualize the banding patterns.

Data analysis

(ANOVA). If there is a significant difference between the treatments, a further test is carried out using Duncan's Multiple Range Test (DMRT) at a 5% significance level. Data obtained from gel electrophoresis visualization were analyzed using qualitative descriptive analysis with visual presentation.

RESULTS AND DISCUSSION

The effect of drought stress and salicylic acid treatment of morphological character

The rice plants be grown under salicylic acid treatments implemented to see its response under drought stress. Salicylic acid plays a role in the response of plant resistance to drought stress as evidenced by the result of plant high, root length, total chlorophyll, hydrogen peroxide content, as well as the expression of responsive genes towards treatment.

Generally, drought stress caused a decrease (Fig. 1) in plant height for all varieties compared to those without stress treatment.

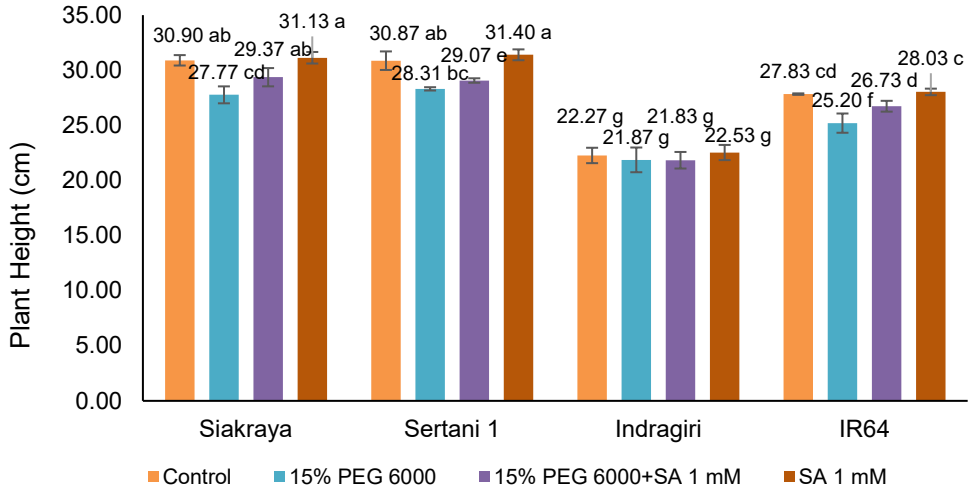

Figure 1. Response of rice plant height under treatment. All parameters were measure after 8 days of treatment. Value presented as the mean ± *SD* ($n = 5$). Different letters indicate significant differences at $p < 0.05$.

The decrease in plant height in the 15% PEG 6000 treatment significantly occurred in the Siakraya variety by 3.13% compared to the control. The decrease in IR64 had a moderate level of resistance to drought stress decreased by 2.63% compared to the control. The decrease in Sertani 1 was 2.56%, compared to the control while the decrease was not significant for the Indragiri by 0.4%. Drought stress causes rice plants to experience a reduced cell size, inhibiting plant vegetative growth and decreasing plant height (Li et al., 2022).

The 15% PEG 6000 + SA 1 mM treatment generally increased plant height compared to the 15% PEG 6000 treatment, namely Siakraya, IR64, and Sertani 1 by 1.6%, 1.53%, and 0.76% respectively, while Indragiri experienced a not significant decrease by 0.04%. Rice plants treated with 15% PEG 6000 + SA 1 mM generally had plant heights that tended to be more stable than those treated with 15% PEG 6000, this was due to the response of the application of salicylic acid which was able to maintain membrane stability during drought stress (Ubaidillah et al., 2016). Salicylic acid also plays a role in regulating plant water relations and the rate of photosynthesis under abiotic stress conditions (Khalvandi et al., 2021).

The response of rice plants to drought stress also can be observed through its root length. Rice plants treated with drought stress generally experienced an increase in root elongation for all varieties compared to those without stress (Fig. 2). Root elongation with 15% PEG 6000 treatment significantly occurred at Siakraya by 3.73%, Sertani 1 by 2.77%, and Indragiri by 2.47% compared to the control treatment. The IR64 variety was not significant with a change occurring by 0.77% compared to the control because the IR64 variety had a moderate level of resistance to drought stress.

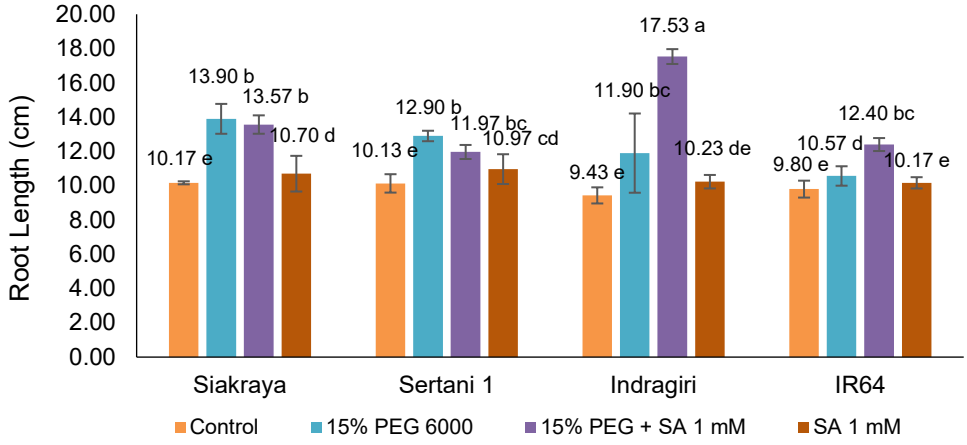

Figure 2. Response of Root Length to Treatment under treatment. All parameters were measure after 8 days of treatment. Value presented as the mean ± *SD* ($n = 5$). Different letters indicate significant differences at $p < 0.05$.

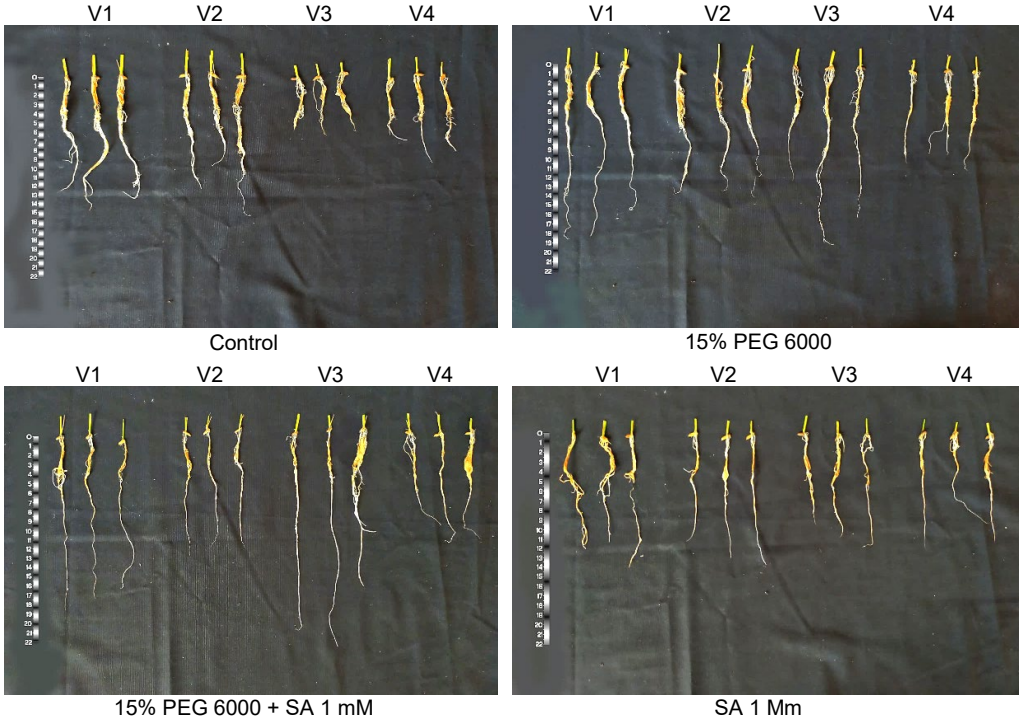

Figure 3. The morphology of root under several treatment condition V1; Siak raya, V2; Sertani, V3; Indragiri and V4; IR64.

Increasing root elongation occurs with the aim of expanding the root absorption area for water and nutrients (Noelle et al., 2018). In the rice variety Siakraya and Sertani 1 (Fig. 3), the 15% PEG 6000 + SA 1 mM treatment reduced root elongation compared to the 15% PEG 6000 treatment by 5.63% and 1.83%. The varieties of Indragiri and IR64

in the 15% PEG 6000 + SA 1 mM treatment increased root elongation by 0.33% and 0.93% compared to the 15% PEG 6000 treatment. Several varieties in the 15% PEG 6000 + SA 1 mM treatment generally experienced an increase in root elongation compared to those treated with 15% PEG 6000 due to a response from the application of salicylic acid which is an endogenous growth regulator. Salicylic acid is phenolic and also a signaling molecule that participates in regulating physiological processes in plants such as root growth, increased root volume, and the rate of photosynthesis (Jayakannan et al., 2015).

The effect of drought stress dan salicylic acid treatment on chlorophyl content and H_20_2

Due to the function of salicylic acid as a regulator of some physiological processes in plants including the rate of photosynthesis, the result of total chlorophyll in rice plant under the drought stress also varied. Rice plants treated with drought stress generally experienced a decrease in total chlorophyll content compared to those without stress treatment (Fig. 4), however, there were certain varieties treated with drought stress but experienced an increase in total chlorophyll content.

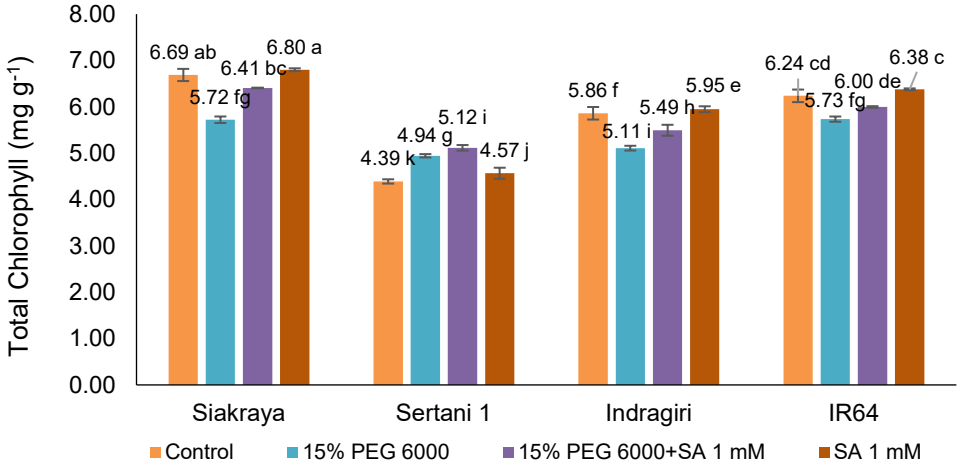

Figure 4. Response of Total Chlorophyll Content under treatment. All parameters were measure after 8 days of treatment. Value presented as the mean ± *SD* ($n = 5$). Different letters indicate significant differences at $p < 0.05$.

The significant decrease of total chlorophyll content in the 15% PEG 6000 treatment occurred in Siakraya by 0.97%, Indragiri by 0.75%, and IR64 by 0.51% compared to the control treatment. The decrease in total chlorophyll content occurs because drought stress may have an impact in the form of accelerating the rate of leaf aging, abscission of old leaves, and inhibited opening of stomata, thereby reducing leaf dilation and inhibiting the rate of photosynthesis. The increase in total chlorophyll content in the 15% PEG 6000 treatment, namely Sertani 1, was 1.39% compared to the control. The increase in total chlorophyll content is due to the stimulus for chlorophyll synthesis in young leaves caused by the activation of enzymes in light-dependent biosynthesis and changes in chlorophyll levels in certain varieties against drought stress (Shin et al., 2021).

Treatment of 15% PEG 6000 + SA 1 mM generally increased the total chlorophyll content compared to the 15% PEG 6000 treatment, namely the Siakraya variety by 0.69%, Indragiri by 0.38%, IR64 by 0.27%, but the increase was not significant in Sertani 1 of 0.18%. The 1 mM SA treatment indicated the same response in general as the control treatment. The increase in total chlorophyll content in the 15% PEG 6000 + SA 1 mM treatment was due to salicylic acid acting to block the flow of electron transfer in photosystem II (PS II). Salicylic acid competes with quinone B (QB) for binding sites in the photosystem II (PSII) reaction center, thereby reducing the rate of electron flow from H_2O to NADP+ via electron carriers in the PS II reaction. Salicylic acid induces the production of singlet and triplet chlorophyll in photosystem II, therefore salicylic acid can increase the content of chlorophyll a, b, and the rate of photosynthesis (Radwan et al., 2019).

Drought stress generally increase the content of H_2O_2 in all varieties of rice plants (Fig. 5). The significant increase in the content of H_2O_2 in the 15% PEG 6000 treatment occurred in Siakraya by 13.21%, Sertani 1 by 7.12%, Indragiri by 5.28% and IR64 by 3.93% compared to the control. The increase in H_2O_2 content occurs because H_2O_2 is not immediately decomposed in the process of photosynthesis resulting in the accumulation of toxic H_2O_2 in plants (Hasanuzzaman et al., 2019).

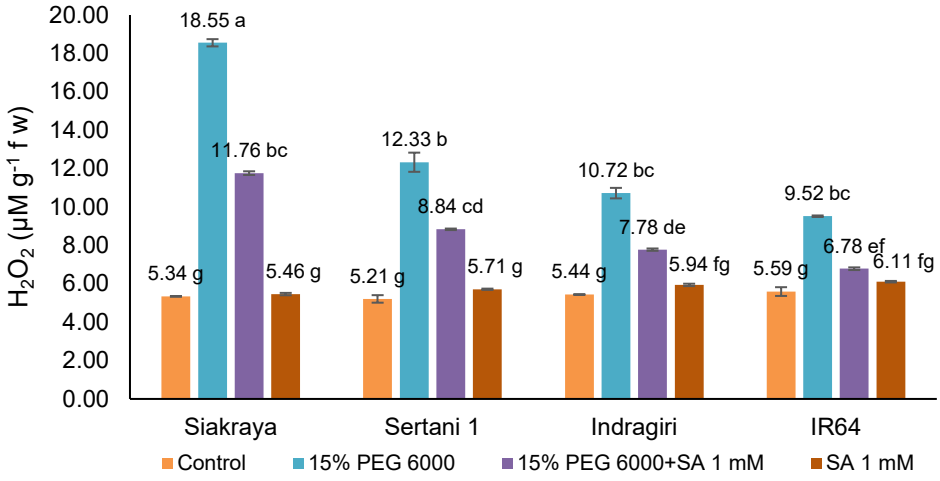

Figure 5. Response of Hydrogen Peroxide Content (H_2O_2) under treatment. All parameters were measure after 8 days of treatment. Value presented as the mean ± *SD* (n = 5). Different letters indicate significant differences at $p < 0.05$.

The 15% PEG 6000 + SA 1 mM treatment generally decreased the content of H_2O_2 compared to the 15% PEG 6000 treatment, the decrease occurred in Siakraya by 6.79%, Sertani 1 by 3.49%, Indragiri by 2.94%, and IR64 by 2.74%. The decrease in the content of H_2O_2 was due to the addition of salicylic acid in the drought stress treatment which helped maintain the stability of the membranes in the plant. Salicylic acid also increases the rate of photosynthesis, increases the synthesis of secondary metabolism, and regulates the activity of antioxidant enzymes so that it is possible to reduce excess ROS production (Hasanuzzaman et al., 2017).

Hydrogen peroxide is one of the ROS product molecules that can cause oxidative damage to proteins, DNA, and lipid peroxidation. One of the main cells responsible for ROS production is the chloroplast (Sarker & Oba, 2020). During photosynthesis, energy from sunlight is fixed and transferred to two light-harvesting complexes (photosystem II and photosystem I) in the chloroplast membrane. In addition, the electron transport components of the thylakoid chloroplasts on the PSI side such as the Fe-S center and thioredoxin can be reduced automatically resulting in a reduction to form superoxide (O_2-) and H_2O_2. The rubisco enzyme, which carboxylatesribulose-1.5-bisphosphate (RuBP) during carbon assimilation, also uses oxygen to oxygenateribulose-1.5-bisphosphate. This reaction produces glycolate which is then transported from the chloroplast to the peroxisomes where they are oxidized by glycolate oxidase to produce H_2O_2. The mitochondrial electron transport chain is also responsible for ROS production under normal conditions, although to a lesser extent than chloroplasts and peroxisomes in the photosynthetic process (Nahar et al., 2016).

The effect of drought stress dan salicylic acid treatment on antioxidant gene and abiotic stress related gene

Gene expression of OsAPX1, OsCATA, Mn-SOD, Cu/Zn-SOD, and APX Cytosolic was used as a research parameter to see the response of antioxidant enzyme activity to drought stress in all varieties when compared to OsACTIN, considering that OsACTIN was used as a housekeeping gene which was used as an internal control for gene expression analysis that had no effect on stress. The increase in gene expression in the 15% PEG 6000 treatment occurred in the expression of the OsAPX1, OsCATA, Mn-SOD, and Cu/Zn-SOD genes compared to the control treatment (Fig. 6), but the *APX Cytosolic* gene expression has a lower expression level in the control treatment.

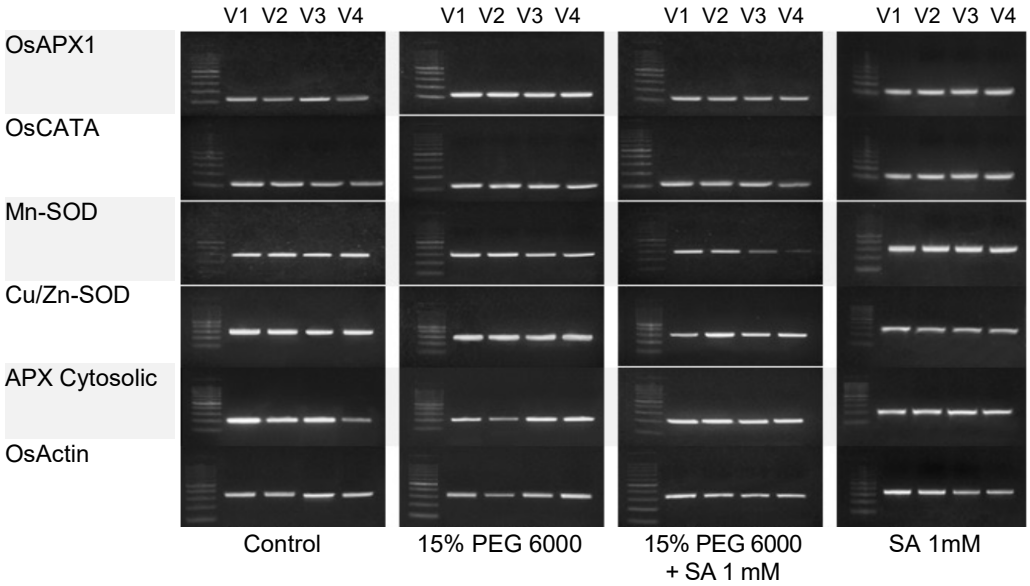

Figure 6. Expression of OsAPX1, OsCATA, Mn-SOD, Cu/Zn-SOD, and APX Cytosolic genes were performed from total RNA samples for PCR analysis were isolated from leaf under several condition at 8 DAT. V1: Siakraya, V2 : Sertani 1, V3: Indragiri, and V4: IR64.

The varieties that had an average volume increase in band thickness were higher in the 15% PEG 6000 compared to the control treatment, namely the Siakraya and Sertani 1 varieties. In the treatment of 15% PEG 6000 + SA 1 mM, high gene expression values were obtained in APX Cytosolic, Cu/Zn-SOD, and OsCATA, but OsAPX1 and Mn-SOD had lower gene expression levels. The Sertani1 had an average volume increase in band thickness higher than other varieties in the 15% PEG 6000 + SA 1 mM treatment. In the 1 mM SA treatment, the expression of OsCATA, Cu/Zn-SOD, and Cytosolic APX genes also had average expression levels that were almost the same as the 15% PEG 6000 + SA 1 mM treatment, but the average expression level increased in OsAPX1 and Mn-SOD genes compared with 15% PEG 6000 + SA 1 mM treatment.

Increased expression of the OsAPX1 and Mn-SOD genes occurred in the 1 mM SA compared to the 15% PEG 6000 + SA 1 mM treatment. This may be because the application of salicylic acid to plants without stress can upregulate several ROS-related genes by activating ROS metabolic pathways, but when salicylic acid is treated on plants with drought stress, salicylic acid responds lower. The addition of salicylic acid to plants can directly increase the activity of antioxidant enzymes in an effort to increase protection against oxidative stress and increase the salicylic acid content in cells (Mostofa et al., 2020).

The sizes of the DNA fragments of the OsCATA and OsAPX1 genes in the control treatment, 15% PEG 6000, 15% PEG 6000 + SA 1 mM, and SA 1 mM, were between 100–200 bp. The sizes of the DNA fragments of the Mn-SOD, Cu/Zn-SOD, and APX Cytosolic genes in the control treatment, 15% PEG 6000, 15% PEG 6000 + SA 1 mM, and SA 1 mM, were between 250–300 bp, 250–350 bp, and 150–250 bp respectively. The sizes of the DNA fragment of the OsACTIN gene in the control treatment, 15% PEG 6000, 15% PEG 6000 + SA 1 mM, or SA 1 mM, were between 300–400 bp.

Rice plants adapting to drought stress require a number of genes to be expressed in plant cells, including genes involved in signaling pathways (Nahar et al., 2016). These genes include OsCATA, OsAPX, Mn-SOD, Cu/Zn-SOD, and Cytosolic APX. In addition, during abiotic stress conditions, salicylic acid becomes a signal for plants to activate the expression of defense genes from stress. Salicylic acid also interacts with other signaling molecules, such as ABA, to coordinate the maintenance of membrane stability in plants under stress conditions. Salicylic acid also increases the rate of photosynthesis, increases the synthesis of secondary metabolism, and regulates the activity of antioxidant enzymes, impacting the activity of ROS which are toxic, so it is possible to reduce excess ROS production due to stress conditions (Hasanuzzaman et al., 2017).

Drought stress causes a reduction in CO_2 intake in plants resulting in an accumulation of O_2 which causes the formation of ROS. ROS may cause severe damage to metabolic processes in photosystem I and photosystem II. Efforts to reduce ROS production in rice plants are by activating signal transduction such as salicylic acid to help produce and activate enzymatic antioxidant systems, such as CAT, APX, and SOD. SOD forms the first line of defense against drought-induced ROS under drought conditions, in which SOD dismutase superoxide radicals into H_2O_2, then CAT reacts with H_2O_2 to catalyze the formation of H_2O and O_2 and APX decomposes H_2O_2 into H_2O by involving GR, MDHAR, DHAR in the AsA/GSH cycle. (Das & Roychoudhurry, 2014; Sarker & Oba, 2018a, 2018b).

Expression of OsAB13 and OsLEA genes is generally used as a research parameter to see whether there is the resistance of rice plants in all varieties to drought stress compared to control treatments.

Based on Fig. 7, the expression of the OsACTIN gene is used as a housekeeping gene which is used as an internal control for the analysis of gene expression that has no effect on stress. The figure shows that several plant varieties have resistance to drought stress on the expression of the OsAB13 gene. The rice variety with high expression of the OsAB13 gene in the 15% PEG 6000 compared to the control treatment was Indragiri, while Siakraya and Sertani 1 had lower gene expression levels than the control treatment, and OsAB13 in 1R64 was not expressed in the 15% PEG 6000 treatment. The expression level of OsAB13 which appeared in the 15% PEG 6000 treatment showed that Indragiri, Sertani 1, and Siakraya varieties had a correlation of resistance to drought stress in the OsAB13 gene. The OsAB13 (abscisic acid insensitive 3) gene has an important role in the development of plant growth against drought stress because its expression increases when plants suffered drought stress (Vashisth et al., 2021).

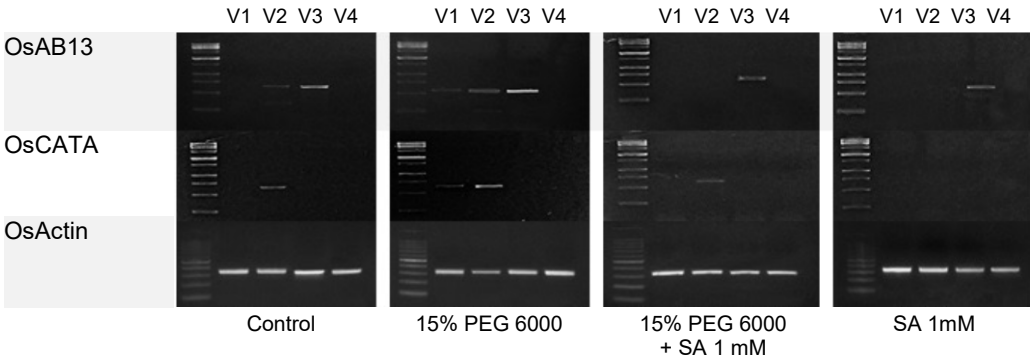

Figure 7. Expression of of OsAB13 and OsLEA genes were performed from total RNA samples for PCR analysis were isolated from leaf under several condition at 8 DAT. V1: Siakraya, V2 : Sertani 1, V3: Indragiri, and V4: IR64.

In the 15% PEG 6000 treatment, only the Sertani 1 variety had a high expression level of OsLEA gene compared to the control treatment, while the gene was not expressed in Siakraya, Indragiri, and IR64 varieties in the 15% PEG 6000 treatment. The expression levels of the OsLEA gene in the 15% PEG treatment 6000 indicate that Sertani 1 has a correlation of resistance to drought stress in the OsLEA gene. The OsLEA (late embryonic proteins abundant) gene has an important role in drought stress. LEA enzymes are important in increasing plant tolerance to drought in correlation with abscisic acid and other hormones as a form of signaling to drought stress (Wang et al., 2007).

CONCLUSIONS

The response to the treatment and variety of rice plants significantly affected plant height, root length, total chlorophyll, and H_2O_2 content. Increased expression of the OsAPX1, OsCATA, Mn-SOD, and Cu/Zn-SOD genes occurred in the 15% PEG 6000 treatment compared to the control treatment. The 15% PEG 6000 + SA 1 mM treatment had high gene expression values in APX Cytosolic, Cu/Zn-SOD, and OsCATA, but had lower gene expression levels of OsAPX1 and Mn-SOD. In the 1 mM SA treatment, the gene expression of OsCATA, Cu/Zn-SOD, and APX Cytosolic had an expression level that was almost the same as that of the 15% PEG 6000 + SA 1 mM treatment, but the expression

level of OsAPX1 and Mn-SOD increased. The rice variety Sertani 1 expressed the OsLEA gene as an indicator of resistance to drought stress, while the OsAB13 gene was expressed in rice varieties Indragiri, Sertani 1, and Siakraya as an indicator of resistance to drought stress. Based on this study, it is necessary to carry out further research by adding other research variables to support current data such as the content of antioxidant enzymes during drought stress or any study related to salicylic acid treatment.

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Germination performance and seedling characteristics of chili pepper after seed priming with leaf extract of *Moringa oleifera*

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Abstract. Germination is the most critical stage of the plant life cycle since it is a principal component of seedling establishment and survival. The germination of chili pepper (*Capsicum annuum*) seeds is typically slow and non-uniform under favorable and unfavorable conditions. To overcome these problems, seed priming with moringa leaf extract is a viable option. The objective of this study was to investigate the effect of moringa leaf extract priming on germination performance and seedling characteristics of chili pepper. A completely randomized design was applied with five replications. Different concentrations of moringa leaf extract (1:30, 1:20, and 1:10) as priming solution were evaluated along with priming with distilled water (hydro priming), whereas non-primed seed was taken as control. The results revealed that hydro priming and moringa leaf extract priming effectively increased final germination percentage, germination rate index, germination index, vigor index, shoot length, and decreasing mean germination time. Conversely, the treatments had no significant effect on root length, while moringa leaf extract-primed seeds significantly increased shoot fresh weight. Furthermore, priming with moringa leaf extract at 1:20 had slightly better results than hydro priming, since it yielded higher germination index (363.60) and germination rate index (13.74), although it was at par with other concentrations. Additionally, it significantly produced the highest root fresh weight (13.56 mg) and lower coefficient of variation of germination time (14.61) than the control. Based on these findings, priming with moringa leaf extract in amount of 1:20 can be suggested for improving germination and seedling growth of chili pepper.

Key words: *Capsicum annuum*, biostimulant, germination index, moringa leaf extract, vigor index.

INTRODUCTION

Chili pepper (*Capsicum annuum* L.) is one of the most important vegetable crops in the world. It belongs to the Solanaceae family and is a warm-season annual crop (Samarah et al., 2020). Pepper fruit is mostly consumed as a fresh vegetable or dried as a spice (Lemos et al., 2019). It is noteworthy that the fruit is an excellent source of

vitamins (A, C, E), phenolic acids, flavonoids, carotenoids, and capsaicinoids, which are essential for human health (Abou-Sreea et al., 2021).

The most critical stage of the plant life cycle is germination (Diel et al., 2019). Seed germination will affect the establishment of crops, which are accordingly related to ultimate crop yield and quality (Boter et al., 2019). It begins with water uptake and is completed when the radicle protrudes outside of the seed coat (Wolny et al., 2018). It has been reported that the germination of chili pepper is slow and non-uniform under favorable as well as unfavorable conditions (Yadav et al., 2011; Barchenger & Bosland, 2016). In order to overcome these issues, the seed priming technique has been widely used by farmers (Chen et al., 2021).

According to Yadav et al. (2011), seed priming is a pre-sowing seed treatment in which seeds are hydrated and induce pre-germination metabolic activities without allowing radicle emergence. Then, the seeds are dried back to their initial moisture content. It was also observed that seed priming has possibility to reduce germination time, increase seed vigor, and enhance germination uniformity (Espanany et al., 2016; Tu et al., 2022).

The process of seed priming can be divided into three phases (Ruttanaruangboworn et al., 2017). In phase I, seeds imbibe water to activate enzymes. Moreover, phase II includes the degradation of food reserve, reorganization of cell membrane, and biosynthesis of starch to promote root protrusion and seedling growth later in the next phase. This phase stops after re-drying the seeds. Finally, phase III occurs when the radicle protrusion can be seen, and afterward, root and seedling growth continues.

Numerous studies have been conducted to evaluate the effect of seed priming with natural growth-promoting substances as a sustainable approach (Masondo et al., 2018; Neto et al., 2020; Mutlu-Durak & Kutman, 2021). Priming with *Moringa oleifera* leaf extract (MLE) has gained attention as a promising method to improve germination performance due to having a higher amount of minerals, cytokinin, GA_3, ascorbic acid, and more (Bibi et al., 2016; El Sheikha et al., 2022). Previously, seed priming with MLE reduced germination time and enhanced final germination percentage (Nouman et al., 2012; Hala & Nabila, 2017). Other reports by Basra et al. (2011) showed that seed priming with MLE was effective to improve seedling growth.

The studies on the priming of seeds with plant extracts are rare in pepper (Pérez et al., 2021). To the best of our knowledge, plant hormones and nutrients are prevalent priming solutions for chili pepper seeds (Aloui et al., 2014; Quintero et al., 2018; Cano-González et al., 2021). They are relatively expensive for poor farmers (Afzal et al., 2012). The use of MLE which possesses considerable amounts of hormones and nutrients can be a cheaper alternative priming agent, but most studies dealing with MLE priming were mainly focused on cereal seed crops (Phiri, 2010; Ahmed et al., 2021). The MLE effect on germination and seedling characteristics of chili pepper remains unknown. Therefore, the objective of this study is to investigate the effect of MLE priming on germination performance and seedling characteristics of chili pepper.

MATERIALS AND METHODS

Experimental Design

The experiment was carried out under laboratory conditions at the Faculty of Agriculture, Universitas Padjadjaran, Indonesia. A completely randomized design was

performed with five replications. Chili pepper seeds of the commercial cultivar 'Tanjung' were subjected to five priming treatments. It comprised of priming with distilled water (hydro priming), priming with moringa leaf extract (MLE) diluted with distilled water for 30, 20, and 10 times (1:30; 1:20; and 1:10 respectively), and the non-primed seed was considered as a control treatment.

MLE Preparation

Fresh, healthy, and mature leaves of moringa were harvested, cleaned with tap water, and stored overnight in refrigerator. The extraction process followed the method of El Sheikha et al. (2022). It was conducted by mixing moringa leaves (30 g) with distilled water (300 mL) in a home blender, and then the mixture was sieved through cheese cloth and Whatman No. 1. Afterwards, the extract was centrifuged at 8,000 rpm for 15 min. The supernatant was collected and diluted with distilled water to achieve the required concentration. MLE used in this study (Fig. 1, a) contains beneficial substances such as P (98.93 mg L^{-1}), K s(410.30 mg L^{-1}), Ca (147.23 mg L^{-1}), flavonoid (151.96 mg L^{-1}), vitamin C (14.09 mg L^{-1}), cytokinin (12.00 mg L^{-1}), and GA_3 (23.00 mg L^{-1}).

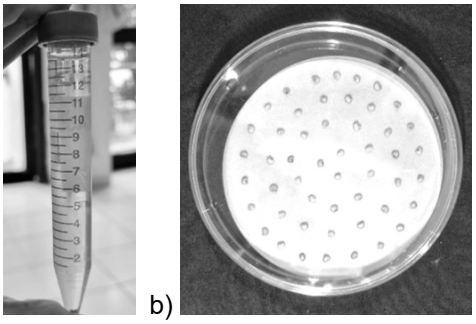

Figure 1. Moringa leaf extract (MLE) applied to germination of chili pepper (a) and germination test of chili pepper seeds in Petri dishes (b).

Seed Priming and Germination Test

The chili pepper seeds were surface sterilized with 1% sodium hypochlorite for 30 s, washed with distilled water, and shade dried. The seeds then were soaked in priming solutions for 24 h and dried back for 48 h at room temperature (26 °C) to reach their original weight. For germination test, 50 seeds per treatment per replication were placed in a 15 cm Petri dish, which contained double layers of Whatman No. 1 filter papers moistened with 5 mL of distilled water (Fig. 1, b). Petri dishes were incubated for 14 d at 26/27 °C day/night temperature and exposed to white LED light at 100 µmol m^{-2} s^{-1} (12 h photoperiod). Germination was observed daily in which the criterion of germination was 2 mm of radicle protrusion.

Data Collection

Final germination percentage (FGP) and mean germination time (MGT) were determined according to Wu et al. (2019). Germination rate index (GRI) and germination index (GI) were analysed using the formula proposed by Shah et al. (2021). Meanwhile, coefficient of variation of the germination time (CV_t) and vigor index (VI) were measured according to Ranal & Santana (2006) and Guragain et al. (2021) respectively. Those parameters were calculated using the following equations:

$$\text{FGP} = \frac{NGS}{NTS} \text{ x } 100 \quad (1)$$

$$\text{MGT} = \frac{\sum (N1T1 + N2T2 + \ldots + NiTi)}{\sum (\text{N1} + \text{N2} \ldots + \text{Ni})} \quad (2)$$

$$\text{GRI} = \frac{N1}{T1} + \frac{N2}{T2} + \ldots + \frac{Ni}{Ti} \quad (3)$$

$$\text{GI} = (10 \text{ x } N1) + (9 \text{ x } N2) + \ldots + (1 \text{ x } Ni) \quad (4)$$

$$\text{CV}_\text{t} = \frac{S_t}{MGT} \text{ x } 100 \quad (5)$$

$$\text{VI} = \frac{\textit{Seedling length} \text{ (cm) x } FGP \text{ (\%)}}{100} \quad (6)$$

where *NGS* is the number of germinated seeds at the end of experiment (14 d after incubation), *NTS* is the number of tested seeds, *Ni* is the number of seeds germinated in the i^{th} time, *Ti* is the time taken for seeds to germinate at i^{th}, S_t is standard deviation of the germination time, *MGT* is mean germination time, and *FGP* is final germination percentage.

Moreover, seedling characteristics were analysed at 14 d after incubation. The measurements consisted of root length, shoot length, root fresh weight, shoot fresh weight, and vigor index. Twenty uniform and normal seedlings from each replication were selected and averaged to measure those parameters.

Statistical Analysis

Data were collected and statistically analysed using analysis of variance (ANOVA) with SPSS v21 software. The differences in treatments were assessed by Duncan's multiple range test ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of Priming on Seed Germination

The presented results revealed that seed priming treatment notably affected the final germination percentage (FGP) (Table 1). Hydro priming and MLE priming showed the same noticeable increment regarding FGP, which increased by up to 7.60% compared to that of non-primed seeds (control). These results are in accordance with Hala & Nabila (2017) that seeds primed with different concentrations of MLE enhanced FGP of sweet pepper due to the role of phytohormones, amino acids, and mineral elements in MLE, which positively influenced this parameter. The percentage of increase in FGP reported by these authors was higher by about 53.52% than our study. Priming treatment promotes the mobilization of seed reserve from endosperm to embryo, resulting in better performance of germination (Majda et al., 2019). Higher FGP in primed seeds also may be attributed to the early metabolic processes during the hydration (Mir et al., 2021).

In the current study, germination rate index (GRI) was significantly influenced by seed priming treatment (Table 1). Compared with the control, all primed seeds exhibited remarkably higher GRI. The highest GRI was observed in MLE 1:20 (13.74), with an enhancement of 11.71%, but it was statistically at par with MLE 1:30 (13.58) and MLE 1:10 (13.56). Previously, seed priming improved the speed of germination, which was the fastest noted in MLE-primed seeds (Yasmeen et al., 2013). Better result in germination rate by MLE application was related to the improvement in metabolic activity (Nouman et al., 2012). Afzal et al. (2012) pointed out that MLE priming induced

the activity of amylase enzymes in seeds, hydrolysing starch into smaller molecules for the growth and development of embryo. Besides, the presence of calcium (Ca) in MLE also acts as an enzyme cofactor and facilitated faster germination (Gunasekar et al., 2017). During germination, Ca modulates the activity of kinase enzymes and certain phosphatase involved in signal translation (Karim et al., 2020). Fast seed germination is considered an essential attribute marking a quick transition to the growth stage in the plant life cycle (De Ron et al., 2016). Seed priming in most horticultural crops has primarily displayed an increase in germination rate (Tu et al., 2022).

According to Table 1, seed priming treatment showed a significant effect on germination index (GI). Priming of seeds with MLE 1:20 displayed the maximum GI (363.60), being higher by about 7.83% as compared to the control (337.20). However, it was statistically similar to other MLE concentrations. According to Hassanein & Al-Soqeer (2017), GI emphasizes both germination percentage and its speed. Afzal et al. (2012) confirmed that MLE priming advanced the germination of maize seeds, providing higher GI values. In MLE-primed seeds, numerous nutrients and vitamins possessed in MLE are transferred to embryo during phase II of seed priming, which ultimately improved GI. Seed germination also is influenced by plant hormones (Vysokova et al., 2019). Gibberellic acid (GA), a growth hormone found in MLE, plays a marked role in stimulating seed germination (Gunasekar et al., 2017). GA triggers the synthesis, activation, and secretion of hydrolytic enzymes, realising reducing sugars and amino acids, which are important for embryo growth (Vieira et al., 2002).

Table 1. Effect of priming chili pepper seeds with moringa leaf extract (MLE) on final germination percentage (FGP), germination rate index (GRI), germination index (GI), mean germination time (MGT), coefficient of variation of germination time (CV_t), and vigor index (VI)

Treatments	FGP (%)	GRI	GI	MGT (day)	CV_t (%)	VI
No priming (control)	90.71 a	12.30 a	337.20 a	4.09 b	19.12 b	5.80 a
Hydro priming	96.64 b	13.11 b	349.60 b	3.81 a	16.05 ab	6.20 b
MLE priming (1:30)	95.98 b	13.58 bc	360.00 bc	3.74 a	15.93 ab	6.59 b
MLE priming (1:20)	96.37 b	13.74 c	363.60 c	3.73 a	14.61 a	6.54 b
MLE priming (1:10)	97.60 b	13.56 bc	361.00 bc	3.75 a	13.33 a	6.45 b
Critical Value	3.66	0.53	11.49	0.13	3.67	0.37

[1] Mean values followed by different letters on the same column indicate significant differences according to Duncan's multiple range test ($P < 0.05$).

Moreover, mean germination time (MGT) responded differently to seed priming treatment, as presented in Table 1. MGT denotes the day at which most seeds have germinated (Talská et al., 2020). Hydro priming and MLE priming at all concentrations tested took less time to germinate, in a range from 3.73 to 3.81 days. These treatments reduced MGT by up to 8.80% as compared to the control. The primed seeds have the ability to accomplish germination in a short time (Nazari et al., 2017). Gilbero et al. (2014) supported these results, reporting that hydro priming and MLE priming were recorded as the most effective treatment in decreasing MGT. During the pre-germination stage, a solution-retention effect occurs in primed seeds and subsequently affects vital metabolic processes before radicle protrusion (Makhaye et al., 2021). Seed priming induces RNA activity and improves adenosine triphosphate (ATP) production, which helps the seeds to germinate (Ahmed et al., 2021).

Data depicts that the impact of seed priming was significant on the coefficient of variation of germination time (CV_t) in this study (Table 1). Seeds primed with MLE 1:20 (14.61%) and MLE 1:10 (13.33%) appreciably decreased CV_t by up to 30.28% in comparison with the control. However, they were statistically the same with MLE 1:30 (15.93%) and hydro priming (16.05%) treatments. CV_t interprets the uniformity or variability of germination, in which lower CV_t values express higher uniformity (Ranal & Santana, 2006). Nonetheless, the relevant study regarding MLE effect on CV_t is still limited. A lower CV_t of wheat seeds has been previously documented due to MLE priming compared to the control (Ahmed et al., 2021) and the percentage of decrease was lower by about 25.49% from our study. El-Katony et al. (2020) described a reduction in CV_t by using another biostimulant such as algae extract. They also proved that the highest CV_t was obtained in non-primed seeds. Uniform seed germination often resulted in a more uniform seedling establishment and healthier plant growth (Wu et al., 2019). In order to achieve uniform harvests, the factors that contribute to the variations in seed germination must be eliminated (Hayashi et al., 2020).

According to Table 1, the priming of seeds varied substantially on the vigor index (VI). A similar enhancement in VI was observed by hydro priming and MLE priming, being higher than in non-primed seeds. These treatments improved VI by up to 13.62%. Basra et al. (2011) mentioned that seed priming with MLE caused most of the N and Ca in MLE appeared to be partitioned to embryo, resulting in higher VI. Similarly, the high contents of Ca and other minerals in MLE might be responsible for increasing VI (Yasmeen et al., 2013). In addition, the hydration state of primed seeds is controlled, and subsequently, seeds can avoid the endoderm to break and produce pre-germination metabolism, which improves the seedling vigor and growth potential of seedlings (Chen et al., 2021). VI determines the potential for uniform and rapid germination as well as development of normal seedlings (Damalas et al., 2019). Vigorous growth together with earlier crop establishment can minimize weed competition, increasing water and nutrient absorption (Karim et al., 2020).

Effect of Priming on Seedling Growth

Fig. 2 represent the characteristics of seedling in the current study. No significant difference was observed in root length among the treatments, ranging from 4.33 to 4.81 cm (Fig. 3, a). In contrast, the treatment had a significant impact on shoot length (Fig. 3, b). The longest shoot was achieved by hydro priming and priming with MLE, which ranged from 1.80 to 1.85 cm and increased by up to 14.91%. Meanwhile, shoot length was significantly lower in the control (1.61 cm). The results in this study are consistent with the findings of Phiri & Mbewe (2010), who reported that the application of MLE as seed priming agents negatively altered the root length of bean and groundnut seedlings. Likewise, MLE-primed seeds had no significant effect on root length of sorghum and wheat (Phiri, 2010). Furthermore, an increase in shoot length by MLE priming has been previously reported by Sarmin (2014). This stimulatory effect of MLE might be linked to the cellular proliferation in shoot apical meristem after priming with this extract (Noor et al., 2016).

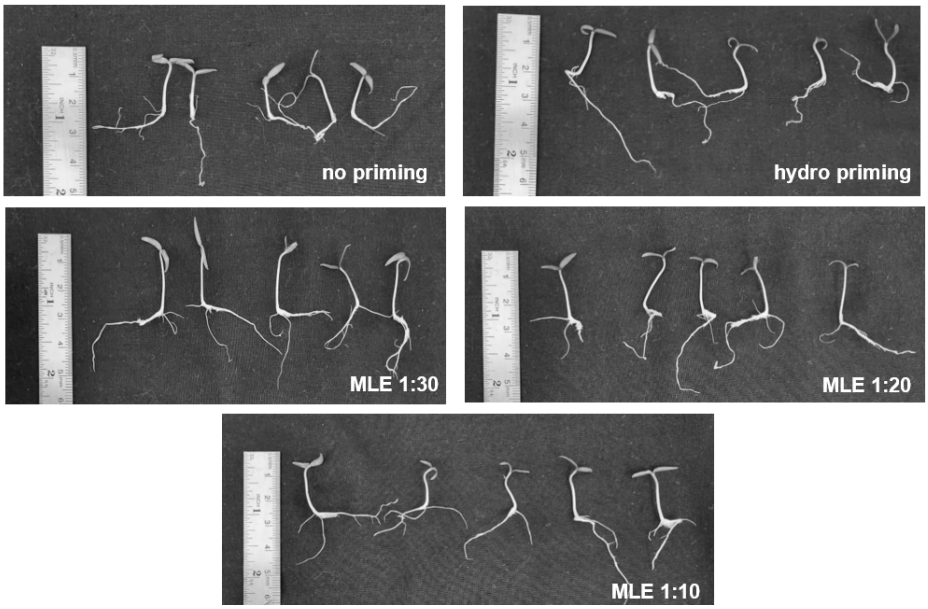

Figure 2. Effect of priming chili pepper seeds with moringa leaf extract (MLE) on seedling characteristics of chili pepper at 14 d after incubation.

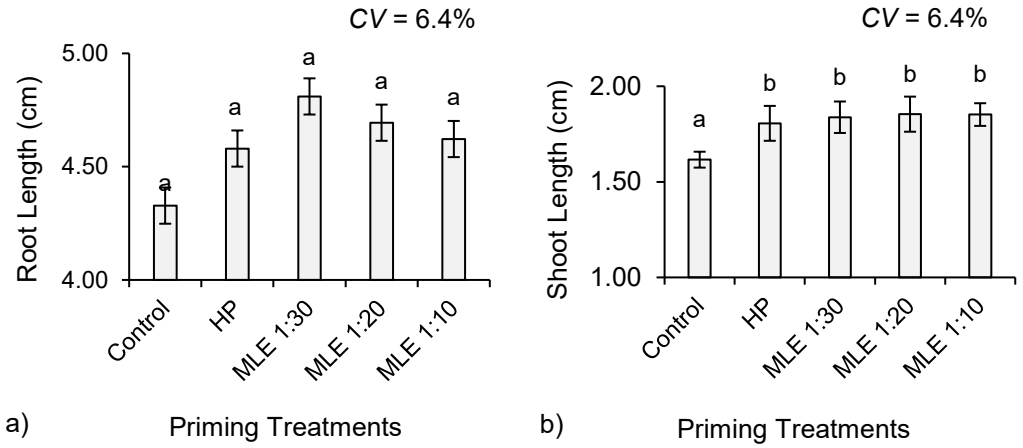

Figure 3. Effect of priming chili pepper seeds with moringa leaf extract (MLE) on root length (a) and shoot length (b) of seedlings (control = no priming, HP = hydro priming, MLE = moringa leaf extract priming). Mean values followed by different letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$). Critical value for root and shoot length is 0.35 and 0.10 respectively. *CV* = coefficient of variation.

Seeds primed with MLE 1:20 (13.56 mg) and MLE 1:10 (13.07 mg) significantly increased root fresh weight by up to 151.11% (Fig. 4, a). Among the treatments, the lowest root fresh weight was recorded in the control (5.40 mg) and hydro priming (6.48 mg). In similar, a significant change was detected in shoot fresh weight of chili pepper seedlings (Fig. 4, b). Seeds primed with MLE 1:30 (17.08 mg), MLE 1:20

(16.63 mg), and MLE 1:10 (16.61 mg) yielded heavier shoot in comparison with the control (14.98 mg). They increased by up to 14.02% from the control but statistically similar to hydro-primed seeds (15.83 mg).

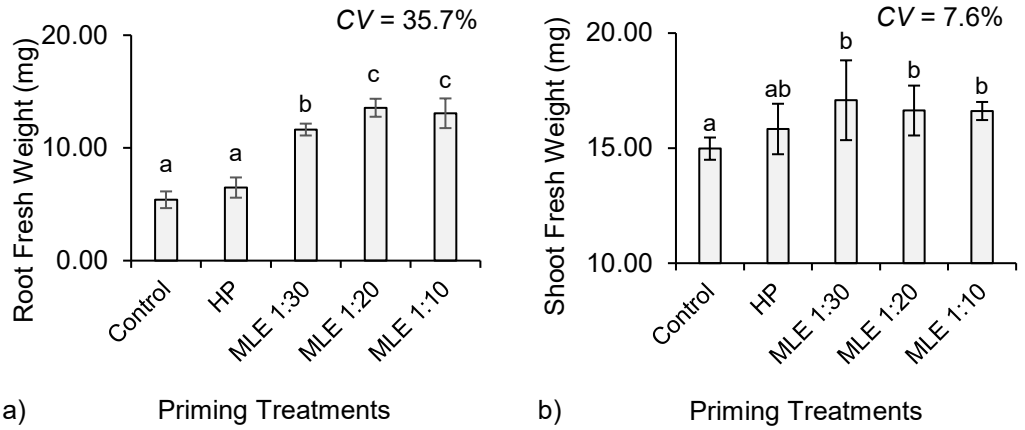

Figure 4. Effect of priming chili pepper seeds with moringa leaf extract (MLE) on root fresh weight (a) and shoot fresh weight (b) of seedlings (control = no priming, HP = hydro priming, MLE = moringa leaf extract priming). Mean values followed by different letters indicate significant differences according to Duncan's multiple range test ($P < 0.05$). Critical value for root and shoot fresh weight is 1.18 and 1.42 respectively. *CV* = coefficient of variation.

In other words, the present study clearly indicated that seedlings fresh weight of pepper was positively affected by MLE treatment. The same trend was observed in rice seedlings due to MLE application, (Khan et al., 2022). Priming treatment repairs membrane damage, decreases physical barriers of the endosperm, enhances immature embryo growth, and leaches germination inhibitors to promote root growth (Chen et al., 2021). In terms of root weight, the data were in line with Yasmeen et al. (2013), who demonstrated the effect of seed priming with MLE on the growth of wheat seedlings. They revealed that MLE-primed seeds showed higher root fresh weight compared to control treatment. Similarly, fenugreek seeds primed with MLE were considerably effective in improving shoot fresh weight under non-stressed conditions (Al Khazan, 2020). These beneficial effects of MLE can be attributed to the presence of zeatin, a naturally-occurring cytokinin, which is a growth-promoting substance (Iqbal et al., 2015). Cytokinin plays a major role to promote cell division in root and shoot systems (Azzam et al., 2022). In the present study, seed priming treatments are effective for improving germination process, yielding more uniform germination, and producing better seedling characteristics of chili pepper under laboratory conditions. The use of MLE as seed priming agent, particularly at the concentration of 1:20, had slightly better results than hydro priming, which was expressed by higher GI, GRI, and root fresh weight. These positive results are in agreement with Yousof et al. (2017) observations that seeds primed with MLE at moderate concentrations displayed better germination parameters and seedling characteristics. Seeds primed with MLE solution trigger several biochemical changes, including enzyme activation, starch hydrolysis as well as dormancy breaking (Hala & Nabila, 2017). It also activates the synthesis of GA and

proteins in cell wall for radicle protrusion, besides promoting antioxidant mechanism, as a protection against DNA damage (Gunasekar et al., 2017). Moreover, MLE contains the beneficial substances such as zeatin, ascorbic acid, Ca, and K, which accelerate seed germination and seedling development (Yasmeen et al., 2013).

CONCLUSIONS

In conclusion, hydro priming and moringa leaf extract (MLE) priming effectively improved the final germination percentage, germination rate index, germination index, shoot length, as well as vigor index of chili pepper seeds and reduced mean germination time. Meanwhile, the treatments did not show any significant effect on root length. Seeds primed with MLE increased shoot fresh weight compared to non-primed seeds (control). Furthermore, MLE priming at the concentration of 1:20 had slightly better effects than hydro priming, since it yielded higher values of germination index (363.60) and germination rate index (13.74), although it was statisticaly similar to other MLE treatments. It also produced the highest root fresh weight (13.56 mg) and more uniform seedlings compared to the control, which was reflected by lower coefficient of variation of germination time (14.61). Future studies may be needed to understand how MLE priming affects the biochemical changes of chili pepper seeds during germination and seedling growth, also the MLE impact on the chili growth and final yield.

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