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Effect of water deficit on maize seeds *(Zea mays* L.*)* during germination

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Abstract. Global climate changes cause the frequent recurrence of droughts, which reduce crop production more than any other environmental factor. This study was conducted to access the effect of water deficit on maize seeds *(Zea mays* L.*)* DKC 5143 hybrid during germination. The tasks were to assess the influence of different rank of osmotic stress on the maize lipid peroxidation (LPO), proline content, catalase and aminotransferases activities, and morphometric parameters during the early stages of maize seeds germination. The maize seeds were exposed to five levels of water availability which produced by PEG-1500 solutions (0, 20, 50, 100, 200 g L^{-1}). Seeds of maize were germinated on Petri dishes for 7 days under controlled parameters. Amounts of TBARS were increased in maize sprouted seeds by 1.9 times, coleoptiles by 1.4 times, and in roots - by 1.9 times under water deficit. Proline content increased by 9.2 times in coleoptiles and by 6.0 times in 7 days maize roots while PEG-1500 (200 g L^{-1}) treatment. An increasing of catalase (CAT), aminotransferases (ALT, AST) activities according to osmotic potential value was also observed. A remarkable development of maize oxidative reaction was associated with a significant reduction in emergence, wet weight and length of water-stressed plants. These results assume that the maize adaptive strategy to osmotic stress during germination was found in the activation of LPO and antioxidant components. The findings provide useful help for correcting the stress state of maize using osmotically active regulators.

Key words: aminotransferases, catalase, drought, germination, lipid peroxidation, maize, osmotic stress, proline.

INTRODUCTION

The water deficit is the most limiting factor of yield formation among the factors that cause plants stress. Drought periods repeated very frequently during the seeding period and have a negative impact on the plants during the vegetation period in the semi-arid and arid part of Ukraine and Europe, in general (Lipper et al., 2014; Vogel et al., 2019; Horváth et al., 2021). Disorders of water balance in the plant organism lead to changes in the photosynthesis intensity, carbohydrate and protein metabolism. Water

stress promotes the accumulation of various toxic products, including activated oxygen metabolites (AOM), lipid peroxides, and oxidized proteins (Kolupaev et al., 2019).

It is known, that an antioxidant system plays a significant role while plants adapt to unfavorable environmental factors. The antioxidant system provides the tolerance of damaging environmental stresses which is correlated with an increased capacity to scavenge or detoxify ROS (Yang et al., 2021). Various enzymatic and nonenzymatic antioxidant components eliminate AOM, inhibit the development of the peroxidation process, and prevent DNA fragmentation (Masoumi et al., 2010). Antioxidative enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) play an important role against drought stress (Habibi & Hajiboland, 2011; Moharramnejad et al., 2019).

Protein metabolism of plants shows significant sensitivity in implementing adaptive reactions. When plant's leaves are under water stress, their protein content decreases. Besides that, the water deficit reduced the pool of total protein in C_3 plants more intensively than in C_4 plants (Ozturk et al., 2021).

Transferases take an important role in these processes participating not only in the synthesis of amino acids and proteins but also in maintaining energy metabolism. It is found that C_4 plants have high activity of aminotransferases (Schlüter et al., 2019). Alanine aminotransferase (ALT) (EC 2.6.1.2) catalyzes the transfer of amino between alanine and glutamate involving α-ketoglutarate and pyruvate. Aspartate aminotransferase (AST) (EC 2.6.1.1) catalyzes the transamination of glutamate and aspartate involving α-ketoglutarate and oxaloacetate. These enzymes are represented by several isoenzymes forms in plant cells and localized in the cytosol, plastids, mitochondria and peroxisomes (de la Torre et al., 2014). Aspartic and glutamic acids play an important role in plant adaptation to adverse factors as a reserve for the synthesis of new amino- and ketoacids. The role of aminotransferase reactions in the detoxification of xenobiotics, heavy metals, and pesticides (Singh et al., 2015) has enough investigated. The changes in aminotransferase activity under the influence of temperature, water, phytohormones, and nutrients were described (Kendziorek et al., 2012; Xue et al., 2021). However, the ontogenetic changes of aminotransferase activity of germinated maize under a water deficit have not been sufficiently investigated.

Water is an activation factor of biochemical and physiological processes that accompany seed soaking and germination. Plants are highly sensitive to even slight depression of water potential at seedling stages. Therefore, the physiologically normal level of free radical processes is disturbed and the synthesis of compatible osmolytes is increased in embryonic axes under osmotic stress conditions (Zivcak et al., 2016; Kolesnikov et al., 2019).

Maize (*Zea mays* L.) is one of the most important cereal crops in the world after wheat and rice. Normally, it needs 500–800 mm of water during its life cycle (80–110 days). Water availability and movement into the seeds are very important to promote germination, initial root growth, shoot elongation, and therefore at the establishment of a uniform stand. The germination starts with seed imbibition as a result of water uptake. This process occurs due to the distinct levels of osmotic potential between the dry seed and water in the substrate of germination. In general, the seed water content of cereal crops must reach at least 35 to 45% of seed dry mass to occur the germination process. The highly negative osmotic potential may affect the seeds' water uptake, making germination not possible. The most common responses of plants to the

reduction of osmotic potential are a delay in initial germination and a reduction in the rate and total germination (Ashraf et al., 2016; Queiroz et al., 2019; Hunter et al., 2021). Under low moisture levels of the steppe, water deficit negatively influences different stages of growth and development of maize and grain crops, in general (Akıncı & Lösel, 2012; Golabadi et al., 2015; Mazhar et al., 2020). Although maize is a drought-resistant crop, it sprouts best when the upper soil layers are well-hydrated and heated (Bhusal et al., 2021; Khaeim et al., 2022).

There are numerous studies noted changes in the activity of the antioxidant system and metabolic components under drought conditions during seed germination, but the obtained results are different and sometimes contradictory. At present, the major focus of maize research is to improve abiotic stress tolerance characteristics. The study of maize resistance to water deficits during seedling stages is important for understanding the mechanism of the stress damage, for finding techniques that optimize metabolic processes, and for the selection of optimum moisture at sowing. As a result of this research, we will be able to develop agrotechnologies for growing maize hybrids in arid conditions without compromising the grain yield under stress conditions.

The present study was performed with a hypothesis that water deficit-induced change in pro-antioxidant system could affect yield and growth of maize plants grown under drought conditions. Therefore, the aim of present study was to evaluate the influence of different water stress levels on the maize lipid peroxidation, proline content, catalase, and aminotransferases activities and morphometric parameters during the early stages of maize seeds germination.

This study provides essential information regarding germination requirements, and it investigates tolerance to drought stress.

MATERIALS AND METHODS

Plant material, experimental procedures

The seeds of maize *(Zea mays* L.*)* DKC 5143 hybrid (produced by Monsanto Company) were used to study the effect of osmotic stress. DKC 5143 is a mid-late maize hybrid (FAO 430) with high plasticity and medium drought resistance. The maize seeds belong to the tooth-shaped type, weight of 1,000 grains is 330–370 g. Maize seeds were placed in growth chamber conditions with a temperature regime of 24 ± 2 °C in the dark. Three days after sowing, the germinated seeds were grown at 14 h of photoperiod, 60% relative humidity (RH), and 100 mmol m^{-2} s^{-1} of photosynthetic photon flux density (PPFD) provided by fluorescent lamps.

The design of the experiment included 5 groups. One group was used for control where maize seeds were germinated on water. The other four groups were used for water deficit treatments by exposure to PEG-1500 (polyethyleneglycole) at concentrations of 20, 50, 100, 200 g L^{-1}. Each concentration produces an osmotic potential (-0.5, -1.5, -3 and -6 bar) accordingly (Michel & Merrill, 1973). Seeds of the control and drought treatments were germinated according to the International Seed Testing Association (ISTA) protocol. For each treatment 250 seeds were placed on five 90 mm diameter Petri dishes (50 seeds on each dish). Two layers of filter papers were moistened with 5 mL of incubation medium (ISTA, 2014).

Dry seeds, endosperm of germinated seeds and embryonic axis (primary roots and coleoptile) were used for determination of biochemical parameters. The endosperm of

germinated seeds was taken at 2, 4, 6, 12, 24 hours after sowing. The embryonic axis was taken on the 3rd and the 7th day after sowing. The seeds did not germinate in the group with 200 g L^{-1} PEG-1500 treatment, so the parameters were not determined in the coleoptiles and roots on the 3rd and the 7th day.

Measurements

Water uptake and growth measurement

The water uptake was measured by putting known weights seeds of maize in water or water solutions of PEG-1500 at different concentrations. After different intervals of time, the weight of the water absorbed by the seeds was ascertained and then weighed. The kinetics of water uptake was monitored in 2, 4, 6, 12, 24 hours from seeds sowing on Petry dishes. Seeds water content was calculated as the difference between the raw weight of soaked seeds (SRW) and their start dry weight (SDW) divided by the SDW according to the Eq. (1) and the result is presented as a percentage (Djébali, 2012).

$$\mathrm{WC} = \frac{\mathrm{SRW} - \mathrm{SDW}}{\mathrm{SDW}} \times 100\% \quad (1)$$

The energy of seed's germination (ESG) was measured after 3 days of growing. The amount of normally germinated seeds was calculated when ESG accounting was conducted. Normally germinated maize kernels have a developed main germinal root, which was larger than half of the seed length and formed sprouts. This index was calculated using the formula (2):

$$\mathrm{ESG} = \frac{50 - n}{50} \times 100\% \quad (2)$$

where, n – number of seeds, which didn't germinate for 72 hours, un.

The laboratory germination (LG), raw weights of roots and coleoptiles, and length of coleoptiles of maize were measured on the 7th day after sowing. The calculation of laboratory germination was made using the ratio (%) of the total number of seeds that were taken for germination to the number of not germinated seeds. The swollen, rotten, and abnormally germinated seeds are referred to as not germinated ones (ISTA, 2014).

Seedlings were separated into roots and coleoptiles on the 7th day after germination. The raw weights (g 100 units) of roots and coleoptiles were quantified using analytical scales. Coleoptile length (cm) was recorded as the distance from the scutellum to where leaf one had broken through the tip of the coleoptile (Hakizimana, 2000).

TBA-reactive substances (TBARS) determination. Plants samples were homogenized in LN with a mortar and pestle at the presence of 100 mmol tris-HCl buffer (pH 7.8) at ratio 1:9 (v/v) under temperature 0–4 °C. The tissue homogenate was centrifuged for 10 min (8000g) and the supernatant liquid was analyzed.

Plant homogenate with a volume of 1.0 mL was incubated in the boiled water bath with 3.0 mL of 0.5% thiobarbituric acid (TBA) on 20% trichloroacetic acid (TCA) solution for 30 min. After that, the reaction mixture was placed on ice and then centrifuged for 10 min at 5,000 g. The supernatant was used for photometric analyses. The absorbance of supernatant was read at 532 nm ('Unico UV-2800') subsequent to subtraction of non-specific absorption at 600 nm. The level of TBARS was measured according to malondialdehyde (MDA) concentration, which was calculated using its extinction coefficient 155 mM^{-1} cm^{-1} and expressed in $\mu M \times g^{-1}$ of raw tissue (Heath & Packer, 1968; Dhindsa et al., 1981).

Proline determination. Proline contents ($\mu g \times g^{-1}$) were measured using the rapid colorimetric method by Bates et al. (1973). Proline was extracted from 1.0 g of raw tissue by grinding in 10 mL of 3% (v/v) sulfosalicylic acid. The mixture was then centrifuged at 4,000 g for 10 min. In a test tube, 2 mL of the supernatant followed by 2 mL of freshly prepared acid-ninhydrin solution and 2 mL of acetic acid was placed. The tube was incubated in a boiled water bath for 1 hour and the reaction was terminated in an ice-bath. Then the reaction mixture was extracted with 4 mL of toluene and shook for 20 sec. After the separation, the toluene phase was collected into a test tube and its absorbance was read at 520 nm. Proline concentrations were determined from a standard curve prepared using analytical grade proline.

Catalase activity determination. Catalase activity (EC 1.11.1.6) was determined by measuring the rate of H_2O_2 conversion to O_2 at room temperature and the result was presented in $mcatal \times mg^{-1}$ protein (Goth, 1991). Plant homogenate with a volume of 0.1 mL, obtained as previously described, was added to 2.0 mL of 0.03% H_2O_2 and incubated for 5 min. The reaction was terminated by 1.0 mL of 4% ammonium molybdate solution. The absorbance of the colored complex was read at 410 nm. Catalase activity was calculated using its extinction coefficient 22,400 $mM^{-1}\ cm^{-1}$. The protein content in plant homogenate was measured by Lowry O.H. et al. (1951) with a help of Pholine reagent.

Alanine- and aspartate- aminotransferase activities determination. The activity of aminotranferase was determined by the colorimetrical method, with 2,4-dinitrophenyl-hydrazine in some modifications, the results being expressed in $\mu mol \times h^{-1} \times mg^{-1}$ of protein (Reitman & Frankel, 1957; Sato et al., 2016). The substrate (0.25 mL) was incubated with 0.05 mL of homogenate at 37 °C for 1 hour. For ALT (EC 2.6.1.2) determination, substrate contains: phosphate buffer 0.1 M, DL-a-alanine 0.2 M, 2-oxoglutarate 2 mM; and for AST (EC 2.6.1.1) determination substrate contains: phosphate buffer 0.1 M, L-aspartate 0.1 M, 2-oxoglutarate 2 mM. The reaction was terminated by adding 0.25 mL of 1mM 2,4-dinitrophenylhydrazine solution in 1M HCl. After 20 min, 2.5 mL of 0.4 M NaOH solution was poured and the mixture was incubated for 10 min again. The absorbance was read at 540 nm.

Statistical analysis

The data were analyzed using Analysis of Variance (ANOVA). All measurement represents the means and standard error (± SE) of five replicas. Statistically significant differences between means were compared at the 0.05 probability level by t-Student's test. Pearson's correlation test was conducted to determine the correlations between biochemical parameters (Edwards, 1976) and correlation coefficient values were determined using Excel.

RESULTS AND DISCUSSION

The water uptake of maize kernels during the soaking process was uneven. In the first hours, the kernels absorbed water vigorously, but as the kernels were saturated with water, the process slowed down. The required humidity was reached slowly in the last hours. Achieving the required humidity occurs after 24–30 hours. As known, the initial water uptake is carried out by seed shells that have a large number of capillaries, pores, and voids serving as a reservoir for the primary water accumulation. Then water

penetrates the seminal membranes, the germ, and the aleurone layer and is bounded by proteins and carbohydrates strongly. Further movement of water is directed inside the endosperm (Agarry et al., 2014).

Maize seeds, germinated on water, are characterized by the fastest water absorption in compare with seeds germinated on PEG-1500 solutions during the first day (Fig. 1).

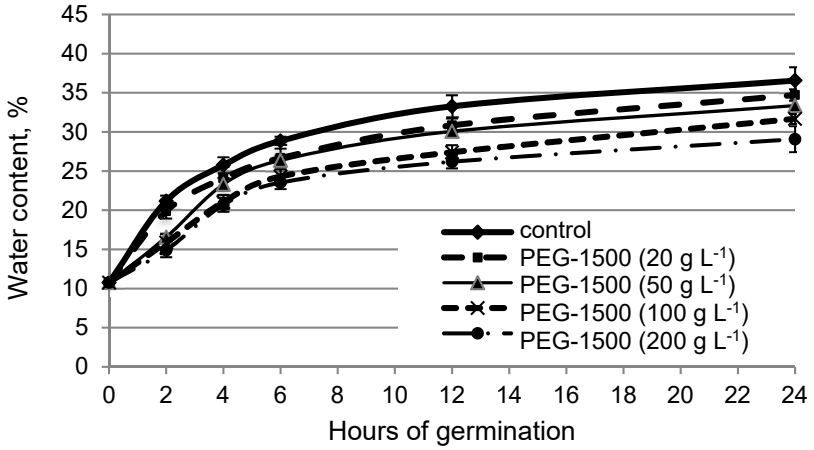

Figure 1. Changes in maize seeds water content during germination under different water deficit. Vertical line – standard error of mean.

Water absorption intensity was decreased while maize seeds germinated under water deficit conditions. Moreover, the reduction of seeds soaking directly depended on the degree of water potential depression in the substratum. A reduction in soaking was observed 2 hours after germination had begun. The most active inhibition of soaking process (up to 7.5%) was observed in seeds that were growing in the solution of PEG-1500 (200 g L^{-1}). The results indicate the formation of water deficit during maize seeds germination under the PEG solutions.

It is known that water absorption is the initiating factor of seed germination. The depression of medium water potential slows the physiological processes of reserve substances hydrolysis and enzymatic activity, which inhibits the activity of the embryonic axis ultimately (Khodarahmpour, 2011; Badr et al., 2020).

The next phase of germination is characterized by the seeds' transition to a saturated steady state water supply. The water content of maize kernels was increased by 25.9% in the control group by the end of the first day of germination. At the same time, the water content of maize kernels germinated on the PEG-1500 (200 g L^{-1}) increased only by 18.3% compared to the SDW. Certainly, differences in seeds water uptake while germination processes cause some peculiarities in metabolic pathways of germinated maize and formation of general resistance of plant (Liu et al., 2015).

The nature of ontogenetic peculiarities of lipid peroxidation (LPO) processes intensity is complex and depends on many factors: the availability of substrate, the quality of the substrates, germination conditions, etc. The LPO processes and antioxidant protection of plants depend on the strength and validity of adverse factors. The data presented in Table 1 show that TBARS content is increased in all of the studied treatments during the six hours after the maize seeds germination has started.

The TBARS content in the endosperm of seeds increased in 1.75–1.89 times during 6 h after sowing under the water deficit. It is necessary to mention, that TBARS level in the seeds` endosperm, which was incubated in water, reached its maximum (7.31 μmol MDA·g^{-1}) in 2 h after sowing and then the TBARS content reduced.

Table 1. TBA-reactive substances content in germinated seeds of maize under water deficit (μmol MDA·g^{-1}, mean ± *SE*)

Time, h	Treatments				
	H_2O	PEG-1500 20 g L^{-1}	PEG-1500 50 g L^{-1}	PEG-1500 100 g L^{-1}	PEG-1500 200 g L^{-1}
0	3.99 ± 0.04	3.89 ± 0.03	4.10 ± 0.06	4.06 ± 0.13	3.87 ± 0.04
2	7.31 ± 0.14	5.72 ± 0.43*	5.81 ± 0.35*	6.84 ± 0.10	4.85 ± 0.22*
4	5.05 ± 0.19	5.42 ± 0.04	5.68 ± 0.12	6.57 ± 0.15*	6.15 ± 0.20*
6	5.70 ± 0.18	7.00 ± 0.02*	7.26 ± 0.14*	7.54 ± 0.04*	7.37 ± 0.13*
12	2.83 ± 0.08	3.78 ± 0.03*	4.03 ± 0.03*	4.11 ± 0.04*	3.96 ± 0.06*
24	4.35 ± 0.17	4.55 ± 0.08	5.18 ± 0.22*	5.21 ± 0.07*	4.70 ± 0.10

* compare to control (H_2O treatment) ($P < 0.05$).

The intensity of peroxidation process was decreased in the maize seeds of all PEG-1500 treated groups from 6 h up to 24 hours after the germination starts. The TBARS content in seeds incubated on PEG solutions of different concentration was higher by 1.23–1.32 times compared to the control group after 6 h of germination. The maximum increase of TBARS content in the maize seeds (by 1.45 times) was recorded under 100 g L^{-1} PEG-1500 treatment in 12 h after sowing. The intensity of LPO in seeds was at the range of 4.35–5.21 μmol MDA g^{-1} under all examined treatments at the end of the first day of germination. Generally, it was noted, that TBARS level in the endosperm of seeds was found higher than the control ones during the first day of germination under PEG-1500 solutions.

The data illustrated in Fig. 2 indicate the significant increase of lipid peroxidation intensity in maize coleoptiles after 3 days exposure in water deficit conditions.

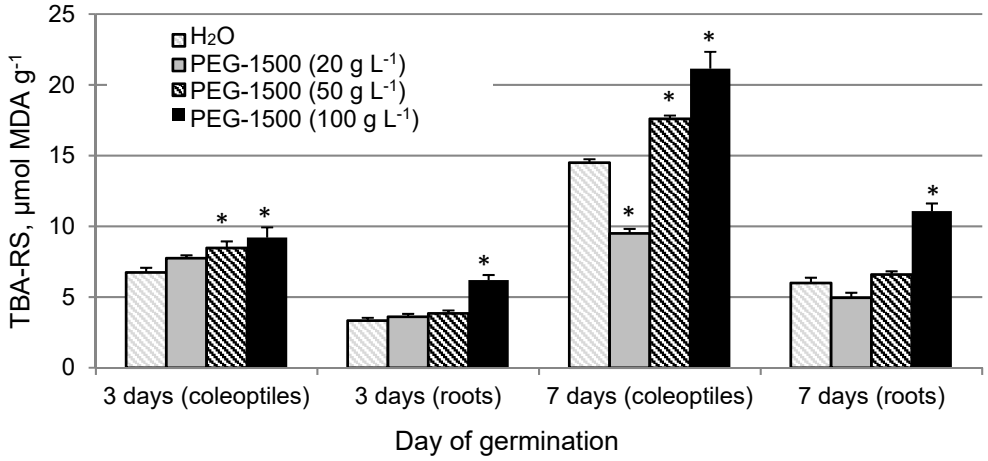

Figure 2. TBA – reactive substances content in coleoptiles and roots of maize under water deficit. Vertical line – standard error of mean; * compare to control (H_2O treatment) ($P < 0.05$).

The rising of TBARS content in seedlings tissues under water deficit gives evidence of a higher oxidative metabolism level in their cells compared with plants that germinated under normal water supply (Pyngrope et al., 2013; Anjum et al., 2017a). Moreover, the level of PEG-1500 (100 g L^{-1}) solution was found to be the most active, increasing TBARS content in maize coleoptile by 36.5% compared to the control. In addition, the TBARS content in 3 days maize roots was almost 2 times higher than in the one germinated on water under the influence of PEG-1500 (100 g L^{-1}).

The embryonic axes organs of 7-days seedlings were characterized by a high content of peroxidation products compared to the previous period of ontogenesis. TBARS level of maize coleoptiles and roots continued to increase significantly under the influence of PEG-1500 solution in concentrations of 50 and 100 g L^{-1}. Thus, TBARS content in coleoptiles and roots of 7-days maize seedlings increased by 46.2% and 84.8% respectively, under PEG-1500 (100 g L^{-1}) and compared with control seedlings. Any significant alteration in the intensity of LPO in 3 days embryonic axis maize organs were indicated under the influence of PEG-1500 (20 g L^{-1}). Although, the intensity of LPO in 7 days maize seedlings was even lower ($P \leq 0.05$) than control values under the light water deficit.

The nature of LPO processes intensification and functioning of antioxidant system depends to a large extent on the strength and duration of the unfavorable factor (Labudda, 2013; Killi et al., 2020).

It should be noted that the proline content in maize seeds decreased in the control variant by 28,6% during the first 4 hours of germination (Table 2).

Table 2. Proline content in germinated seeds of maize under osmotic stress (μg·g^{-1}, mean ± *SE*)

Time, h	Treatments				
	H_2O	PEG-1500 20 g L^{-1}	PEG-1500 50 g L^{-1}	PEG-1500 100 g L^{-1}	PEG-1500 200 g L^{-1}
0	18.89 ± 0.37	18.15 ± 0.35	19.45 ± 0.50	19.50 ± 0.85	18.94 ± 0.46
2	17.77 ± 0.41	17.70 ± 0.40	16.92 ± 0.62	16.50 ± 0.69	20.30 ± 0.81*
4	13.54 ± 0.35	13.54 ± 0.41	18.61 ± 0.56*	16.50 ± 0.63*	19.88 ± 0.75*
6	16.92 ± 0.55	16.90 ± 0.43	18.61 ± 0.68	21.15 ± 0.95*	21.57 ± 0.92*
12	19.88 ± 0.93	20.73 ± 1.05	22.00 ± 0.88	22.10 ± 1.12	22.20 ± 1.06*
24	21.15 ± 0.98	24.53 ± 0.95	24.75 ± 1.02*	24.11 ± 1.38	25.38 ± 1.11*

* compare to control (H_2O treatment) ($P < 0.05$).

However, the proline content in seeds varied slightly over the period under the influence of osmotic stress. Further, the proline content increased in the maize seeds of all groups up to the 24-hour germination period. Seeds were characterized by a consistently higher proline content compared to control seeds during the first germination day under water deficiency conditions. Thus, the proline content of maize seeds, which were treated with PEG solutions of different concentrations during the first day, exceeded the control values by 14–20% ($P \leq 0.05$).

The entry of water into the cells is the result of a concentration gradient. The accumulation of osmolytes precedes the phase of cell expansion in the germinal axis, when roots and seedlings are forming. The accumulation of osmolytes and proline, in particular, continues to support the water flow to the cell in order to keep the osmolality of the cell matrix (Naser et al., 2010; Anjum et al., 2017b). Therefore, the gradation of

the osmotic stress influence was observed more evidently in the organs of the maize germinal axis.

The proline concentration increased by 1.44 and 1.19 times in coleoptiles and roots of 3-day maize germinated under PEG-1500 solution (20 g L^{-1}), respectively (Fig. 3).

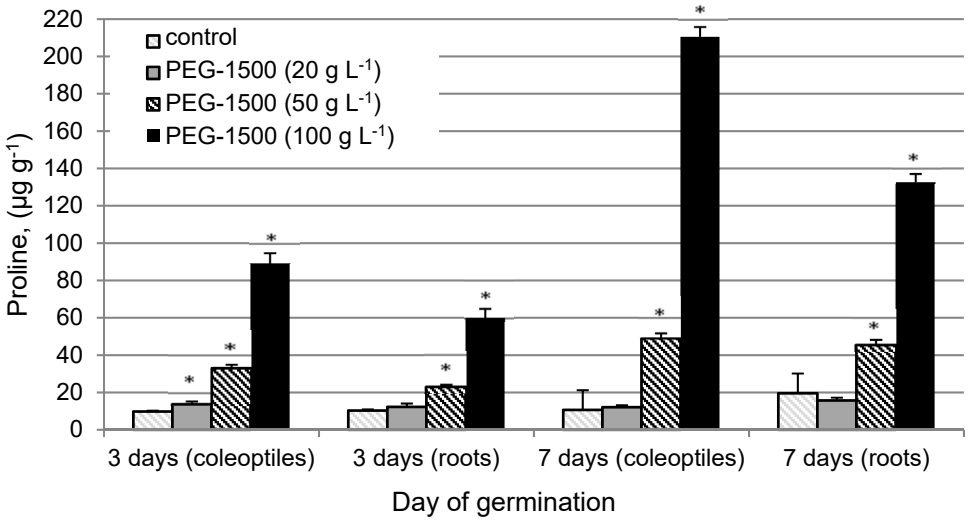

Figure 3. Proline content in coleoptiles and roots of maize under water deficit. Vertical line – standard error of mean; * compare to control (H_2O treatment) ($P < 0.05$).

Whereas, the proline content increased by 9.2 and 5.9 times respectively in the experimental tissues under the action of PEG-1500 (100 g L^{-1}) solution and compared with the control seeds. A similar trend was observed in 7-day seedlings of maize, when excessive activation and accumulation of proline in coleoptiles and roots were recorded, the content of which one was in 19.8 and 6.8 times higher than the concentration of proline in control seedlings, respectively.

A close direct correlation ($r = 0.86 \div 0.91$) between PEG-1500 concentration (osmotic potential) and tissue proline content was found out.

The accumulation of osmotic active substances is a universal adaptive response of plant organism to osmotic stress. Low molecular weight compounds and amino acids, in particular, provide the regulation of osmotic potential, the detoxification of free ammonia, and the normalization of energy metabolism under water deficiency conditions. Proline is referred to the so-called 'stress' amino acids, and the ability of plants to accumulate proline provides their osmotolerance (Tarighaleslami et al., 2012; Hosseinifard et al., 2022). The obtained data confirm the research on different varieties of maize, where it was shown that drought stress had a negative impact on chlorophyll and proline content. (Pawar et al., 2020).

In a series of papers (Raymond & Smirnoff, 2002; Verslues & Sharma, 2010), the synthesis of proline by the glutamate synthase pathway during the stress reaction was proved. It was found that there is a direct correlation between the content of proline and TBARS in germinating maize tissues. The correlation coefficient between these indexes is stronger in the maize roots ($r = 0.95$) than in the coleoptiles ($r = 0.70$) of 3- and

7-days seedlings. This fact probably indicates a higher adaptive capacity of the maize root system in the early stages of germination to osmotic stress.

Antioxidant system plays a key role in eliminating oxidative stress products. Catalase is one of the key enzymes involved in plant organism protection from free radical oxidation of biomolecules (Gomes & Garcia, 2013; Mafakheri et al., 2019).

The initial stage of maize germination is marked by enzymatic activity increased due to the hydro-stimulating initiation of protein complexes. It was observed an increase of CAT activity in the control group from 0.688 to 1.912 mcatal·mg^{-1} protein during the first 24 hours from seeds sowing (Table 3).

Table 3. Catalase activity in germinated seeds of maize under water deficit (mcatal·mg^{-1} protein, mean ± *SE*)

Time, h	Treatments				
	H_2O	PEG-1500 20 g L^{-1}	PEG-1500 50 g L^{-1}	PEG-1500 100 g L^{-1}	PEG-1500 200 g L^{-1}
0	0.688 ± 0.008	0.675 ± 0.010	0.693 ± 0.009	0.680 ± 0.011	0.626 ± 0.007
2	1.237 ± 0.019	1.396 ± 0.011*	1.412 ± 0.010*	1.291 ± 0.005	1.190 ± 0.005
4	1.047 ± 0.055	1.290 ± 0.027*	1.231 ± 0.020*	1.324 ± 0.006*	1.225 ± 0.026*
6	1.466 ± 0.023	1.524 ± 0.006	1.524 ± 0.013	1.754 ± 0.014*	1.818 ± 0.071*
12	1.224 ± 0.002	1.372 ± 0.012*	1.861 ± 0.021*	2.834 ± 0.037*	2.859 ± 0.079*
24	1.912 ± 0.019	1.756 ± 0.057	2.035 ± 0.059	2.236 ± 0.057*	2.578 ± 0.075*

* compare to control (H_2O treatment) ($P < 0.05$).

Changes in seeds CAT activity varied from 3.8 to 14.1% after 2 hours of incubation under the influence of PEG-1500 solutions compared with control. CAT activity in maize seeds of all PEG-1500 treated groups exceeded activity one in the control group after 4 hours from sowing (from 17 to 26%) and after 6 hours (from 4 to 24%). A significant increase of CAT activity in 1.20 and 1.24 times ($P < 0.05$) was proven only under the 100 and 200 g L^{-1} PEG-1500 treatment after 6 hours from seeds sowing, respectively. The maximum increase of CAT activity by 1.24, 2.34 and 1.35 times ($P < 0.05$) was recorded under PEG-1500 (200 g L^{-1}) treatment at 6, 12, and 24 hours from seeds sowing, respectively, and compared with control.

The increase in CAT activity is consistent with the increase of the TBARS content values in endosperm during the 12 hours of germination and can be considered as an adaptive response to the effect of osmotic stress.

PEG-1500 (20 g L^{-1}) solution caused a decrease in coleoptiles CAT activity of both 3- and 7-day seedlings of maize. A non-significant decrease of CAT activity in coleoptiles is associated with a decrease of LPO processes under the influence of PEG-1500 (20 g L^{-1}) solution, which does not create a pronounced effect of water deficit. Maize roots are more sensitive to water stress. An increase of CAT activity in 3- and 7-days maize roots was recorded even under PEG-1500 (20 g L^{-1}) solution. Miller et al. (2010) research believe that the change in CAT activity can be explained by the change in the content of hydrogen peroxide in coleoptiles. It was noted, that hydrogen peroxide accumulates under water stress in plant tissues (Kang et al., 2022). The activation of CAT in coleoptiles and maize roots, incubated with PEG-1500 solutions of higher concentrations, was noted. The highest stimulation of CAT activity was observed under the effect of PEG-1500 (100 g L^{-1}) solution. Thus, CAT activity in 3- and 7-days

maize coleoptiles increased in 1.8 and 1.4 times ($P \leq 0.05$), respectively, under water deficit. A sharp leap of CAT activity in roots was registered when activity one increased in 13.7 and 12.3 times ($P \leq 0.05$) (Fig. 4).

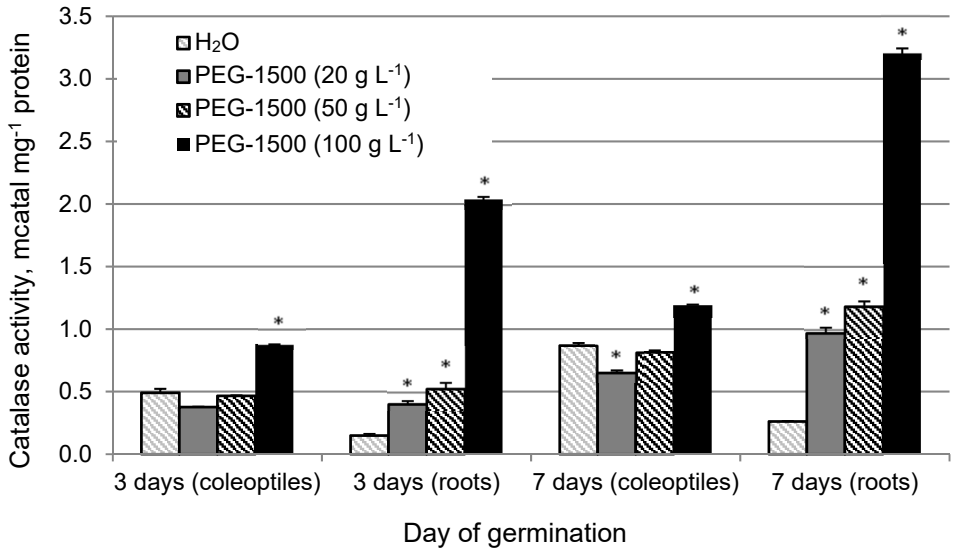

Figure 4. Catalase activity in coleoptiles and roots of maize under water deficit. Vertical line – standard error of mean; * compare to control (H_2O treatment) ($P < 0.05$).

Plants have the ability to avoid oxidative stress and may efficiently scavenge harmful ROS by up-regulating antioxidant enzymes, not only CAT but SOD, POX, and APX (Yang et al., 2021). Moharramnejad et al. (2019) reported a higher level of CAT in drought stressed maize seedling, which coordinates with SOD, POX activity. Numerous studies have reported that higher levels of antioxidants are associated with plant drought tolerance (Habibi & Hajiboland, 2011; Ashraf, 2016; Moharramnejad et al., 2019).

Probably, one of the dominant regulator of LPO processes is catalase or peroxidase in the root system, whereas in the aboveground part of plant, the regulation of peroxidation processes is carried out by low molecular weight antioxidants (Eriyamremu & Lolodi, 2010). Excessive activation of CAT in maize roots compared to coleoptiles allows keeping the TBARS content in roots at lower level than in coleoptiles under the condition of model water deficit. Similar activation of CAT was recorded in model experiments with water deficit of plants (Rafiee et al., 2011; Anjum et al., 2017a; Moharramnejad et al., 2019).

There is a high correlation between the content of peroxidation products and CAT activity in coleoptiles ($r = 0.85$) and roots ($r = 0.89$) of 3- and 7-days maize seedlings. The analysis of these dependences indicates that the root adaptation response was more active than the coleoptiles one under water deficiency.

The effect of any external factors is reflected in the protein metabolism in plants, which is associated with the ALT and AST enzymes. Alanine aminotransferase catalyzes the reversible reaction of the conversion of alanine and 2-oxoglutarate into pyruvate and glutamate. Aspartate aminotransferase catalyzes the reversible transfer of an α-amino

group between aspartate and glutamate. The aminotransferase activity of maize seed endosperm increased during the first germination day. The seeds` ALT activity increased in 2.65 times and AST activity - in 1.58 times (Fig. 5).

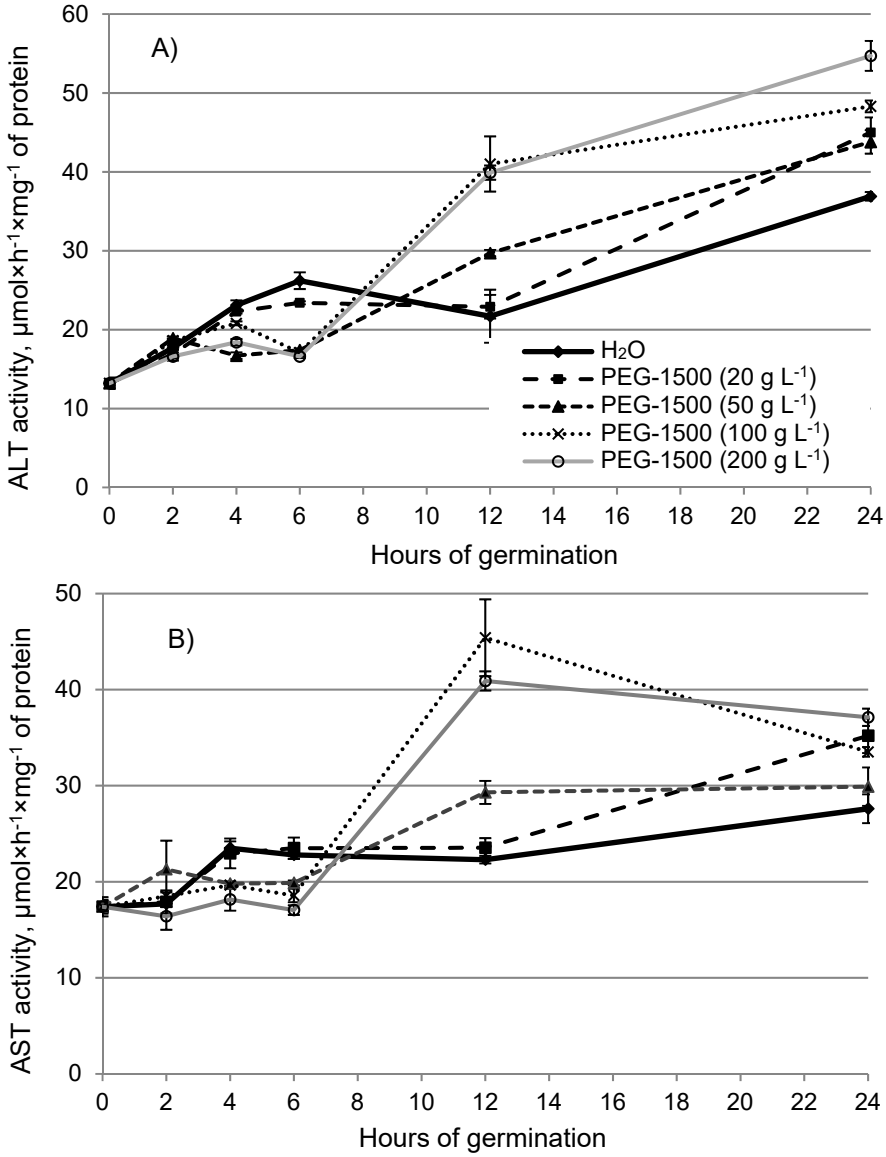

Figure 5. Alanine– (A) and aspartate– (B) aminotransferase activities in germinated seeds of maize under water deficit. Vertical line – standard error of mean.

The activity of aminotransferases under water deficiency was slightly lower than the control parameters during the phase of seed swelling. In the next phase of seed ringing, intensive division and stretching of germinal cells begins, accompanied by an increase in the aminotransferase activity of the seed endosperm under the condition of

water deficiency. Moreover, the rise of ALT and AST activity of maize endosperm had a direct correlation with the osmotic potential of PEG-1500 solutions during the 12- and 24-hour seed germination periods. It was marked a close correlation ($r = 0.76 \div 0.98$) between AST and ALT activity of maize seed endosperm during the first day of germination.

The obtained data indicate that the exposure of seedlings caused a significant increase in ALT activity under conditions of model water deficit (Fig. 6).

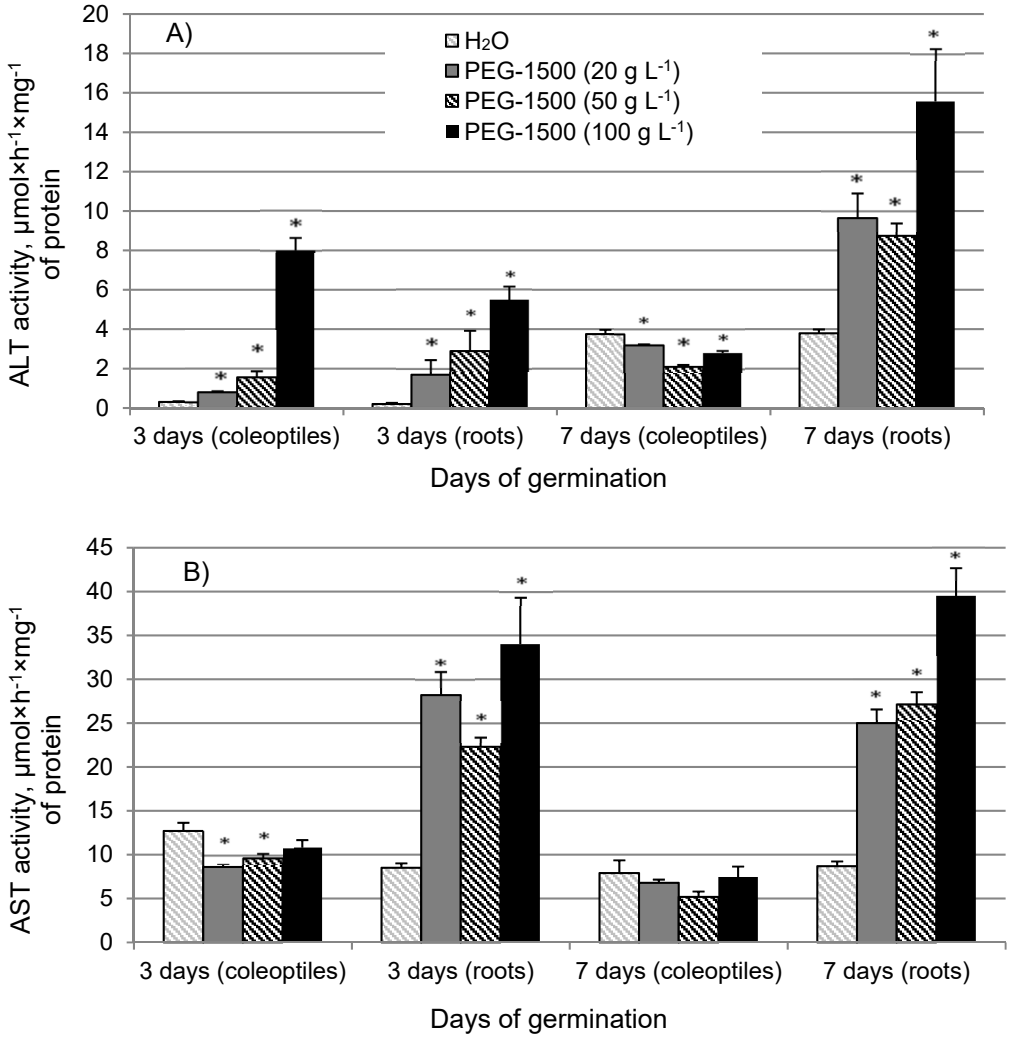

Figure 6. Alanine– (A) and aspartate– (B) aminotransferase activities in coleoptiles and roots of maize under water deficit. Vertical line – standard error of mean; * compare to control (H_2O treatment) ($P < 0.05$).

The most active stimulation of ALT activity in 3-day maize coleoptiles was observed in 25.8 times and roots - in 27.4 times when incubated in PEG-1500 (100 g L^{-1}) solution. It should be noted that the activity of ALT remains high in maize roots in 4.1

times, compared with control seedlings after 7 days seed germination under the water deficit, while some inhibition of the activity of this enzyme in the coleoptile was detected. Pyruvic acid, which is formed during the ALT reaction, is a necessary substrate for gluconeogenesis, that's why the activation of this enzyme contributes to the carbohydrate synthesis activation, which is required for further root growth. The accumulation of glutamate is a result of increased activity of transaminases, which allows to direct metabolic transformations towards the formation of proline, which together with carbohydrates is an important osmoprotector in water stress conditions (Kendziorek et al., 2012; Mostafa et al., 2021).

AST activity of 3- and 7-days maize roots incubated in PEG-1500 solutions exceeded its activity in the roots of control seedlings significantly. Thus, the activity of AST in the roots increased in 2.9–3.3 times even under the influence of PEG-1500 (20 g L^{-1}) solution. The maximum AST activation in 4.0–4.6 times was noticed in the maize roots when incubated in 10% PEG solution. However, the stimulation of AST enzymatic activity in maize coleoptiles under water stress was not observed and remained at the control level. There was a closer correlation between ALT and AST activities ($r = 0.97$) in the weekly maize roots than in coleoptiles ($r = 0.76$) under the effect of different strength water deficit.

ALT and AST play a key role in the metabolism of alanine, aspartate and glutamate, from which asparagine and glutamine are synthesized. Therefore, increasing of their activity is a prerequisite for the amide pool accumulation, as NH^{4+} donors for protein synthesis (Beatty et al., 2013). There is a lack of sufficient nitrate in maize seeds, so asparagine and glutamine may be the only source of ammonium while germination. On the other hand, keto acids that are formed in transamination reactions are also required for the renewal of the free amino acid pool and are necessary for the growth and seedlings development (Hildebrandt et al., 2015; Yu et al., 2015; Xin et al., 2018).

This fact is confirmed by the morphometric indicators of maize seedlings (Liu et al., 2015). Water deficiency adversely affected the germination of maize seeds, so the germination energy and laboratory germination of the seeds declined monotonically and according to the increased osmotic potential of the incubated solution. The most significant reduction of maize germination energy by 56.1% and laboratory germination by 50.0% was observed under PEG-1500 (100 g L^{-1}) effect (Table 4).

Table 4. The germination rate of seeds and biometrical parameters of maize's coleoptiles and roots under water deficit, (mean ± *SE*)

Treatments	Energy of germination, %	Laboratory germination, %	Raw weight of 100 un, g		Length of coleoptiles, cm
			coleoptiles	roots	
control H_2O	86.40 ± 3.24	94.17 ± 0.83	10.56 ± 0.08	13.36 ± 1.84	3.58 ± 0.09
PEG-1500 (20 g L^{-1})	87.78 ± 3.04	93.33 ± 1.36	7.97 ± 0.93*	14.12 ± 1.80	3.04 ± 0.12
PEG-1500 (50 g L^{-1})	74.44 ± 3.77*	81.67 ± 2.15*	2.77 ± 0.10*	7.70 ± 0.51*	1.31 ± 0.02*
PEG-1500 (100 g L^{-1})	30.28 ± 6.14*	44.17 ± 2.85*	1.70 ± 0.30*	3.12 ± 0.05*	0.48 ± 0.03*

* compare to control (H_2O treatment) ($P < 0.05$).

The drought induced by PEG is due to osmotic stress resulting from its higher molecular weight than any other similar compounds. The maize seeds cultivated on PEG-1500 (200 g L^{-1}) solution didn't emerge at all, although the swelling of the seeds was observed. It should be noted, that a similar fact was recorded when using PEG-6000 (Maga et al., 2019; Raj et al., 2020).

Osmotically dependent inhibition of growth processes in maize seedlings under water deficiency was observed. If the raw weight of maize coleoptiles is decreased by 24.5% under the influence of PEG-1500 (20 g L^{-1}) solution, the raw weight of roots is decreased by 42.4% only when incubated with PEG-1500 (50 g L^{-1}) solution. The water deficit solution leads to 6.2 times decrease of the raw weight of the coleoptile, and to 4.3 times decrease in the root's raw weight while using PEG-1500 (100 g L^{-1}).

The results of the study indicate a negative correlation between the germination rate of maize seeds and the intensity of peroxidation, the accumulation of proline, and enzyme activity. The inhibition of the growth processes of maize, cereals seedlings, and fruit trees under drought conditions has been shown in a number of papers (Khodarahmpour, 2011; Golabadi et al., 2015; Gerasko et al., 2019; Pawar et al., 2020).

CONCLUSIONS

In conclusion, the development of oxidative stress was observed under conditions of model water deficiency in maize seedlings. The most active inhibition of the soaking process (up to 7.5%) was observed in seeds that were growing in the solution of PEG-1500 (200 g L^{-1}). From the above findings, it can be concluded that TBARS level in the endosperm of seeds, roots and coleoptiles of maize was found higher under water stress affect. The proline content in maize seeds, roots, and coleoptiles maximum exceeded the control values when PEG-1500 (100 and 200 g L^{-1}) was used. It was confirmed that the activation of catalase in coleoptiles in 1.4–1.8 times and roots of maize seedlings in 12.3–13.7 times under water deficit. ALT and AST activities of maize seeds and roots exceeded their activity in the control seedlings significantly under PEG-1500 treatment. These changes should be seen as an adaptive strategy to water deficit.

The result also shows that an increase in water stress level decreased the germination percentage, raw weight of coleoptiles and roots, and coleoptile length of maize. The intensity of maize growth processes in the early stages of development correlates with the level of water deficits. Generally, the maximum reduction in germination, coleoptile length, raw weight of root and coleoptile was observed in the highest water stress given.

The results of this study reveal that further research is required to determine the responses of plants under environmental stresses because plants live simultaneously under the effect of multiple stress factors in natural habitats or fields.

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