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RESEARCH ARTICLE

Cross-stress Tolerance (Cold and Salt) in Plants Have Different Seed Nutrient Content (Maize, Bean and Wheat)

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ABSTRACT

The aim of this study was to determine cross-stress tolerance in plants have different seed nutrient content (maize, bean and wheat). For this purpose, salt (50 and 100 mM NaCl) and cold stress (12/7ºC) separately or in combinations (cross stress) were applied and studied the alterations of root and stem growth, total soluble protein content and antioxidant enzyme activities (superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX)) associated with induction of cold hardiness by salt stress. Salt and cold stress and its combinations caused inhibition of root and stem growth, and antioxidant enzyme activities (SOD, CAT, POD and APX) were significantly increased or decreased due to both salt, cold stress and its combinations. The soluble protein content increased in maize and wheat while decreased in bean in all applications. Cross-stress, on the other hand, decreased the soluble protein content according to alone salt or cold stress in all plants. As a result, there is not determined any relationship among cross-stress tolerance and growth, soluble protein content, antioxidant enzyme activities or plants have different energy sources. For example; while the highest increase in SOD, CAT, POD and APX activities were observed in maize, root-stem growth was most decreased in maize.

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Introduction

Plants are immobile and is faced with abiotic environmental stresses such as cold, temperature, soil salinity, drought and nutrient deficiency or excess throughout their life. Abiotic stress factors limit 50% or more productions of world agriculture (in point of yield- plant growth and development) (Yasuda, 2017). Plants have cellular and/or molecular responses that increase tolerance to stress in response to abiotic stresses. Although salt, water and cold stresses are clearly different from each other and each of them certain plant responses arise, also activates some common reactions. Low temperature, drought and salinity represent stress factors associated with plant cell dehydration.

Salinity is one of the most important stress factors that reduce the growth and efficiency of plants in various climates. It is an important problem especially in arid and semi-arid regions. Approximately 22% of the agricultural land in the world is salted (F.A.O. 2010). Biochemical and physiological responses to salt stress in plants vary and affect almost all plant processes. High salinity includes both ionic (chemical) and osmotic (physical) component. Salt stress usually cause water stress, ionic toxicity, nutritional imbalance (presence of nutrients in the soil, receive and transport in plant), oxidative stress, changes in metabolic processes (photosynthesis, lipid metabolism and protein synthesis), membrane disturbance, slow cell division and growth and genotoxicity, thus affect plant growth. These effects depend on the stage of the plant

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growth, the duration and density of stress. The most important effect of salt stress is the prevention of plant growth by reducing enzyme activities and biochemical components (Akladious and Mohamed, 2018; Kaleem et al., 2018).

Low temperatures cause typical symptoms of chilling such as senescence, inhibition of growth, water imbalance, mineral nutrition, respiratory and photosynthesis (at least decrease in chlorophyll and damage to the photosynthetic apparatus) (Erdal et al., 2015). It can lead to a higher cold tolerance that lead to molecular, biochemical and physiological changes when a plant exposure to a cold temperature and this called as acclimation (Mutlu et. al., 2016). Low temperature can lead to immediate mechanical stresses, changes in the activity of macromolecules and reduced osmotic potential in the cellular environment. Cold stress responses in plants are extremely complex events that alter the biochemical composition of cells to prevent damage caused by low temperatures. In addition, cold stress has a significant effect on plant morphology, which leads to decrease in growth and low productivity. One of the cold resistance factors is the ability of the antioxidant enzymes to maintain their activity during cold stress and to recover relatively quickly after the plants are transferred to hot conditions. (Eremina et al., 2016; Zhang et al., 2014). Therefore, it is vital to develop products that are resistant to salt and cold stress in order to feed the growing population in the world

Plants are usually not only subject to a continuous stress factor that potentially damages the plant at the same density, but at same or different times of their lives are exposed to various stress factors (Foyer et al., 2016). It may vary from time to time in the variety and intensity of the stress throughout the life of the plant. Plants are often exposed to multiple stresses such as drought and heat, or drought and cold. The combination of several stresses can cause more damage than alone stress. While answers to a single stress such as cold, drought and heat have been extensively researched, little is known about plant response to multiple simultaneous stresses (Lee and Back, 2016). Cross-stress tolerance can be achieved when the response to stress by the plants increases the performance of these individuals in another type of stress. This tolerance, therefore, means that plants are exposed to different stress factors at different times, which is usually seen in plants in nature. Cross-tolerance to environmental stress is a common phenomenon in plants, and exposure to some form of stress provides an overall increase in resistance to a range of different stresses (Yasuda, 2017). Cross stress tolerance is important in the daily life of plants in nature. Therefore, plant plasticity is necessary to overcome such stresses that occur repeatedly in the life of plants; this creates acclimation mechanisms that allow them to react better to single or multiple stresses of the same or different nature at all times. Cross-tolerance occurs due to synergistic co-activation of non-specific stress-sensitive pathways that cross biotic-abiotic stress limits (Yasuda, 2017). Cross stress tolerance is a sophisticated molecular process involving morphological, anatomical, physiological and biochemical changes in cell, organ and all plant levels. Cross-tolerance events are often associated with the regulation of gene expression and increased ROS (reactive oxygen species) production by H_2O_2, oxidative signal and redox signaling (Munne-Bosch and Alegre, 2013).

Although some mechanisms associated with stress memory in plants are known in addition to epigenetic modifications and morphological changes, such as accumulation of specific transcription factors or protective metabolites, cooperation among ecologists, physiologists, biochemists and molecular biologists in the present and near future is very important to understand cross-stress tolerance and stress "memory".

In this study, it is aimed to investigate whether exposure to salt stress (NaCl) will increase the cold stress resistance of plants and are there any relationship between different seed nutrient content (maize, bean and wheat) and stress tolerance. Cross-stress tolerance has evaluated by determining changes in the root-shoot growth, total soluble protein content and antioxidant enzyme (SOD, CAT, POD and APX) activities.

Materials and Methods

Plant Material and Growth Conditions

Before germination, seeds of maize (*Zea mays* L. cv. Hido), bean (*Phaseolus vulgaris* cv. Kızılhaç) and wheat (*Triticum aestivum* L. cv. Bezostoja-1) were surface sterilized by using (0.01% w/v) sodium hypochlorite solution, and washed with sterile dH_2O. Seeds have different nutrient content were grown in control (25/20°C), salt stress (50 and 100 mM NaCl, 25/20°C, they were selected with preliminary studies), cold stress (12/7 ºC), cold (12/7 ºC)+salt stress (50 mM NaCl) and cold (12/7 ºC)+salt stress (100 mM NaCl) conditions for 7 days in petri dishes. On the 7th day, all the plants were harvested (the tissues were rinsed three times in distilled water after harvested) and analyzed.

Measurement of Root and Stem Length of Plants

The root and stems of the plants were cut with the help of the bust of the joints and the lengths were measured with the help of the millimetric ruler. Roots measured based on the length of the main root (Bozcuk, 1978). The lengths of the root and stem of the applications were separately measured and divided by the number of plants, and mean root and stem length were calculated as cm / plant.

Determination of Total Soluble Protein Content

Total soluble protein contents in the root and stem were measured according to the method of Smith et al. (1985). Total soluble protein content was determined as µg protein/gr tissue.

Determination of Antioxidant Enzyme Activities

For the enzyme assays (SOD, CAT, POD and APX), leaf tissues (0.5 g) were homogenized in liquid nitrogen, and 5 ml 10 mmol l^{-1} K-P buffer (pH 7.0) containing 4% (w/v) polyvinylpyrrolidone (PVPP) and 1 mmol l^{-1} disodium ethylenediamine tetraacetic acid (EDTA) was added. The homogenates were centrifuged at 12,000xg and 4 ºC for 15 min, and the supernatant was used to determine enzymes activities. SOD activity was measured according to Elstner and

Heupel (1976). CAT activity was measured according to Gong et al. (2001). POD activity was determined according to Yee et al. (2002). APX activity was determined according to Nakano and Asada (1981).

Results and Discussion

In the study, the change in growth, which is an important indicator of the plant's vital functions and the evaluation of plant conditions, has been chosen as a criterion. All stress conditions (50 and 100 mM NaCl and cold) were determined to inhibit root and stem elongation of maize, bean and wheat (Table 1). These results are consistent with studies reporting that salt and cold stress reduce plant growth (Mohamed and Latif, 2016; Zhou et. al., 2018). Suppression of plant growth under environmental stress may be due to decreased cell division, elongation and apical meristem activity. Decrease in shoot and root length in salt stress may be due to either the inhibitory effect of water shortage on growth-promoting hormones or the reduction in water absorption and activity of metabolic events (Akladious and Mohamed, 2018; Akladious and Hanafy, 2018; Mutlu et. al., 2016). This inhibitory effect may possibly be due to the decrease in intracellular CO_2 concentration and the effect of salt on the stoma and photosynthesis due to the deterioration of photosynthetic enzymes, chlorophyll and carotenoids (Zhou et. al., 2018). At the same time, in the case of cross stress (Cold + NaCl (50 and 100 mM), these inhibitions were determined to be more (Table 1). It was observed that root-stem growth more decreased as salt concentration increased in cold stress. Exposure of a plant to a single stress can lead to a broad spectrum of abiotic stresses and, in some cases, to tolerance to biotic stresses, and this is known as cross-stress tolerance. Gaining abiotic stress tolerance or cross stress tolerance requires activation of mechanisms that minimize cellular damage or deterioration. Antioxidant systems play a vital role in triggering cross-tolerance through ROS scavenge and / or signal functions (Hossain et. al., 2018). However, in this study, although the ROS scavenge capacity maximum increased in the maize among seeds have different seed nutrient content in stresses (salt and cold) and cross stress conditions, the root-stem growth was the most negatively affected in maize plant. There was no significant improvement observed in cross stress applications according to stress alone. Even the severity of stress increased.

Table 1. Effects of salt (50 and 100 mM NaCl), cold stress (12/7ºC) and cross stress (Cold + NaCl (50 and 100 mM)) on plant root and stem lengths (cm) with different seed nutrient content

	MAIZE				BEAN				WHEAT			
Applications	**Root**	**Change %**	**Stem**	**Change %**	**Root**	**Change %**	**Stem**	**Change %**	**Root**	**Change %**	**Stem**	**Change %**
Control	12.6		6.8		9.5		2.4		12.8		11.4	
50 mM NaCl	9.8	-22.2	5.2	-23.5	9.2	-3.2	1.9	-20.8	10.4	-18.8	9.8	-14
100 mM NaCl	6.4	-49.2	3.7	-45.6	6.3	-33.7	1.4	-41.7	8.0	-37.5	6.3	-44.7
Cold (12/7ºC)	7.8	-38.1	5.2	-23.5	6.9	-27.4	1.9	-20.8	9.7	-24.2	8.3	-27.2
Cold+50 mM NaCl	5.9	-53.2 -39.8 -24.4	3.2	-53 -38.5 -38.5	5.7	-40 -38 -17.4	1.6	-33.3 -15.8 -15.8	8.0	-37.5 -23.1 -17.5	7	-38.6 -28.6 -15.7
Cold+100 mM NaCl	3.9	-69 -39 -50	2.5	-63.2 -32.4 -52	4.6	-51.6 -27 -33.3	1.4	-41.7 0 -26.3	6.2	-51.6 -22.5 -36.1	5.0	-56 -20.6 -39.8

The soluble protein amounts in maize and wheat increased in all applications (salt, cold and cross stress (salt + cold)) (Table 2). It has been reported that the soluble protein amounts in maize and potatoes increases with increasing salt concentration (Abd El-Samed et. al., 2004; Ryu et. al., 1995). In previous studies it was determined that the soluble protein amount in wheat leaves increased significantly under cold stress (Turk et al., 2014). Protein changes have proven to be a key feature of cold resistance. The soluble protein amount in bean plant reduced in all applications (salt, cold and cross stress (salt + cold)) (Table 2). Öztürk et al. (2012) reported that salt stress reduces the soluble protein amount in the pea. Reduction in protein content is a common response to salinity stress. The reason for this is that amino acids in proteins react with active radical and degrade. High concentrations of NaCl (100 and 150 mM) caused a significant reduction in the total soluble protein content of cowpea leaves according to their controls. Protein degradation in a saline environment may originate from reduction in protein synthesis, increased proteolysis, and reduction in amino acid availability, and denaturation of enzymes involved in protein synthesis, or the reaction of amino acid of proteins with active radical and degradation. Although the highest increase in the soluble protein amount was seen in maize plants in stress conditions, the highest growth inhibition was also observed in maize. As a result, there was no correlation between the degree of maize susceptibility and the soluble protein amount in the root and stems in all stress conditions.

Table 2. Effects of salt (50 and 100 mM NaCl), cold stress (12/7ºC) and cross stress (Cold + NaCl (50 and 100 mM)) on the soluble protein content (mg g^{-1} FW) of plants with different seed nutrient content

	MAIZE		BEAN		WHEAT	
Applications	**Protein**	**Change %**	**Protein**	**Change %**	**Protein**	**Change %**
Control	1.146		1.996		1.270	
50 mM NaCl	1.576	37.5	2.000	0.2	1.463	15.2
100 mM NaCl	1.690	47.5	1.987	-0.5	1.499	18
Cold (12/7ºC)	1.562	36.3	1.990	-0.3	1.527	20.2
Cold+50 mM NaCl	1.490	30 -5.5 -4.6	1.975	-1.1 -1.3 -0.8	1.431	12.7 -2.2 -6.3
Cold+100 mM NaCl	1.550	35.3 -8.3 -0.8	1.982	-0.7 -0.3 -0.4	1.435	13 -4.3 -6

Under normal conditions, ROS is effectively scavenged by antioxidant systems. Nevertheless, very low concentrations of ROS assume a positive function as signaling molecules that cause the formation of defense responses in plants. However, when plants are exposed to environmental stresses such as salinity and cold, the antioxidant system cannot prevent the formation of excess ROS (such as superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-)). Excess ROS produced in cells triggers phytotoxic reactions such as lipid peroxidation, protein degradation and DNA mutation causing cellular damage (Bezirganoglu et. al., 2018). The capacity of plants to withstand stress conditions often depends on their ability to activate an adequate antioxidant response. Preservation of the redox balance is a prerequisite for the development of tolerance against both biotic and abiotic stresses (Jiang *et al.*, 2012). Some plant species have the potential to protect cellular systems from the effects of this ROS by increasing the activity of enzymatic (SOD, CAT, POD) enzymes and non-enzymatic (ascorbate and glutathione) substances (Agarwal and Pandey, 2004).

Superoxide dismutase (SOD, EC 1.15.1.1) is a metallic enzyme that catalyzes the conversion to the less toxic H_2O_2 by protecting the plant against the harmful effects of superoxide radical ($O_2^{\bullet -}$) in chloroplast ($2O_2^{\bullet -} + 2H^+ \rightarrow H_2O_2 + O_2$). Compared to plants in terms of SOD activity in 50 mM NaCl stress; SOD activity increased in maize and wheat, while decreased in bean. SOD activity increased in maize while decreased in bean and wheat in 100 mM NaCl stress (Table 3). These results are consistent with studies showing that SOD activity increases in tomato, wheat and pea plants under salt stress (Doğan et. al., 2010; Jahantigh et. al., 2016; Öztürk et. al., 2012). The increase in SOD activity in the leaves exposed to salt stress may be due to activation of pre-existing SOD or synthesis of new SOD under salt conditions. SOD activity in cold stress increased in maize while decreased in bean and wheat. Previous studies have reported increased SOD activity in cold stress (Erdal et. al., 2015; Bezirganoglu et. al., 2018). It is reported that SOD activity decreases in cold tolerant barley and increases in cold sensitive barley (Mutlu et. al., 2016). The decrease in SOD activity and excessive $O_2^{\bullet -}$ formation may be one of the main factors which causes metabolic deterioration in cold stress. In the case of cross stress (Cold + NaCl (50 and 100 mM)), SOD activity increased in maize compared to control while decreased in bean and wheat. In the case of cross stress, SOD activity was reduced in maize, beans and wheat according to alone 50 mM NaCl stress. It increased in bean but decreased in maize and wheat according to alone 100 mM NaCl stress. In cold+50 mM NaCl cross stress condition, SOD activity was decreased bean, maize and wheat according to cold stress alone (12/7 ºC). In cold+100 mM NaCl cross stress condition, SOD activity increased in bean while decreased in maize and wheat according to cold stress alone (12/7ºC).

Table 3. Effects of salt (50 and 100 mM NaCl), cold stress (12/7ºC) and cross stress (Cold + NaCl (50 and 100 mM)) on the SOD activities (U mg−1 protein) in plants with different seed nutrient content

	MAIZE		BEAN		WHEAT	
Applications	**SOD Activity**	**Change %**	**SOD Activity**	**Change %**	**SOD Activity**	**Change %**
Control	195		340		208	
50 mM NaCl	262	34.6	315	-7.4	213	2.4
100 mM NaCl	268	37.4	296	-13	182	-12.5
Cold (12/7ºC)	267	37	305	-10.3	168	-19.2
Cold+50 mM NaCl	251	28.7 -4.2 -6	290	-14.7 -8 -5	145	-30.3 -32 -13.7
Cold+100 mM NaCl	213	9.2 -20.5 -20.2	308	-9.4 4.1 1	109	-47.6 -40.1 -35.1

Catalase (CAT, EC 1. 11.1.6) is the most effective enzyme to prevent oxidative damage by breaking down H_2O_2 in peroxisomes and glioxisomes in all living things. CAT converts H_2O_2, H_2O and molecular O_2 in peroxisomes. Since CAT is an unstable enzyme, it is determined that it can be inhibited by H_2O_2 when exposed to high light intensity and stress. All stress conditions (Cold, 50 and 100 mM NaCl and Cold + NaCl (50 and 100 mM)) were determined to increase CAT activity in maize leaves. It was determined that CAT activity increased as salt concentration increased in cold stress (Table 4). Previous studies have reported that salt and cold stress increase CAT activity (Demir and Öztürk, 2004; Esim et. al., 2014; Bezirganoglu et. al., 2018). All stress conditions (Cold, 50 and 100 mM NaCl and Cold + NaCl (50 and 100 mM)) were generally determined to inhibit CAT activity in bean leaves (except for cold + 50 mM NaCl). It was reported that salt and cold stress inhibits CAT activity (Mutlu et. al., 2013; Öztürk et al., 2012; Keles and Oncel, 2002). It was determined that CAT activity decreased as salt concentration increased in cold stress. Stress conditions (Cold, 50 and 100 mM NaCl) increased CAT activity in wheat leaves. But CAT activity in cases of cross stress (Cold + NaCl (50 and 100 mM)) decreased as salt concentration increased in cold stress.

Table 4. Effects of salt (50 and 100 mM NaCl), cold stress (12/7ºC) and cross stress (Cold + NaCl (50 and 100 mM)) on the CAT activities (U mg−1 protein) in plants with different seed nutrient content

	MAIZE		BEAN		WHEAT	
Applications	**CAT Activity**	**Change %**	**CAT Activity**	**Change %**	**CAT Activity**	**Change %**
Control	0.020		0.344		0.100	
50 mM NaCl	0.045	125	0.326	-5.2	0.125	25
100 mM NaCl	0.084	320	0.290	-15.7	0.120	20
Cold (12/7ºC)	0.037	85	0.287	-16.6	0.110	10
Cold+50 mM NaCl	0.051	155 13.3 37.8	0.302	-12.2 -7.4 5.2	0.103	3 -17.6 -6.4
Cold+100 mM NaCl	0.070	250 -16.7 89.2	0.255	-26 -12.1 -11.2	0.105	5 -12.5 -4.6

Peroxidase (POD, EC 1.11.1.7) is an enzyme involved in various defense mechanisms, including lignification, auxin metabolism, salt tolerance and heavy metal stress, and protects the cell against oxidative damage. Therefore, POD is often used as a parameter to determine changes in growth and metabolism under environmental stress conditions. It has been determined that the activity of peroxidases, which are related to physiological events and played an active role in metabolism, have increased its activity under very different stresses. Stress conditions (Cold, 50 and 100 mM NaCl) increased POD activity in maize leaves but significantly reduced activity in cross stress conditions (Cold + NaCl (50 and 100 mM)) according to controls. It was determined that POD activity decreased as salt concentration increased in cold stress. It was determined that 50 and 100 mM NaCl stress inhibited POD activity in bean leaves, and cold stress alone caused an increase whereas cross stress (Cold + NaCl (50 and 100 mM)) caused generally a significant decrease in the activity according to controls. It was determined that POD activity decreased as salt concentration increased in cold stress. Stress conditions (Cold, 50 and 100 mM NaCl) inhibited POD activity in wheat leaves, and cross stress conditions (Cold + NaCl (50 and 100 mM)) showed statistically significant more decrease in the activity according to controls. It was determined POD activity decreased as salt concentration increased in cold stress (Table 5). Previous studies have reported that salt and cold stress increase POD activity (Demir and Öztürk, 2004; Öztürk et. al., 2012; Turk et. al., 2014). It was suggested that POD activity reduced in cold stress (Erdal et. al., 2015).

Table 5. Effects of salt (50 and 100 mM NaCl), cold stress (12/7ºC) and cross stress (Cold + NaCl (50 and 100 mM)) on the POD activities (U mg−1 protein) in plants with different seed nutrient content

	MAIZE		BEAN		WHEAT	
Applications	**POD Activity**	**Change %**	**POD Activity**	**Change %**	**POD Activity**	**Change %**
Control	1460		57.500		1424	
50 mM NaCl	2394	64	46.000	-20	1280	-10.1
100 mM NaCl	3052	109	32.700	-43.1	928	-34.8
Cold (12/7ºC)	1940	33	58.600	2	703	-50.6
Cold+50 mM NaCl	2130	46 -11 9.8	34.900	-39.3 -24.1 -40.4	700	-50.8 -45.3 -0.43
Cold+100 mM NaCl	1880	28.8 -38.4 -3.1	32.800	-43 0.3 -44	776	-45.5 -16.4 10.4

Ascorbate peroxidase (APX) (EC 1.11.1.11) has an important role in defense against ROS in many organisms (such as plants, algae, whips). APX family has a higher affinity to H_2O_2 than CAT. APX protects cells against H_2O_2 not only under normal conditions, but also under stress conditions (Öztürk et al., 2012). It was determined that all stress conditions (Cold, 50 and 100 mM NaCl and Cold + NaCl (50 and 100 mM)) increased APX activity in maize leaves. In the cold stress, APX activity increased as salt concentration increased. In general, stress conditions (50 and 100 mM NaCl and Cold + NaCl (50 and 100 mM)) inhibited APX activity in bean leaves (except for cold stress) according to controls (Table 6). It was also found out that APX activity decreased as salt concentration increased in cold stress. It was determined that salt stress (50 and 100 mM NaCl) increased APX activity in wheat leaves, and cold stress inhibited the activity. Cross stress conditions (Cold + NaCl (50 and 100 mM)) resulted in a significant decrease in activity relative to salt stress and an increase in cold stress. APX activity was inhibited as salt concentration increased in cold stress. Previous studies have explained increased APX activity in salt and cold stress conditions (Doğan et. al., 2010; Turk et. al., 2014; Erdal et. al., 2015; Bezirganoglu et. al., 2018). Reduced APX activity in salt and cold stress conditions has also been reported in previous studies (Öztürk et. al., 2012; Lukatkin, 2002).

Table 6. Effects of salt (50 and 100 mM NaCl) and cold stress (12/7°C) on the APX activities (U mg−1 protein) in plants with different seed nutrient content

	MAIZE		BEAN		WHEAT	
Applications	**APX Activity**	**Change %**	**APX Activity**	**Change %**	**APX Activity**	**Change %**
Control	0.179		0.257		0.250	
50 mM NaCl	0.236	31.8	0.196	-23.7	0.306	22.4
100 mM NaCl	0.283	58.1	0.190	-26.1	0.326	30.4
Cold (12/7°C)	0.242	35.2	0.283	10.1	0.211	-15.6
Cold+50 mM NaCl	0.245	37 3.8 1.2	0.185	-28 -5.6 -34.6	0.270	8 -11.8 28
Cold+100 mM NaCl	0.292	63.1 3.2 20.7	0.175	-32 -8 -38.2	0.255	2 -21.8 21

Conclusion

As a result; salt, cold and cross stress inhibited root-stem growth of maize, bean and wheat plants. Cross stress has more inhibitory effect than alone salt or cold stress. On the basis of the plant variety, the most inhibition was seen in maize, wheat and bean, respectively. The total soluble protein content increased in maize and wheat while decreased in bean in all applications. Cross-stress, on the other hand, decreased the soluble protein content according to alone salt or cold stress in all plants. No cross-tolerance was observed between the amount of total soluble protein and root-stem growth. Antioxidant enzyme activities (SOD, CAT, POD and APX) increased in maize while decreased in bean. In wheat, CAT and APX activities increased while SOD and POD decreased. In the cases of cross stress, it has shown increases and decreases compared to plant types. No cross-tolerance was observed among root-stem growth, antioxidant enzyme activities and different seed nutrient content in all plants. For example, the highest increase in SOD, CAT, POD and APX activities was observed in maize, while root-stem growth decreased most in maize.

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