Agronomy Research **23**(X), xxx–ccc, 2025
https://doi.org/10.15159/AR.25.053

Evaluation of salt tolerance in sugarcane mutant clone *M4* through the application of a rhizobacterial consortium

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Received: January 13th, 2025; Accepted: June 9th, 2025; Published: June 21st, 2025

Abstract. Sugarcane is one of the most important commodities in the world, with average global production reaching 1.9 billion tonnes in 2022. However, the availability of productive land does not meet the high production demand. This encourages farmers to utilize suboptimal land to meet global sugar cane needs. The aim of this study was to utilise a rhizobacterial consortium to identify the sugarcane mutant clone *M4*'s salt tolerance, which was compared to the widely used *Bululawang* variety. The experiment was conducted using a hydroponic system with salt treatment (150 mM NaCl) and varying concentrations of the rhizobacterial consortium (2, 4, and 6 mL). Morphological and physiological parameters were measured to assess the response to salt stress. The findings showed that the *M4* clone significantly improved plant height, root length, and total chlorophyll content compared to *Bululawang*. The application of the rhizobacterial consortium significantly enhanced salt tolerance in both genotypes, with the *M4* clone showing a stronger response. Overall, the *M4* clone displayed greater potential for cultivation in high-salinity soils, particularly when supported by the application of a rhizobacterial consortium. These findings provide valuable insights for the development of sugarcane varieties with enhanced tolerance to abiotic stress, potentially improving agricultural productivity in suboptimal lands.

Key words: Abiotic stress, bululawang variety, rhizobacterium, salt stress, sugarcane.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is an economically valuable agricultural commodity (Nguyen et al., 2022). In 2022, global sugarcane production reached approximately 1.9 billion tonnes, with Indonesia contributing over 2% of the total production (Karp et al., 2022). However, the lack of suitable agricultural land is making it increasingly challenging to expand sugarcane production. Consequently, farmers often resort to cultivating crops on suboptimal lands (Shrivastava & Kumar, 2015). Salt stress

is estimated to affect 20% of the world's irrigated land and can reduce crop yields by up to 50% (Munns & Tester, 2008).

Salt stress adversely impacts sugarcane plants through various physiological and biochemical mechanisms. When soil salinity levels are high, plants experience osmotic stress (Rani et al., 2024). Moreover, the accumulation of sodium (Na^+) and chloride (Cl^-) ions in plant cells causes ionic toxicity, disrupting nutrient balance by inhibiting the uptake of essential ions such as potassium (K^+). Potassium is vital for various physiological processes, including photosynthesis and enzymatic activity (Ferreira et al., 2017). Consequently, photosynthesis efficiency declines because Na^+ can substitute K^+ in chloroplasts, thereby reducing photosynthesis and ATP production in plants (Wang et al., 2024). Furthermore, salinity induces the formation of reactive oxygen species (ROS), which damage cell membranes, proteins, and DNA, accelerating cell aging and death (Arif et al., 2020). One environmentally friendly effort of mitigating the adverse effects of salt stress is the application of exogenous salicylic acid (SA), through increased proline biosynthesis as an osmolyte that helps maintain cellular osmotic balance under stress conditions (Apon et al., 2023). However, the effectiveness of SA is considered less sustainable, as it strongly depends on the application method, salinity level, solution concentration and requires repeated treatments to sustain the plant's physiological response (Poór et al., 2019; Silvia et al., 2020).

On the other hand, the availability of sugarcane varieties tolerant to salinity stress is very limited. Therefore, developing new varieties capable of thriving in saline conditions while maintaining high productivity is essential for the sustainability of sugarcane farming in suboptimal lands (Cherubin et al., 2021). Previous research has developed the agronomically superior Bululawang variety into several mutant clones through the Ethyl Methane Sulfonate (EMS) technique, namely the M2, M3, and M4 genotypes, which demonstrated greater agronomic performance than the parent variety (Faesol et al., 2022). Among these, the M4 genotype exhibited the most favourable morphological response to abiotic stress (Buqori et al., 2022). These findings suggest that M4 possesses strong adaptive potential under abiotic stress, although its performance under saline conditions remains unclear.

Nowadays, rhizobacteria have been reported as a promising, environmentally friendly alternative to enhance sugarcane resistance against both biotic and abiotic stresses (Santos et al., 2020). One primary biotic stress in sugarcane is *Fusarium* wilt, caused by *Fusarium sacchari*, which can be controlled using *Pseudomonas spp*. through various mechanisms, including the production of antibiotics, siderophores, and hydrolytic enzymes that inhibit pathogen growth, as well as the induction of systemic resistance in plants (Chetan et al., 2024). Besides that, rhizobacteria can enhance plant resistance to salinity stress through various mechanisms, including phosphate solubilisation, production of growth hormones (IAA and cytokinins), and enzyme synthesis (Gupta & Pandey, 2019; Dos Santos et al., 2020; Swiontek Brzezinska et al., 2022). Other studies indicate that *Bacillus* spp. and *Pseudomonas* spp. significantly enhance plant growth and yield under saline conditions by improving root architecture and nutrient uptake (John & Radhakrishnan, 2018). In sugarcane, *Bacillus* and *Azospirillum* species have been shown to improve nutrient uptake and antioxidant production, thereby enhancing growth and yield under salt stress (Moutia et al., 2010). Therefore, it is necessary to evaluate the use of a rhizobacterial consortium to improve

salt tolerance in the M4 mutant clone, which has demonstrated superior agronomic traits. Such an evaluation is crucial for developing adaptive, sustainable, and environmentally friendly strategies to enhance sugarcane productivity on salt-stress-affected land.

This study aims to evaluate the physiological and agronomic responses of the *M4* mutant clone and *Bululawang* to salt stress and to assess the effectiveness of a rhizobacterial consortium in enhancing plant resilience as part of a sustainable and environmentally friendly biointensive approach.

MATERIALS AND METHODS

Plant Materials

This study evaluated two sugarcane genotypes: *Bululawang* and the mutant clone *M4*. *Bululawang* is a commercial sugarcane variety that has been cultivated for over 20 years in Indonesia. The mutant clone *M4* was derived from EMS-induced mutagenesis. Mutagenesis in mutation breeding can be carried out using physical or chemical mutagens. Ethyl methane sulfonate (EMS) is a chemical widely used to induce mutations that can regulate economically essensial traits (Siddique et al., 2020). Several studies have shown that EMS is widely used in research to enhance agronomic traits (Espina et al., 2018). Based on reseatch that has been conducted, EMS mutagen led the production of three selected genotypes, namely the mutant clone *M4* (M1.4, M2.4, and M3.4) which is showed the highest agronomic performance and yield and recommended as new stable superior genotypes (Faesol et al., 2022). On other hand, the rhizobacterial species used in this study included *Actinomycetes*, *Azotobacter* sp., *Azospirillum* sp., *Rhizobium* sp., *Pseudomonas* sp., *Lactobacillus* sp., *Bacillus* sp., and *Streptomyces* sp. (Table 1). The bacterial consortium that has been used was BioToGrow liquid organic fertilizer with doses of 4 mk L^{-1}, 6 mL/L, and 8 mL L^{-1}.

Table 1. The type of bacteria and the number of colonies used in the research

Microorganisms	Composition (CFU mL^{-1})
Actinomycetes	1.4×10^{8}
Azotobacter sp.	3.4×10^{10}
Azospirillium sp.	2.7×10^{9}
Rhizobium sp.	6.15×10^{11}
Pseudomonas sp.	8.35×10^{11}
LactoBacillus sp.	6.8×10^{7}
Bacillus sp.	1.0×10^{8}
Streptomycete sp.	7.2×10^{5}

Media and Plants Preparation

The medium used was a 1:1 ratio of soil and sand in three 60×35 cm trays, each containing 48 seedlings. The single-buds used as seedlings were counted with an average of eight internodes per stem from the lowest internode. The single-bud seedlings were soaked in warm water for 1 hour to break the dormancy. Then, the seedlings will be planted in pots filled with media and covered with plastic for one week. Watering as a form of seedling care is also carried out every day for 60 days.

Experimental Design

The experimental design was a factorial completely randomised design consisting of two factors and three replications. The first factor was the sugarcane genotype, consisting of *Bululawang* and the mutant clone *M4*. The second factor comprised

salinity treatments: 150 mM NaCl, 150 mM NaCl + 2 mL rhizobacterial consortium, 150 mM NaCl + 4 mL rhizobacterial consortium, 150 mM NaCl + 6 mL rhizobacterial consortium, and a control without stress

Physiological Assay at the Early Growth Stage

The experiment was conducted in the greenhouse of the Faculty of Agriculture, University of Jember, Indonesia, from December 2023-January 2024. An optimal hydroponic system with a photoperiod of 16 hours of light and 8 hours of dark was used to cultivate the two sugarcane genotypes at the seedling growth stage. The genotypes were separately planted in trays containing 20 L of Hoagland nutrient solution. The nutrient solution was replaced every two days, and the pH was controlled. Salinity treatments began at the third leaf stage by adding NaCl with a dose 1,000 ppm and the rhizobacterial consortium at the designated doses. After three weeks of salinity treatment, sugarcane seedlings were sampled for morphological and physiological response assays.

Morphological Analysis

The morphological traits observed included plant height, root length, number of primary roots, fresh weight, dry weight, and stem diameter. Plant height was measured from the base to the plant`s apex using a measuring tape. Root length was determined by straightening the roots and measuring from the root base to the tip. The number of primary roots was counted for each sample. Fresh weight was measured by immediately weighing harvested plant samples using an analytical balance. Dry weight was measured after drying the plant samples in an oven at 70 °C for 72 hours. Stem diameter was measured at the base of the plant stem using calipers for precision.

Total Chlorophyll

Approximately 0.1 g of fresh leaves was ground with 10 mL of 80% acetone using a mortar and pestle. The extract was filtered to remove solid particles, and the liquid was analysed using a spectrophotometer at 345 nm and 663 nm (Lichtenthaler, 1987).

Hydrogen Peroxide Assay

Approximately 0.5 g of fresh leaf tissue was ground in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) solution. The mixture was centrifuged at 12,000 rpm for 15 minutes at 4 °C. A 500 µL aliquot of the supernatant was combined with 500 µL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M potassium iodide. The colour intensity of the solution was measured at 410 nm using a spectrophotometer. Hydrogen peroxide levels were determined using a standard curve based on known concentrations (Christou et al., 2014).

Proline Content

Approximately 0.5 g of fresh leaf tissue was ground in 10 mL of 3% sulfosalicylic acid solution. The mixture was centrifuged at 12,000 rpm for 15 minutes. A 2 mL aliquot of the supernatant was mixed with 2 mL of acid ninhydrin solution and 2 mL of glacial acetic acid. The mixture was heated in boiling water for 1 hour, cooled on ice, and then mixed with 4 mL of toluene. The toluene layer was separated, and its colour intensity

was measured at 520 nm using a spectrophotometer. Proline content was calculated using a standard curve (Bates et al., 1973).

Data Analysis

The data were analysed using ANOVA, and Tukey's test was applied to compare mean values at a 5% significance level. All statistical analyses were performed using *IBM SPSS Statistics* version 25.

RESULTS AND DISCUSSION

Morphological parameters

The morphological response shown by *Bululawang* sugarcane and mutant clone *M4* to salinity stress and bacterial application is illustrated in Fig. 1. In both genotypes of the sugarcane plants, the control treatment with salinity stress exhibited the smallest morphological results, particularly in the plant height parameter. Meanwhile, the treatment with 6 mL of bacterial consortium + 150 mM NaCl/L demonstrated better plant height and morphological appearance for both genotypes.

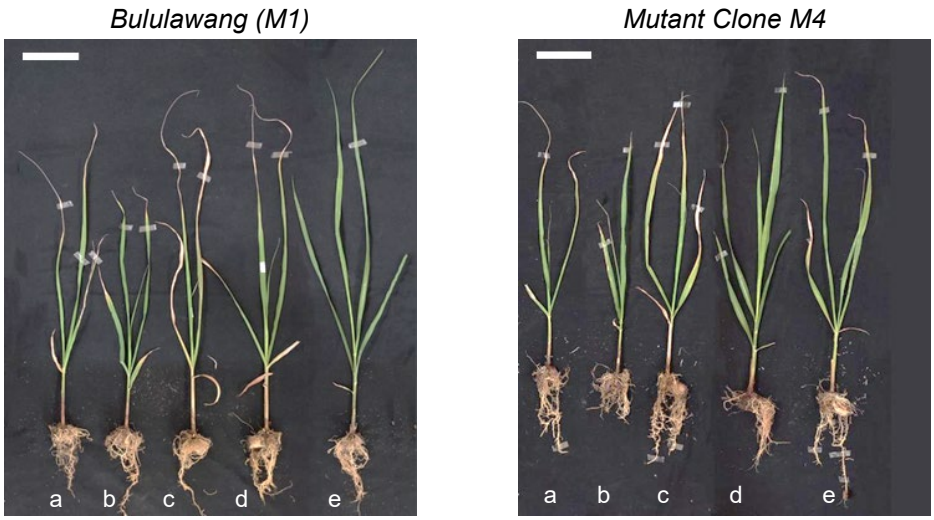

Figure 1. Morphological differences in sugarcane plants among various treatments for each genotype. (a: control without stress; b: control + saline stress; c: 2 mL rhizobacterial consortium + saline stress; d: 4 mL rhizobacterial consortium + saline stress; e: 6 mL rhizobacterial consortium + saline stress (scale bar = 2.54 cm).

The effects of various treatments on plant height and root length for the *Bululawang* genotype and mutant clone *M4* sugarcane are illustrated in Fig. 1. Under control conditions without NaCl, plant height and root length were the lowest for both genotypes.

As shown in Fig. 2, under the control treatment with 150 mM NaCl, both genotypes exhibited a significant reduction in plant height and root length compared to the control without NaCl, demonstrating the adverse effects of salinity stress. As the concentration

of the rhizobacterial consortium increased, significant improvements in both plant height and root length were observed. For the *Bululawang* genotype, plant height increased by nearly 40% with the application of 6 mL rhizobacterial consortium + 150 mM NaCl compared to the control ($p < 0.05$). For the *Bululawang* genotype, plant height increased by nearly 40% with the application of 6 mL rhizobacterial consortium + 150 mM NaCl compared to the control ($p < 0.05$). Similarly, mutant clone *M4* exhibited an approximate 35% increase in plant height under the same treatment conditions ($p < 0.05$). The root length for Bululawang increased most significantly with 4 mL rhizobacterial consortium + 150 mM NaCl, showing an increase of about 50% compared to the control. Mutant clone *M4* demonstrated a nearly 60% increase in root length with 4 mL rhizobacterial consortium + 150 NaCl compared to the control.

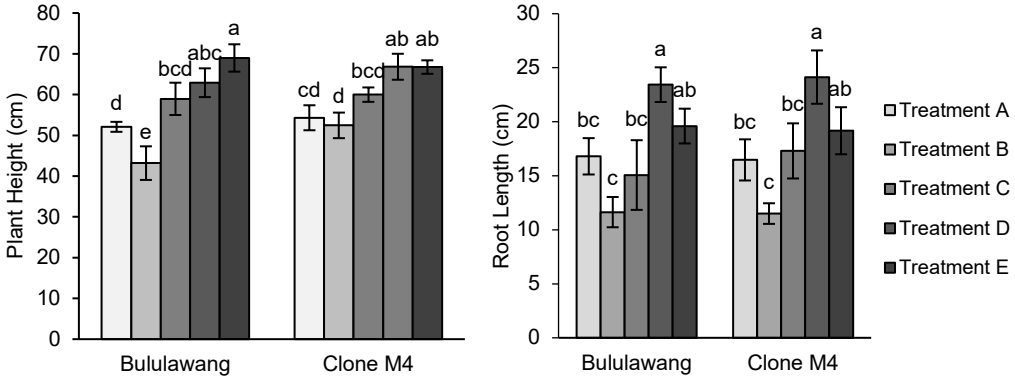

Figure 2. Plant height and root length in *Bululawang* and *M4* sugarcane genotypes under salinity stress with and without rhizobacterial treatment. Treatment A: Control + 0 mM NaCl; Treatment B: Control + 150 mM NaCl; Treatment C: 150 mM NaCl + 2 mL rhizobacterial consortium; Treatment D: 150 mM NaCl + 4 mL rhizobacterial consortium; Treatment E: 150 mM NaCl + 6 mL rhizobacterial consortium.

The results also demonstrate the effects of different treatments on the number of primary roots and stem diameter in the *Bululawang* genotype and the mutant clone *M4* sugarcane (Fig. 3). Data reveal that under the control treatment with 150 mM NaCl, there was a notable decrease in the number of primary roots and stem diameter in both genotypes. Under control conditions without NaCl, the number of primary roots was the lowest for both genotypes. Increase concentrations of the rhizobacterial consortium, significant increases in both parameters were compared by treatment control with 150 mM NaCl (B). For the *Bululawang* genotype, the number of primary roots is highest with the application of 4 mL of rhizobacterial consortium compared to the control with salinity (B). Mutant clone *M4* also exhibited an increase in the number of primary roots under the same treatment, reflecting a positive response to the rhizobacterial treatment. Stem diameter in *Bululawang* increased most significantly with the application of 4 mL of rhizobacterial consortium compared to the control with salinity (B). Mutant clone *M4* showed similar results, with significant increases in stem diameter across all

rhizobacterial treatments compared to the control. Overall, mutant clone *M4* demonstrated superior salt stress tolerance compared to *Bululawang* when treated with the rhizobacterial consortium.

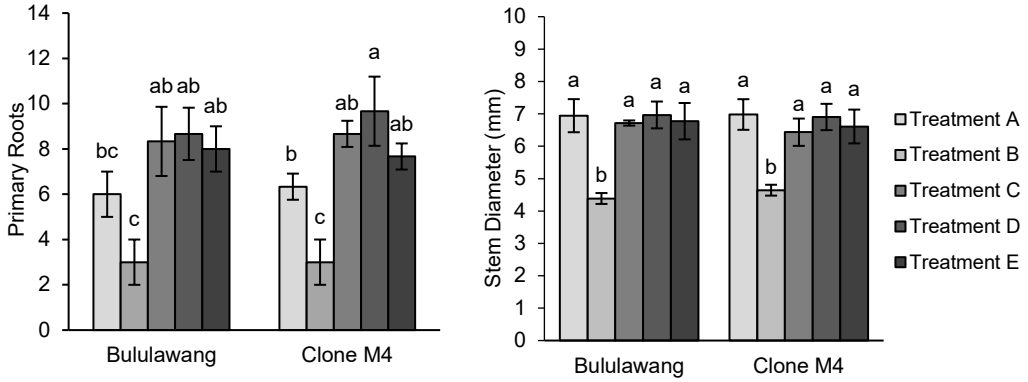

Figure 3. Primary roots and steam diameter in *Bululawang* and *M4* sugarcane genotypes under salinity stress with and without rhizobacterial treatment. Treatment A: Control + 0 mM NaCl; Treatment B: Control + 150 mM NaCl; Treatment C: 150 mM NaCl + 2 mL rhizobacterial consortium; Treatment D: 150 mM NaCl + 4 mL rhizobacterial consortium; Treatment E: 150 mM NaCl + 6 mL rhizobacterial consortium.

The effects of various treatments on fresh weight and dry weights in the *Bululawang* genotype and mutant clone *M4* sugarcane highlight clear differences between the treatments and control (Fig. 4). Under control conditions with NaCl, both fresh and dry weights were the lowest for both genotypes. The control treatment with 150 mM NaCl resulted in a significant reduction in both fresh and dry weights, illustrating the detrimental effects of high salinity on plant biomass.

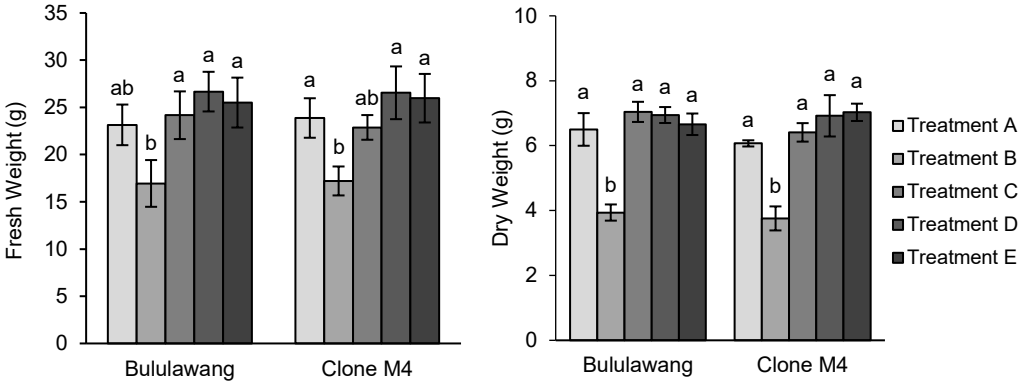

Figure 4. Fresh and dry weight in *Bululawang* and *M4* sugarcane genotypes under salinity stress with and without rhizobacterial treatment. Treatment A : Control + 0 mM NaCl; Treatment B : Control + 150 mM NaCl; Treatment C : 150 mM NaCl + 2 mL rhizobacterial consortium; Treatment D : 150 mM NaCl + 4 mL rhizobacterial consortium; Treatment E : 150 mM NaCl + 6 mL rhizobacterial consortium.

However, higher concentrations of the rhizobacterial consortium demonstrates fluctuative but remains the highest value in both fresh and dry weights. For the *Bululawang* genotype, fresh weight increased significantly, with the highest increase observed under the treatment with 4 mL of rhizobacterial consortium, resulting in an increase compared to the control. Similarly, dry weight showed a notable improvement under the same treatment compared to the control for the fresh weight parameter. However in dry weights, treatment E clone *M4* showed the highest value among other treatments. Mutant clone *M4* demonstrated a various response to the rhizobacterial consortium treatments. Fresh weight increased under the treatments with 4 mL and 6 mL of rhizobacterial consortium compared to the control. Dry weight in mutant clone *M4* showed similar enhancements, with an increase under the same treatments compared to the control.

Total Chlorophyll

Under control conditions without NaCl, total chlorophyll content in both genotypes was higher compared to the control + 150 mM NaCl (Fig. 5), indicating the negative effect of salt stress on chlorophyll.

The increase in rhizobacterial consortium concentration showed a positive trend and was in line with the increase in total chlorophyll content in the M4 clone mutant genotype. While the *Bululawang* genotype showed that the increase in total chlorophyll use of the rhizobacterial consortium was not in line (Fig. 5). In the *Bululawang* genotype, total chlorophyll has demonstrated differences from every treatment; the highest number, with 4 mL of rhizobacterial consortium, shows the highest rise in chlorophyll content compared to the control treatment without salinity (B). Mutant clone *M4* had a total chlorophyll value that increased gradually from 2 mL to 6 mL consistently. Comparison of the value of control with 150 mM NaCl with the provision of rhizobacteria showed a significant value. This indicates that the provision of a rhizobacteria consortium can effectively increase resistance to salt stress.

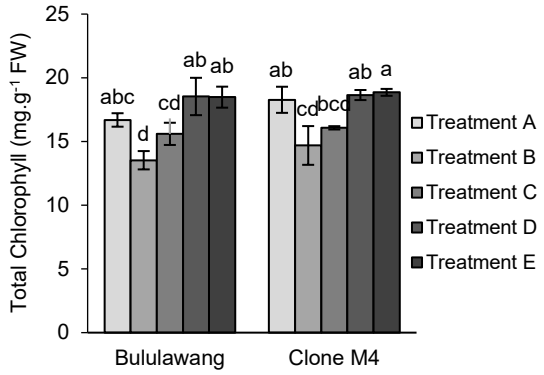

Figure 5. Total chlorophyll in *Bululawang* and *M4* sugarcane genotypes under salinity stress with and without rhizobacterial treatment. Treatment A : Control + 0 mM NaCl; Treatment B : Control + 150 mM NaCl; Treatment C : 150 mM NaCl + 2 mL rhizobacterial consortium; Treatment D : 150 mM NaCl + 4 mL rhizobacterial consortium; Treatment E : 150 mM NaCl + 6 mL rhizobacterial consortium.

Hydrogen Peroxide

Fig. 6. illustrates the changes in hydrogen peroxide (H_2O_2) accumulation in the *Bululawang* genotype and the *M4* mutant clone under various treatments, including a control without salinity, a control with salinity stress, and the application of a

rhizobacterial consortium at different levels (2, 4, and 6 mL) under salinity stress. In the *Bululawang* genotype, salinity stress resulted in an increase in H_2O_2 accumulation by more than 50% compared to the control without salinity. However, the application of the rhizobacterial consortium in various treatment variably reduced H_2O_2 accumulation, with the most significant reduction observed in the 6 mL treatment, which decreased H_2O_2 levels by nearly 20% compared to salinity stress without the rhizobacterial treatment.

The *M4* mutant clone exhibited a similar pattern, where salinity stress increased H_2O_2 accumulation by approximately 75% compared to the control without salinity. The application of the rhizobacterial H_2O_2 accumulation in this clone, with the greatest reduction occurring in the 4 mL and 6 mL treatment, lowering H_2O_2 levels compared to salinity stress without the rhizobacterial treatment. These results highlights that the application of the rhizobacterial consortium mitigated oxidative stress by salinity in both genotypes, with a more pronounced effect observed in the *M4* mutant clone, which is less influence by the treatments because its variably result induced.

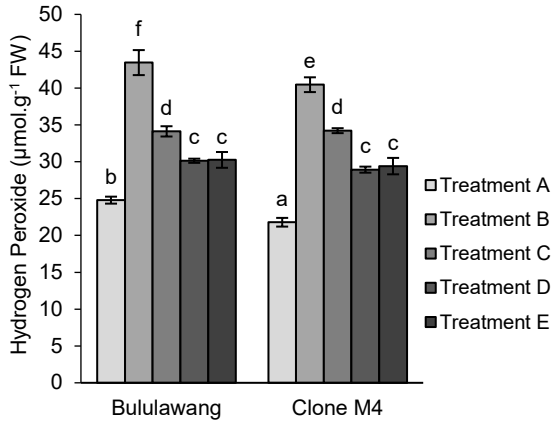

Figure 6. Accumulation of hydrogen peroxide in *Bululawang* and *M4* sugarcane genotypes under salinity stress with and without rhizobacterial treatment. Treatment A : Control + 0 mM NaCl; Treatment B : Control + 150 mM NaCl; Treatment C : 150 mM NaCl + 2 mL rhizobacterial consortium; Treatment D : 150 mM NaCl + 4 mL rhizobacterial consortium; Treatment E : 150 mM NaCl + 6 mL rhizobacterial consortium.

Proline Content

In the *Bululawang* genotype, exposure to salinity stress resulted in a marked increase in proline accumulation, nearly doubling the levels observed in the control without salinity. This substantial rise highlights the genotype's osmotic adjustment mechanism in response to salinity. Furthermore, the application of the rhizobacterial consortium under salinity conditions led to an additional increase in proline content. Notably, the highest concentration of the consortium was 6 mL in the *Bululawang* genotype, and 2 mL in the mutant clone *M4* genotype had brought about proline accumulation with the highest number. That indicates the consortium may enhance salinity stress tolerance by promoting osmoprotectant synthesis.

The mutant clone *M4* exhibited a similar yet more pronounced increase in proline levels compared to the non-stressed control, suggesting a strong osmotic adjustment response potentially associated with its mutational background. The application of the rhizobacterial consortium further elevated proline accumulation, although the differences

among the various consortium concentrations were relatively subtle. The 6 mL treatment yielded the highest proline level in the *Bululawang* genotype, while the 2 mL treatment was most effective in the mutant clone *M4* (Fig. 7). These findings indicate that the rhizobacterial consortium enhances proline accumulation, which may serve as a key mechanism for mitigating salinity stress in both genotypes. Moreover, a high increase in proline in the *M4* mutant from control + 150 mM NaCl treatment with rhizobacteria consortium demonstrates a strong response could highlight to its potential advantage under salinity stress due to genetic modifications that enhance its stress resilience.

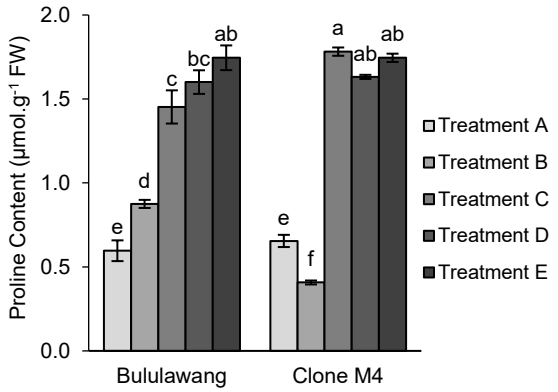

Figure 7. Comparative proline accumulation in *Bululawang* and *M4* sugarcane genotypes under salinity stress with and without rhizobacterial treatment. Treatment A : Control + 0 mM NaCl; Treatment B : Control + 150 mM NaCl; Treatment C : 150 mM NaCl + 2 mL rhizobacterial consortium; Treatment D : 150 mM NaCl + 4 mL rhizobacterial consortium; Treatment E : 150 mM NaCl + 6 mL rhizobacterial consortium.

Discussion

Sugarcane under salinity stress may experience inadequate growth and declines in germination due to ion imbalance and difficulties in water uptake (Apon et al., 2023). Although, *Bululawang* has been cultivated under abiotic stress, such as drought, floods, and high temperatures for 18 years, its superiority could not defeat the M4 Mutant when placed in unfavorable environments. Based on the findings, it is provides that the *M4* mutant clone demonstrates superior performance under salinity stress compared to the *Bululawang* variety across several key parameters.The concentration gradient did not significantly affect the stress tolerance of both genotypes. However, the application of rhizospheric bacterial consortium can help sugarcane plants adapt to salinity stress through the mechanism of increasing photosynthetic capacity, reducing oxidative stress, and increasing adaptive responses responses by manipulate the processes and components in soil (Van Veen et al., 1997). The *M4* clone exhibits greater plant height and stem diameter, suggesting more robust growth under saline conditions. Additionally, the *M4* clone develops a greater number of roots than the *Bululawang* variety, likely contributing to enhanced nutrient and water uptake, thus improving overall resilience. Furthermore, the *M4* clone maintains higher total chlorophyll content under salinity stress, indicating a more efficient photosynthetic capacity despite adverse conditions. Importantly, the *M4* clone also shows lower hydrogen peroxide levels compared to *Bululawang*, reflecting reduced oxidative stress and better cellular protection mechanisms. The strong response of the *M4* mutant clone under salinity stress highlights its potential superiority, likely due to genetic modifications that have enhanced its stress tolerance. Previous studies mentioned that, the *M4* clone showed the highest agronomic and yield performance compared to *Bululawang* (Faesol et al., 2022). The mutant *M4* were identified to have high yield stability in the multi-location trials, and adaptive

species to the environment, hence, they were recommended as new stable superior genotypes. Plant growth promoting bacteria present in rhizosphere of crop plants can directly modulate phytohormones production and metabolism (Bal et al., 2013). Moreover, the application of a bacterial consortium significantly improved the salinity tolerance of both the *M4* clone and the *Bululawang* variety, further supporting the role of beneficial microbes in mitigating stress effects and enhancing plant resilience (Kakar et al., 2016; Khan et al., 2022). These findings collectively underscore the potential of the *M4* clone as a promising candidate for cultivation in saline environments, supported by both its intrinsic genetic advantages and the external application of stress-mitigating bacterial treatments.

In light of prior research on salinity stress, the performance of the *M4* mutant clone under such conditions signifies a substantial advancement in sugarcane's adaptive responses, highlighting the progress achieved through targeted genetic modification. Previous research has consistently demonstrated that salinity imposes substantial challenges to plant growth, typically leading to reduced plant height, decreased chlorophyll content, and increased oxidative stress markers like hydrogen peroxide (Shrivastava & Kumar, 2015). Studies on traditional sugarcane varieties, such as *Bululawang*, have emphasised these vulnerabilities, particularly their limited root development and compromised photosynthetic efficiency under saline conditions. However, the *M4* clone challenges these established trends by not only maintaining but even enhancing growth metrics like plant height and stem diameter under stress, deviating from commonly reported outcomes. Moreover, while earlier studies have underscored the detrimental effects of salinity on chlorophyll content and oxidative stress, the *M4* clone's ability to sustain higher chlorophyll levels and lower hydrogen peroxide accumulation indicates a more robust photosynthetic machinery and superior oxidative stress management. The increase in total chlorophyll in plants treated with PGPR under salinity conditions indicates that PGPR is able to increase the photosynthetic efficiency. PGPR such as *Bacillus* sp., *Pseudomonas* sp. and *Azospirillum* sp. play a role in this improvement. *Bacillus* sp. and *Pseudomonas* sp. are known to increase the uptake of nutrients such as nitrogen and iron, which are important for biosynthesis and as a promising biocontrol organisms for plants (Chaugan et al., 2013; Meena et al., 2016). *Azospirillu*m sp. also contributes to chlorophyll increase through increased nutrient uptake. In addition, PGPR helps maintain ion homeostasis by reducing Na+ uptake and increasing K+ uptake, which supports chloroplast membrane integrity and photosynthetic function under saline conditions. Research has also shown that PGPR can increase the activity of enzymesinvolved in chlorophyll biosynthesis and prevent chlorophyll degradation that is induced by oxidative stress. This stark contrast suggests that the genetic modifications present in the *M4* clone, particularly those induced through EMS mutagenesis, confer an adaptive advantage. EMS mutagenesis, known for its potential to generate significant genetic diversity, has likely played a crucial role in developing these beneficial traits, positioning the *M4* clone as a potentially transformative option for sugarcane cultivation in saline environments (Chen et al., 2023). The augmentation of these intrinsic advantages by the application of a bacterial consortium, which further enhances the salinity tolerance of both the M4 clone and the *Bululawang* variety, aligns with the growing body of research advocating for the integration of microbial treatments to improve plant resilience. This comparison not only

highlights the innovative potential of the *M4* clone but also emphasises the broader implications of utilising EMS mutagenesis to create new genetic variations that can be exploited to combat salinity stress in crops.

The enhanced salinity tolerance observed in the *M4* mutant clone is likely attributable to a combination of genetic modifications and physiological adaptations. Increased plant height, stem diameter, and chlorophyll content under saline conditions suggest that the *M4* clone possesses a more efficient photosynthetic apparatus and structural integrity, essential for maintaining growth and productivity in challenging environments. This is supported by the superior root development observed in the *M4* clone, which likely facilitates improved water and nutrient uptake, thus mitigating the osmotic stress typically associated with high salinity. Moreover, the reduced accumulation of hydrogen peroxide in the *M4* clone compared to the *Bululawang* variety indicates a more effective antioxidant defence mechanism. This suggests that the genetic modifications introduced through EMS mutagenesis may have up-regulated pathways responsible for scavenging reactive oxygen species ROS, thereby reducing cellular damage and maintaining metabolic functions under stress (Dalvi et al., 2021). The observed benefits are further augmented by the application of a bacterial consortium, which likely enhances the plant's natural defence systems by promoting beneficial microbial interactions and stress-alleviating compounds. PGPR such as *Bacillus* sp., *Pseudomonas* sp., *Rhizobium* sp. and *Streptomyces* sp. play a role in this process. *Bacillus* sp. and *Pseudomonas* sp. are known to reduce oxidative stress through increased activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD) (Egamberdieva & Lugtenberg, 2014). PGPRs also contribute to the reduction of H2O2 and protection against oxidative stress through the activation of antioxidant defense system (Nivetha et al., 2021). This mechanism is very important because H2O2 can damage cellular structures and inhibit plant growth (Munns & Tester, 2008). Specifically, the bacterial consortium enhances salinity tolerance through several mechanisms, including the production of phytohormones such as indole-3-acetic acid (IAA), which promotes root elongation and proliferation, improving the plant's ability to access water and nutrients under saline conditions (Gupta & Pandey, 2019; Khan et al., 2022; Swiontek Brzezinska et al., 2022). Inoculating rhizobacteria also improves growth properties such as phosphate, zinc, and potassium solubilisation, enhancing growth parameters in sugarcane (Patel et al., 2022). Additionally, certain bacteria in the consortium may produce exopolysaccharides, which help to stabilise soil structure around the roots, reducing salt accumulation in the root zone and mitigating osmotic stress. Furthermore, the consortium may increase the availability of essential nutrients, such as phosphorus, by solubilising phosphates, thus supporting plant growth under nutrient-limited, saline conditions (Aliyat et al., 2022). Some bacteria within the consortium might also produce ACC deaminase, an enzyme that lowers plant ethylene levels, typically elevated under stress conditions and known to inhibit root growth. This synergistic interaction between genetic resilience and microbial support not only underscores the *M4* clone's potential in saline environments but also highlights the broader applicability of integrating genetic and microbial strategies to improve crop stress tolerance.

CONCLUSIONS

The significant potential of EMS mutagenesis to generate new genetic diversity is vividly demonstrated in this study, particularly through the enhanced salinity tolerance observed in the *M4* mutant clone. This research not only highlights the efficacy of EMS-induced mutations in developing crops with improved stress tolerance but also underscores the broader applicability of this approach in breeding programmes aimed at enhancing resilience in other crop species. By coupling these genetic advancements with microbial treatments, such as the bacterial consortium used in this study, there is a promising opportunity to create sustainable agricultural systems that can thrive under increasingly challenging environmental conditions. As global agricultural landscapes face the dual threats of climate change and soil salinisation, the integration of genetic and microbial innovations presents a viable strategy for ensuring food security and promoting sustainable farming practices. The findings of this study, therefore, provide a compelling case for the continued exploration and application of EMS mutagenesis and microbial interventions in the development of resilient, high-yielding crops capable of with standing environmental stressors. Despite that, further research is needed on the salinity stress tolerance of both varieties in mature plants in the field.

ACKNOWLEDGEMENTS. We would like to thank the Head of the Agronomy Department in the Faculty of Agriculture, University of Jember. We are also grateful to the technicians who assisted us with the project.

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