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DYNAMICS OF SEED GERMINATION, SEEDLING GROWTH AND PHYSIOLOGICAL RESPONSES OF SWEET CORN UNDER PEG-INDUCED WATER STRESS

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Abstract

Stress induced variations in seed germination of various crops has been well reported but germination potential of sweet corn seeds under osmotic stress with relation to time dynamics is still elusive. Present study explored the water absorption, germination potential and physiological indices and of sweet corn seeds exposed to five different levels of PEG-induced water stress i.e., 0, –0.3, –0.6, –0.9 and –1.2 M Pa water potential (Ψ_w) with respect to time dynamics. Results showed that enhanced water stress for prolonged time period (96 h) led to substantial reduction in water absorption and seed moisture contents, seed germination and vigor index as well as seedlings growth and fresh and dry biomass. Osmotic stress triggered antioxidant defense system like super-oxide dismutase (SOD), peroxidase (POD) and catalase (CAT) and accumulation of soluble sugars, proline and protein contents considerably. Initially, activities of SOD and CAT were higher but then reduced as stress persisted, however, POD showed a linear increase with respect to stress exposure time. Water stress also increased MDA contents up to 36 h then declined. Further, α-amylase activity and soluble protein showed significant correlations with maize seed germination. Overall, germination potential decreased with increase in osmotic stress in sweet corn seeds.

Key words: Anti-oxidants, Corn, Seed germination, Water potential.

Introduction

Water stress is one of the major implications in plant production systems that negatively affect agricultural productivity. Imposition of cyclic or erratic drought spells limited the agricultural productivity to one-third of the world's arable land (Chaves & Oliveira, 2004). Among other abiotic stresses, water stress is the single most important factor that limits crop yields by 50% or over (Wang *et al.,* 2003). It affects plant in multiple ways; however, roots are the first to respond through chemical signaling via stem to leaves to induce stomatal aperture regulations to avoid further water loss (Schachtman & Goodger, 2008). It further imbalances the plant water relations from cell to organ levels and disrupts important plant structures (Farooq *et al.,* 2009a, b). Imposition of water stress lowers the soil water potential, thus halted plant growth and development from seedlings to harvest, induced alterations in plant physiological mechanisms and reduced fresh and dry plant biomass (Hu *et al.,* 2007; Ashraf *et al.,* 2016). Earlier growth stages are more vulnerable to drought-induced damages as it affects cell division and differentiation activities (Anjum *et al.,* 2003). Drought induced significant reductions water absorption in maize seeds that led to poor germination and seedling growth (Achakzai, 2009).

In response to limited water conditions, plant produces secondary metabolites and other organic or inorganic solutes in order to maintain cell water status and to protect its structural and functional activities (Seki *et al.,* 2007). Production of osmolytes especially proline not only involved in osmotic adjustments but also provide protection to sub-cellular structures by scavenging free radicals and buffering cellular redox potential (Ashraf & Foolad, 2007; Ashraf *et al.,* 2015; George *et al.,* 2015). Furthermore, induction of oxidative stress via generation of reactive oxygen species (ROS) may cause cell death by deactivating the cell metabolic activities and targeting the cellular structures and important cell organelles (Mittler, 2002). However, plants have established well-organized antioxidant defense systems to survive even under stress conditions and to avoid oxidative damage. The cleansing of ROS is directly related to productions of antioxidants both enzymatic like superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) and some other non-enzymatic like phenolics, carotenoids, proline, anthocyanins etc. (Ashraf, 2009; Weng *et al*., 2015; Ashraf *et al*., 2017).

Sweet corn seed response to drought stress is necessary for both qualitative and quantitative estimation of morpho-physiological and biochemical response at germination stage. However, little research has been done on water exchange and absorption in sweet corn seeds and their early response concerning time dynamics under water stress conditions. So, this study was therefore planned to investigate the seed germination and growth response, diffusional water transport in intact seeds and modulation of some physiological characteristics in sweet corn seeds experiencing osmotic solution mediated water stress with respect to time dynamics. Additionally, consequences of osmotic blockade due to PEG-induced water stress at seed germination stage might be helpful to assess the germination loss and poor stand establishment of maize under actual field conditions with reduced soil moisture contents and soil water deficit.

Materials and Methods

Treatment and growing condition: Seeds of '*Zea mays* L. cv Zhengtian 68' were collected from Guangdong JinZuo Agricultural Science and Technology co., LTD, Guangzhou, China) having ≥85% initial germination with ≤13.0% moisture content. Polyethylene glycol (PEG) with molecular weight of 8000 g mol^{-1} was obtained from Biosharp, Wuhan, China was used to made solutions with different water potential. Twenty homogenous seeds of the sweet corn were placed in each sterilized petri dish with 10 ml solutions having five levels of water potential (Ψ_w) i.e., 0, −0.3, −0.6, −0.9 and −1.2 MPa denoted by T0, T1, T2, T3 and T4, respectively. Previously, Khayatnezhad *et al*. (2010) used 0, −0.2, −0.4, −0.6 and −0.8 MPa while 0.1, −0.2, −0.4, −0.6 MPa water potential were also studied by Zhao *et al*. (2014) regarding their effects on maize seed germination, however, this water stress conditions were still not in a wide range, therefore, we used a bit wide range PEG concentration (0 to −1.2 MPa) for investigating the dynamics in maize seed germination under a wide range of water stress with respect to time. The solution concentrations were prepared as described by (Michel *et al.*, 1983). Solutions were applied after every 48h. All the treatments were placed at room conditions (25ºC) with light/dark period of 12/12 h and relative humidity of 50±5%.

Determination of water absorption: The water absorption of the seeds was measured at 2, 4, 6, 8, 10, 12, 24, 36, 48, 96 h after immersion. After attaining certain times, the seeds were removed from the petri dishes and placed on a paper to exclude excess water and weighed. The water absorption duration was measured according to McWatters *et al.* (2002):

$$Wa = \frac{Wf - Wi}{Wi} \times 100$$

where, *Wa* is water absorption (%), *Wi* and *Wf* is weight of seeds (g) before and after immersion, respectively.

Seed germination and morphological characters: At 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6 and 7days after sowing (DAS), the germination rates were measured with three replicates. Germination was conceived to have ensued when the radicals were half of the seed length. Germination index (C) (Anon., 1983), and vigor index were calculated according to Li *et al.* (2014). Seedlings were harvested at 7 DAS and shoot length and fresh weight were measured immediately after seedling harvest and then dried in oven at–80ºC to record dry weight.

Physiological characters: At 12, 24, 36, 48, 72, 96 h after sowing (for T0, the sampled time was only at 12, 24, 36, and 48 h), the seeds from their respective treatments were sampled for measurement of the physiological characters.

The α-amylase activity was measured according to the method of Zou (2000).The absorbance of the supernatant was measured at 620 nm. Proline contents were measured by method of Bates *et al.* (1973) using ninhydrin. The soluble protein content was measured based on the method of Bradford (1976) using G-250, and expressed as mg/g. The soluble sugar and antioxidant enzyme activities (superoxide dismutase (SOD), peroxidase (POD,) and, catalase (CAT) were measured according to Chen & Wang (2006). The SOD activity was measured using nitro blue tetrazolium (NBT) method, after reaction, the colour change was measured at 560 nm, and one unit of SOD activity is equal to the volume of extract needed to cause 50% inhibition of the colour reaction. For POD activity, enzyme extract (50 µl) was added to the reaction solution system containing 1 ml 0.3% H_2O_2, 0.95 ml 0.2% guaiacol, and 1 ml 50 mmol/L pH 7.0 PBS, the absorbance change of the brown guaiacol at 470 nm was recorded for calculating POD activity. One POD unit of enzyme activity was defined as the absorbance increase because of guaiacol oxidation by 0.01 (U/g). For CAT activity, enzyme extract (50 µl) was added to the reaction solution system containing 1 ml 0.3% H_2O_2 and 1.95 ml H_2O, the absorbance change at 240 nm was recorded for calculating CAT activity. One CAT unit of enzyme activity is defined as the absorbance decrease by 0.01 (U/g).

The malondialdehyde (MDA) content was measured by the method of Chen & Wang (2006). MDA reacted with thiobarbituric acid (TBA), the absorbance of the reaction solutions was recorded at 532 nm, 600 nm, and 450nm. The MDA content of the reaction solutions was calculated as: MDA content (µmol/L) = $6.45(OD_{532}–OD_{600})–0.56OD_{450}$, and final result of MDA was expressed as µmol/g.

Statistical analysis: Petri dishes were arranged in completely randomized design (CRD) in triplicate. Analysis of variance and correlation analyses were performed using Statistix version 8 (Analytical, Tallahassee, Florida, USA). Mean comparisons were made using the least significance difference (LSD) test at $p<0.05$.

Results and Discussion

Water absorption, germination dynamics and seedling growth of sweet corn seeds: Water stress severely reduced the water absorption of sweet corn seeds. Treatment with lowest water potential (T4) significantly reduced uptake and accumulation of water within the seeds thus hampered seed germination. Overall the seeds water absorption was recorded as: T0>T1>T2>T3>T4. After 48 h, T3 and T4 absorbed less water while T0, T1 and T2 still showed the ability to accumulate much water for seed germination (Fig. 1). Osmotic solutions affected the germination pattern of the seeds of sweet corn. T4 resulted in significant reductions seed germination and seedling growth however, higher water absorption in control led to maximum germination % and seedling growth. Time-induced alterations in germination patter can easily be differentiated in Fig. 2 (A-C), where seeds after 60 h sowing period have developed well than 12h after sowing.

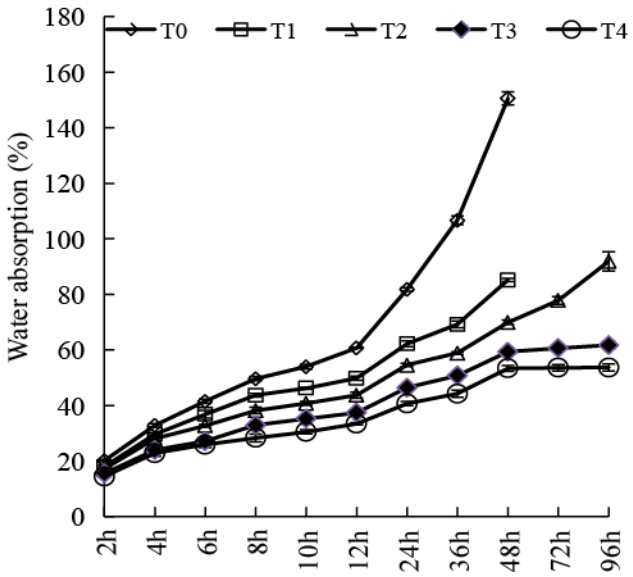

Fig. 1. Dynamics in water absorption of sweet corn seeds under different water stress treatments with respect to their exposure time. Capped bars above means represent S.E. of three replicates. T0: 0 MPa; T1: –0.3 MPa, T2: –0.6, T3: –0.9; T4: –1.2 MPa.

Moreover, osmotic stress modulated the germination period of sweet corn seeds where T0 took minimum time to germinate (~3.5 days) followed by T1 and T2 where seeds took 4, 6 days to germinated, respectively. Furthermore, T3 had less than 20% germinated seeds, while seeds in T4 were not able to even germinate. Thus, the final germination rate of the seeds under water treatments was T0>T1>T2>T3>T4. Moreover, the germination index of the sweet corn seeds was significantly reduced with the levels of water stress. Similarly, water stress at germination stage hampered seed vigor index. The pattern of reduction in vigor index is almost similar to other germination indices, highest in T0 and lowest in T4. Overall, it indicated that threshold value for the water stress for seed germination was around T2, i.e.,–0.6MPa (Fig. 3A-D). Seedling growth and their biomass accumulation were noticeably decreased when seeds were exposed to water stress conditions. Increase in water stress led to signnificant reductions in seedlig height and their fresh and dry weight whilst tallest and healthy seedlings were harvested where seeds were treated with pure water (T0) (Fig. 4 A-C). Loss of seed moisture contents and reduced water absorption rates under osmotic stress possibly due to adverse effect of water stress on aquaporin gating (the most expected mechanism reported in short-term studies) (Ionenko *et al.*, 2012). Reduced amount and functionality of aquaporins on plasma membrane under osmotic stress were also reported some previous experiments (North *et al.*, 2004; Ionenko *et al.*, 2006). However, cellular mechanisms that triggers osmotic stress induced response are still unclear but some scientists explained inhibited activity of water channels at cell membranes through cohesion-tension model where osmolyte exclusion from aquaporin induce tension in it which in turn close the pore by reversible deformation (North *et al.*, 2004; Ehlert *et al.*, 2009). Moreover, PEG-induced reduction in seed germination, seed size and shape abnormalities were also reported by Emmerich & Hardegree (1990).

Enzymatic activities: Water stress in sweet corn seeds induced variations in α-amylase, SOD, POD and CAT activities with respect to time dynamics. For example, in T0, α-amylase activity increased linearly with time from 12 to 48 h afterwards, a sudden increase was observed in seeds exposed to Ψw = –0.3than all other treatments. Poor water absorption in seeds exposed to lowest water potential also apprehended SOD, POD and CAT activities. Pertaining to time period, SOD activity was increase up to 36 h after sowing then declined in all treatments except T0 where maximum SOD activity was observed after 12 h of sowing. Furthermore, a linear increase in POD and CAT activities was noted in all treatments with time but CAT activity was sudden decreased after 72 h of sowing (Fig. 5). Higher activities of SOD at earlier periods reduced O_2^- to H_2O_2, a front line protection against ROS (Alscher *et al.*, 2002) that is further neutralized in to water by POD and CAT activities. Similar findings were also found in the literature (Radhakrishnan & Lee 2013; Mahesh *et al.*, 2013; Huang *et al.*, 2014). Further, prolonged water stress impaired SOD and CAT activities that might reduce their ROS scavenging functions severely, indicating higher capacity of POD than CAT to reduce H_2O_2 engendered by SOD as reported by Khan *et al.* (2002) in the roots of rice plants under salinity stress. Water stress-induced regulations in water relations, photosynthetic pigments, and anti-oxdiants' activities in maize was also reported by Ahmad *et al.* (2017).

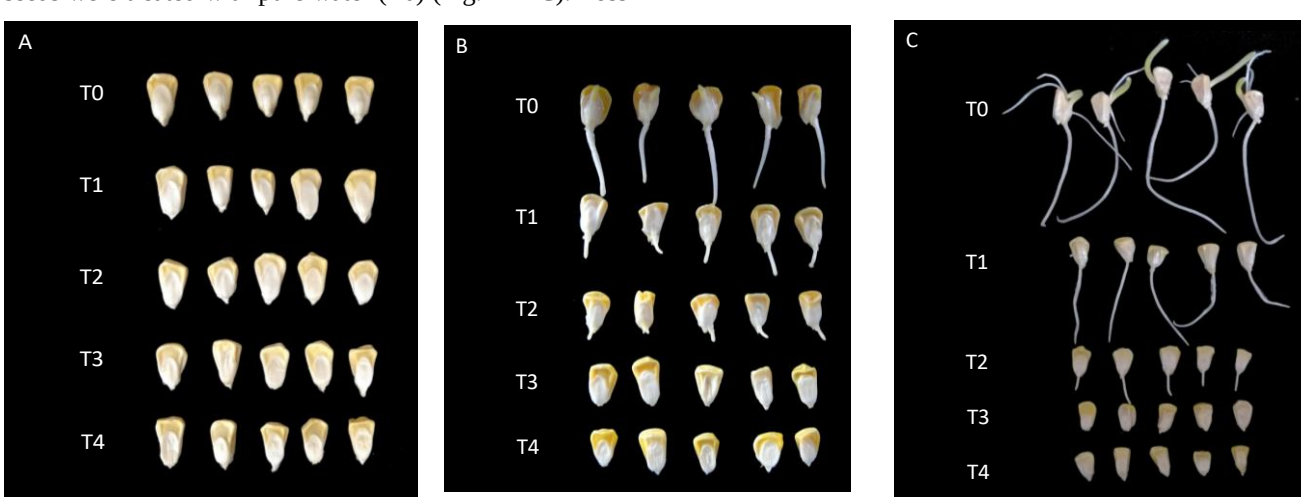

Fig. 2. Appearance of the sweet corn seeds under the influence of PEG-induced water stress. (A) 12h after stress, (B) 36h after stress, and (C) 60 h after stress. T0: 0 MPa; T1: –0.3 MPa, T2: –0.6, T3: –0.9; T4: –1.2 MPa.

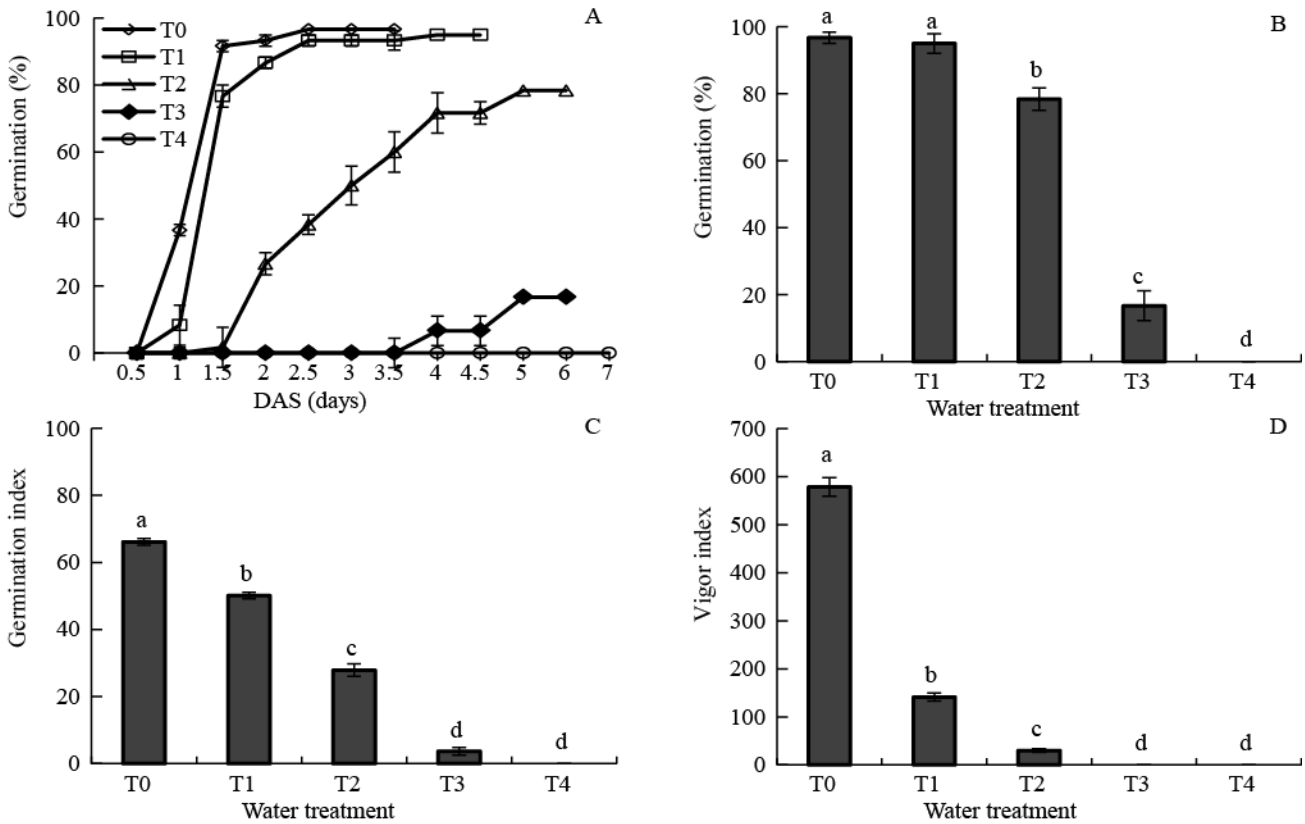

Fig. 3. Dynamics in (A) seed germination, (B) final germination percentage, (C) germination index, and (D) vigor index of sweet corn under different water stress treatments. Different lowercase letters denote statistical differences among treatments at the 5% level according to LSD test. Capped bars above means represent S.E. of three replicates. T0: 0 MPa; T1: –0.3 MPa, T2: –0.6, T3: –0.9; T4: –1.2 MPa.

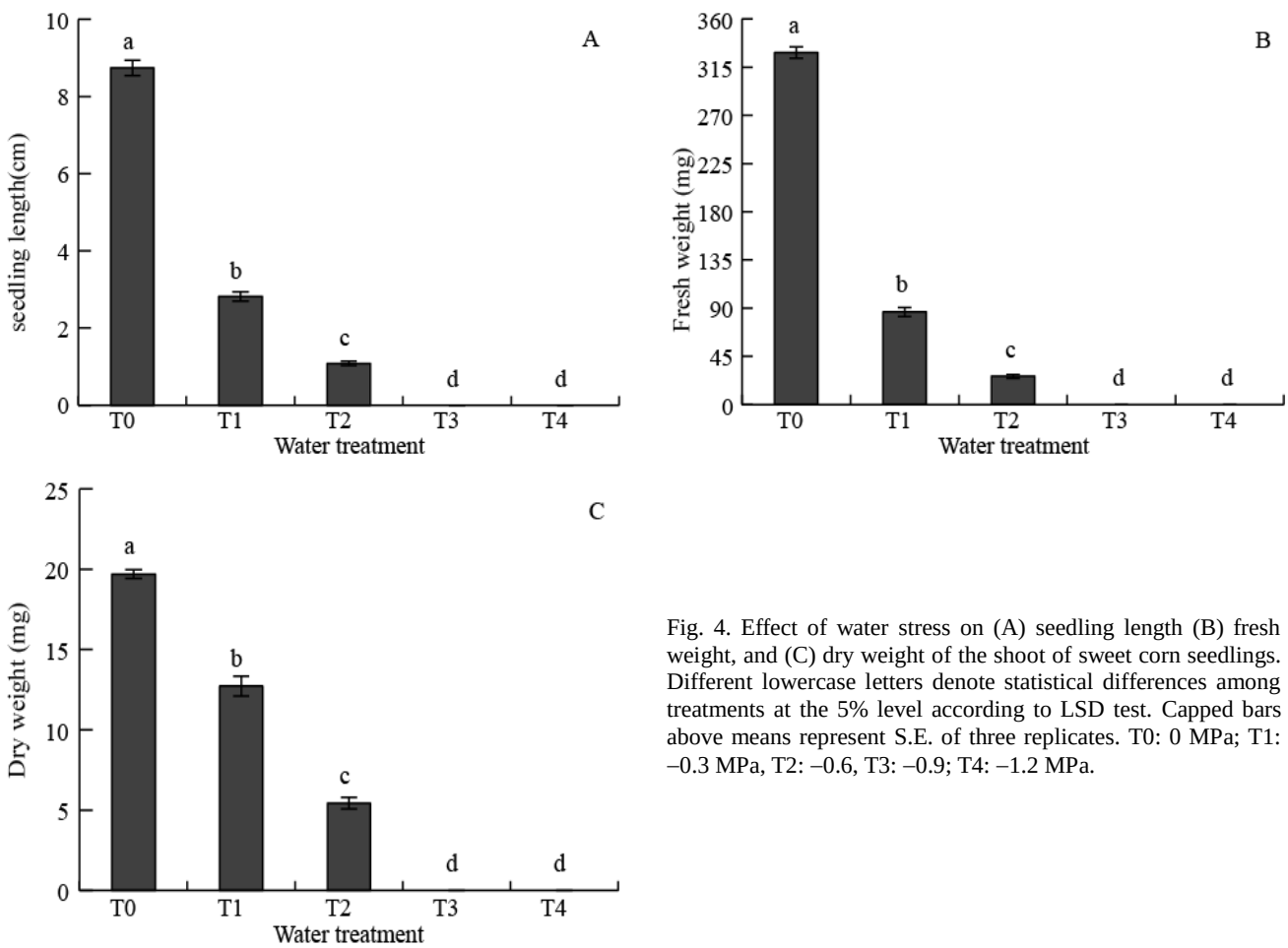

Fig. 4. Effect of water stress on (A) seedling length (B) fresh weight, and (C) dry weight of the shoot of sweet corn seedlings. Different lowercase letters denote statistical differences among treatments at the 5% level according to LSD test. Capped bars above means represent S.E. of three replicates. T0: 0 MPa; T1: –0.3 MPa, T2: –0.6, T3: –0.9; T4: –1.2 MPa.

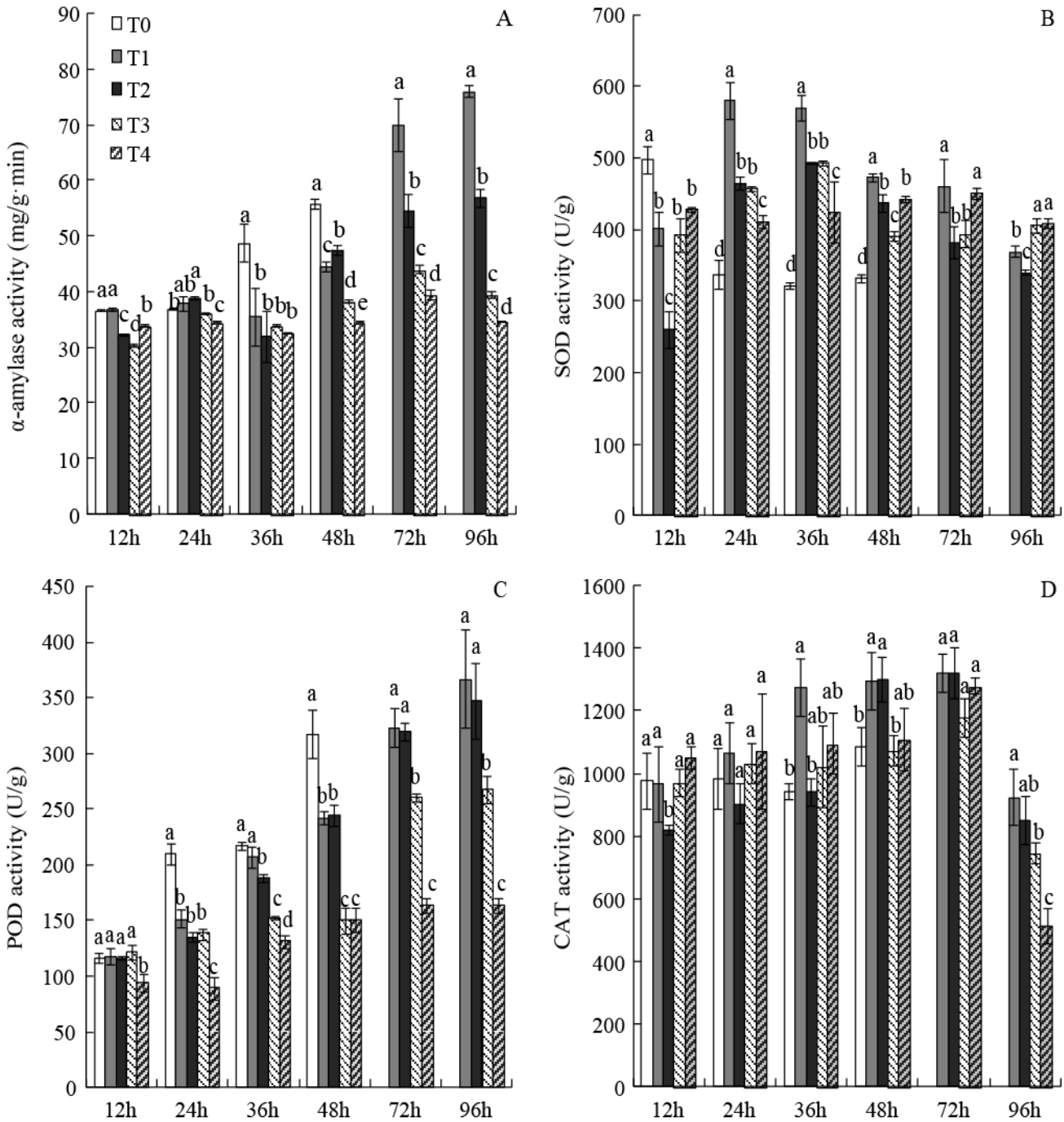

Fig. 5. Activities of (A) α-amylase, (B) super oxide dismutase (C) per-oxidase and (D) catalase in sweet corn seeds under the influence of water stress and its exposure time. U/g = Units per gram of Fresh Weight. Different lowercase letters denote statistical differences among treatments at the 5% level according to LSD test. Capped bars above means represent S.E. of three replicates. T0: 0 MPa; T1: –0.3 MPa, T2: –0.6, T3: –0.9; T4: –1.2 MPa.

Soluble sugar, proline, protein, and MDA and correlation analysis: Osmotic stress regulated the production of protective osmolites but activated the lipid peroxidation process within the seeds. For first 48 h, soluble sugar increased linearly for T0, T1 and T2 while decreased in T3 till 48h, then increased and reached peak at 72 h. For T4, contents of soluble sugars reached peak at 24 h, then remain unchanged. The soluble protein content showed a linear decrease for T0 treatment. Seeds under T1 showed a little increase in protein contents at 24 h, and then decreased linearly, while T2, remained consistent around 60 mg/g ~ 70mg/g till 72 h, then declined to about 40 mg/g. However, T3 and T4 remained higher than 60 mg/g (Fig. 6A and B). Additionally, proline content increased for all the treatments but levels were raised dramatically after 48 h of stress. Protein contents peak reached in seeds applied with normal and water with Ψw = –0.3 Mpa at 36 and 72h, respectively while for T2, T3, and T4, maximum proline contents were observed at 96 h or maybe 96 h later (Fig. 6C). Furthermore, water stress disrupted membrane stability significantly that led to increased

MDA contents in sweet corn seeds. MDA contents increased linearly up to 36 h of stress exposure then started to decline. Maximum MDA contents were recorded in T4 (Fig. 6D). Correlation analyses indicated thatα- amylase activity and soluble protein showed significant correlations with maize seed germination while anti-oxidant enzymes (SOD, POD and CAT), MDA contents, proline and soluble sugar contents showed non-significant associations with seed germination (Table 1). Contributions of osmolytes to osmotic adjustments under water limited conditions prevent water exclusion retained within the seeds. This corroborates with the findings of Tan *et al.* (2006) who observed physio-biochemical changes in *Radix astragali* induced by water deficit conditions at seeding stage. Furthermore, higher MDA contents show increase in lipid peroxidation while accumulation of osmolyte helps to relive oxidative stress under water stress conditions (Sairam *et al.*, 2000; Mika *et al.*, 2005; Ali *et al.*, 2007). Hence, increased accumulation of organic osmolytes in stress exposed seeds implies their protective role against membrane damage due to lipid peroxidation, sheltering ROS clean up system and contributing to maintain cell structures and functions.

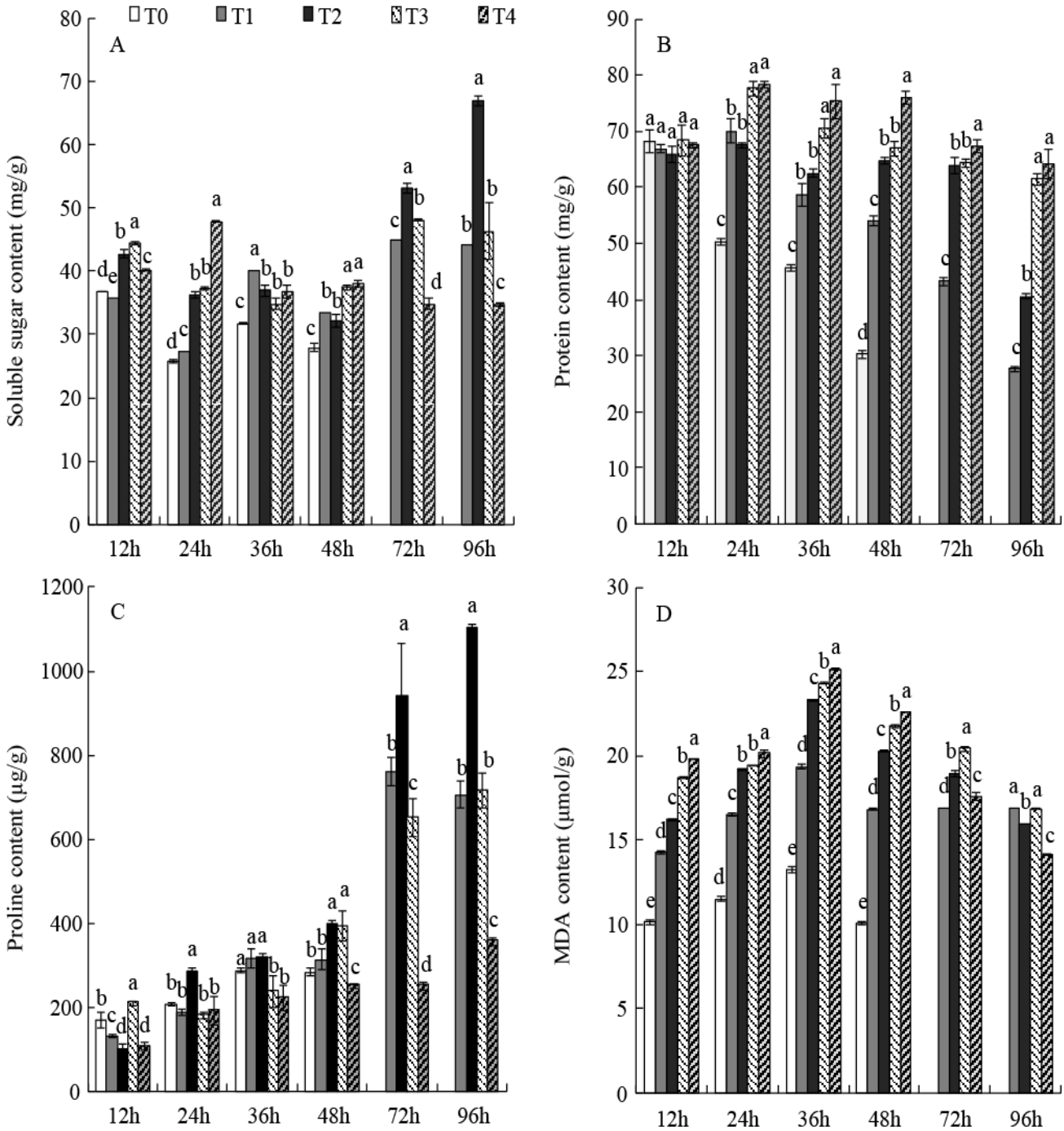

Fig. 6. Accumulation of (A) Soluble sugar, (B), protein, (C) proline, and (D) MDA content of sweet corn under the influence of water stress and its exposure time. Different lowercase letters denote statistical differences among treatments at the 5% level according to LSD test. Capped bars above means represent S.E. of three replicates. T0: 0 MPa; T1: –0.3 MPa, T2: –0.6, T3: –0.9; T4: –1.2 MPa.

Table 1. Correlation of maize seed germination with the physiological traits.

Indices	Equation	R^2	P value
α- amylase activity	y = 6.4739x - 227.12	0.8747*	0.0196
Soluble sugar content	y = -4.5188x + 219.26	0.3186	0.3215
Soluble protein content	y = -4.2806x + 303.76	0.9502**	0.0048
SOD activity	y = 0.039x + 28.292	0.0013	0.9543
POD activity	y = 0.8324x - 119.62	0.6818	0.0850
CAT activity	y = 0.3664x - 334.84	0.2638	0.3760
MDA content	y = -9.1165x + 203.54	0.6400	0.1041
Proline content	y = 0.0124x + 40.149	0.0014	0.9531

*Significant at $p<0.05$; **Significant at $p<0.01$

Conclusion

In conclusion, osmotic stress in sweet corn seeds baldy affected seed germination and affected linearly with prolonged stress periods and stress severity levels. The water stress threshold value for seed germination was around T2 (ΨSw = −0.6MPa). However, osmotic stress induced higher antioxidative activities and more accumulation of organic osmolytes. Overall, the final germination, germination index, vigor index, and seedling growth parameters were recorded as: T0>T1>T2>T3>T4.

Acknowledgements

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