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RESEARCH ARTICLE

Assessment of Morphological, Antioxidant, Biochemical and Ionic Responses of Salt-Tolerant and Salt-Sensitive Okra (*Abelmoschus esculentus)* under Saline Regime

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ABSTRACT
A pot experiment was executed with the objective to observe the response of four okra cultivars under saline regime at biochemical, antioxidant and ionic levels. Sand culture was employed with Hoagland solution as nutrient medium to replenish the nutrient requirement of emerging seedlings of two salt-tolerant (OH-713 and OH-139) and two salt-sensitive (Sitara-9101 and Okra-7080) cultivars. Twenty days old seedlings were subjected to various salt concentrations i.e. 0, 2, 4, 6 and 8 dSm^{-1}). Salinity imposed severe reduction in morphological attributes including shoot length, plant fresh weight and leaf area, being more drastic in salt-sensitive cultivars. High contents of chlorophyll, proline and glycine betaine were observed in salt-tolerant cultivars as compared to salt-sensitive ones. Enhancement in salt stress conferred an ascending trend in terms of antioxidants like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) in all the tested okra cultivars. However the tolerant okra cultivars exhibited the highest levels of these antioxidant enzymes. A synergistic relationship was obvious between glycine betaine and antioxidant enzymes. The ionic response of okra cultivars under salt stress revealed that tolerant cultivars restricted sodium and chloride ions in their roots so less sodium and chloride contents were noted in the leaves of salt-tolerant cultivars.

INTRODUCTION

Salinization of underground water resource is a major problem that contributes in fixing the agricultural productivity (Munns, 2005). Salinization of good arable land in Pakistan is creating a problem with immense socio-economic losses, because 70% of the population depends for its livelihood on these lands, which are gradually dwindling.
The salt stressed plants have shown stunted growth pattern, with minimal life span of leaves and net productivity. Salt stress causes multifarious drastic effects in plants and among these factors production of reactive oxygen species (ROS) is a common phenomenon (Ashraf, 2009). These ROS are highly reactive because they can interact with a number of cellular molecules and metabolites and ultimately lead to cellular damage (Banu et al., 2009). Production of antioxidant enzymes like SOD, POD and APX is an important adaptation to scavenge the ROS (Siaram et al., 2000).
An adaptation against salt stress is marked as the accumulation of compatible solutes. Proline signifies its role as an inducer of salt tolerance in plant species, growing under stress conditions (Ronde et al., 2000). Glycinebetaine encourages the production of enzymes which can scavenge the ROX species, hence plays an indirect role in protection against the drastic effects of reactive oxygen species.
Salt stress is mainly contributed by increased level of some ions especially Na^+ (Rahnama and Ebrahimzadeh, 2004) and Cl^- (Abbas et al., 2010) in soil solution, to an extent that become deleterious to plant growth and productivity. Such increment of ions in soil solution causes the higher uptake of these ions resulting in ionic imbalance and nutritional deficiencies of the plant. The tolerant plants can be categorized as sodium ion accumulators, based on their ability to confine sodium ions in their roots (Blumwald, 2000).
Okra (*Abelmoschus esculentus* L. Moench) is a very popular vegetable of the tropical and subtropical areas,

grown as an annual crop. Okra like other crops in Pakistan faces a dual menace of biotic and abiotic stresses. The objective of the presented study was to assess the response of okra cultivars on the basis of their morphological, antioxidantal, biochemical and ionic attributes under salt stressed conditions for their utilization on saline lands.

MATERIALS AND METHODS

Plant material and growth conditions

Seeds of two salt tolerant (OH-713 and OH-139) and two salt sensitive (Sitara 9101 and Okra-7080) genotypes (salt tolerance was checked in a separate screening experiment) were sown in plastic pots containing Astatula fine sand (hyperthermic, uncoated typicquartzipsamments) individually as a growth medium. The sand had pH of 6.0-6.5, with field capacity 7.2% and incipient wilting at 1.2% (Volume basis), respectively. Half strength Hoagland solution was used as a nutrient solution. The seeds were watered @ 250 mL per pot.

Salinity applications

Sodium chloride (NaCl) at different concentrations (0, 2, 4, 6 and 8 dS m^{-1}) was applied after 20 days of sowing. To avoid osmotic shock, the seedlings were adjusted to their final NaCl level by imposing the salinity in intervals (two days) while the control was without salt stress and irrigated only with half strength Hoagland's solution.

Growth attributes

Shoot length was measured at the end of the experiment with the help of measuring tape in centimeters (cm) from the base of stem to the tip of the shoot and average value for replicates was computed. After fifty days of growth, three plants were selected from each replicate, which were up rooted with care and sand particles were removed by washing with distilled water. After washing, in order to absorb any drop of water present on leaves and shoots, these plants were wrapped with filter paper. Average fresh weight of each replicate was noted with digital balance. Six randomly selected leaves from two plants per replication were separated. These sampled leaves were placed on an electronic leaf area meter (Ll-3100; LI-COR, Inc., Lincoln, Nebr.) to calculate the leaf area (LA) in centimeters. Average area per leaf was worked out according to Michael et al. (2002).

Biochemical attributes

Chlorophyll contents were measured following the method adopted by Arnon (1949). Proline and glycine betaine contents in the salt affected leaf samples of okra genotypes were analyzed using the methods described by Bates et al. (1973) and Grieve and Grattan (1983), respectively.

Antioxidants enzyme activity

The antioxidant enzymes activity like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) was measured by following the methods of Giannopolitis and Ries (1977) and Chance and Maehly (1955).

Measurement of ionic attributes

Sodium contents in leaf and root samples of okra genotypes were measured using the procedure reported by Wolf (1990) for sample digestion and digested samples were analyzed by Flame Photometer (Jenway PFP-7, UK). The chloride contents in leaf and root samples were measured with the help of analyzer (Corning-920, Germany).

Statistical analysis

The experiment was laid out in factorial design (salinity and genotypes) and treatment means were grouped on the basis of honestly significant difference (HSD, Tucky test) at the 0.05 level of probability by using the statistical software, statistica 8.1.

RESULTS

Growth attributes

Salt stress significantly reduced the shoot length in all tested okra cultivars but plants experienced higher salt stress exhibited maximum reduction in shoot length as compared to those grown under low salinity levels (Figure 1). The cultivars, OH-139 and OH-713 presented less percentage reduction in shoot length as compared to the Sitara-9101 and Okra-7080 under +NaCl conditions. Plant fresh weight of both tolerant and non-tolerant cultivars was reduced by salt stress but maximum reduction was recorded in plants grown under 8.0, followed by 6.0, 4.0 and 2.0 dS m^{-1}NaCl stress (Figure 1). However, salt tolerant cultivars (OH-713 and OH-139) maintained maximum shoot fresh weight than non-tolerant cultivars (Sitara-9101 and Okra-7080) in response to salt stress. Leaf area was decreased in all the tested okra cultivars under saline conditions (Figure 1). The percent reduction in leaf area was high under 8.0 dS m^{-1}NaCl stress. The tolerant cultivars occupied the top position by having less reduction in leaf area as compared to the non-tolerant ones

Biochemical attributes

Total chlorophyll contents were decreased in all the tested okra cultivars under saline conditions (Figure 2). The tolerant cultivars (OH-713 and OH-139) were found to be top ranked by expressing least percent reduction in total chlorophyll contents as compared to non-tolerant ones (Sitara-9101 and Okra-7080). Data regarding proline indicated that salinity had significant ($P \leq 0.05$) increasing effect on proline contents (Figure 2). Minimum proline contents were recorded in the plants grown under non-saline conditions, which were

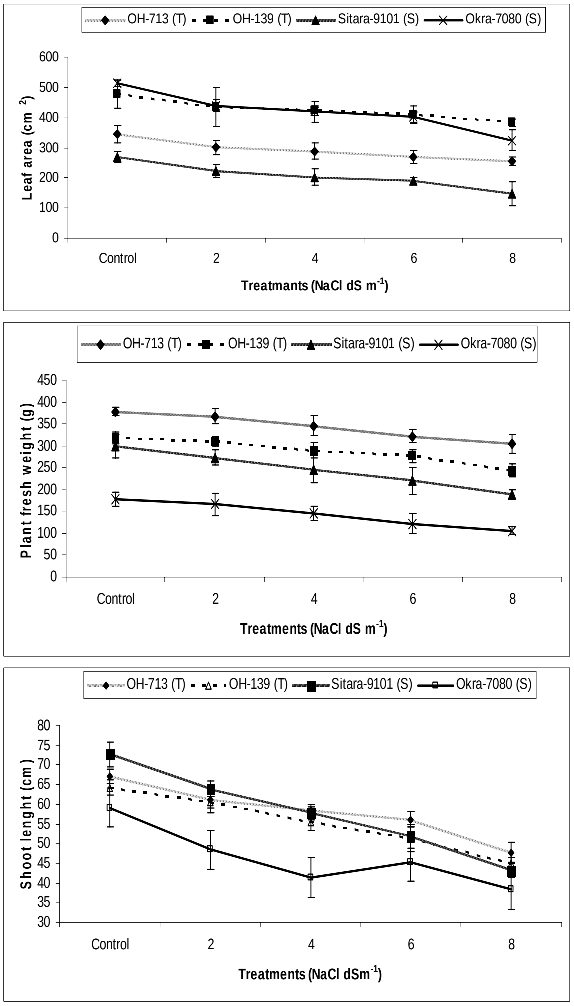

Fig. 1: Effect of salt stress on growth attributes of okra cultivars under saline regimes

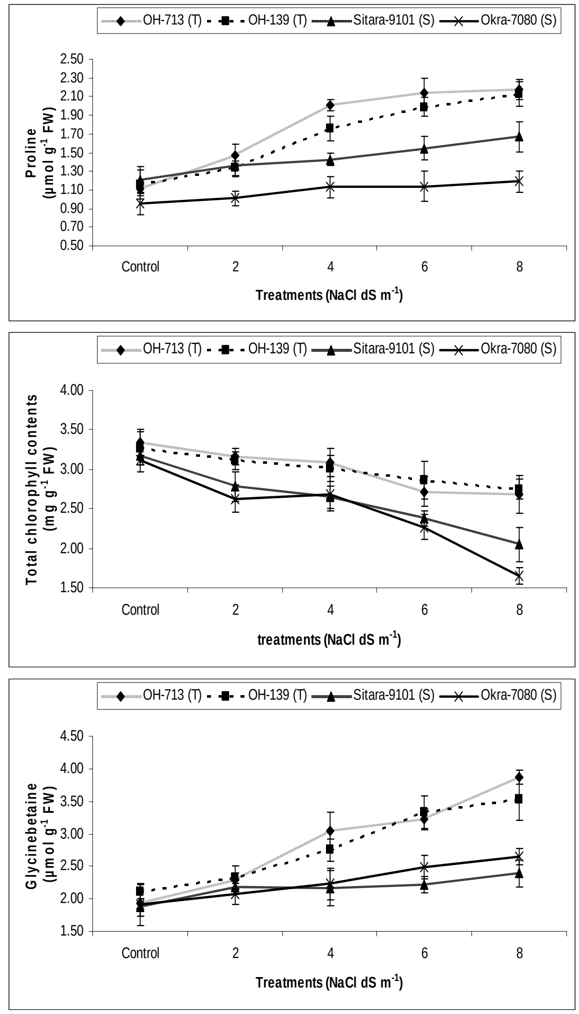

Fig. 2: Effect of salt stress on biochemical attributes of okra cultivars under salt stress

found to be increased with the increasing salinity level. The comparison among cultivars showed variations in values for proline contents. The salt tolerant cultivars (OH-713 and OH-139) gave good performance due to high percentage increase in proline contents in response to NaCl stress. Salt stress induced a significant elevation in glycine betaine contents of all the tested okra cultivars (Figure 2). Minimum glycine betaine contents were noted for plants grown under non saline condition, which was gradually increased at 2.0, 4.0, 6.0 and 8.0 dS m^{-1} NaCl, respectively. The salt tolerant showed good performance by exhibiting high glycine betaine contents while salt sensitive cultivars showed comparatively less improved glycine betaine contents, under increasing salt regime.

Antioxidant enzymes activity

Superoxide dismutase activity (SOD) of all the investigated cultivars was significantly enhanced under saline conditions (Figure 3). Maximum increase in SOD was recorded in plants treated with high NaCl concentration (8.0 dS m^{-1}) followed by 6.0, 4.0 and 2.0 dS m^{-1}. The salinized plants of OH-713 and OH-139 exhibited maximum percent increase in SOD as compared to Sitara-9101 and Okra-7080 with respect to the control. Salt stress induced a marked acceleration in peroxidase activity (POD) of all tested okra cultivars (Figure 3). Minimum POD contents were noted for plants grown under non saline condition which was found to be gradually increased with increasing salinity levels. The salt tolerant cultivars showed good performance by exhibiting high POD while salt sensitive cultivars showed comparatively low POD. The maximum CAT activity was recorded in plants exposed to saline conditions (8.0 dS m^{-1}), followed by 6.0, 4.0, 2.0 and control (Figure 3). The salt tolerant cultivars gave good performance due to high percentage increase in CAT activity in response to NaCl stress.

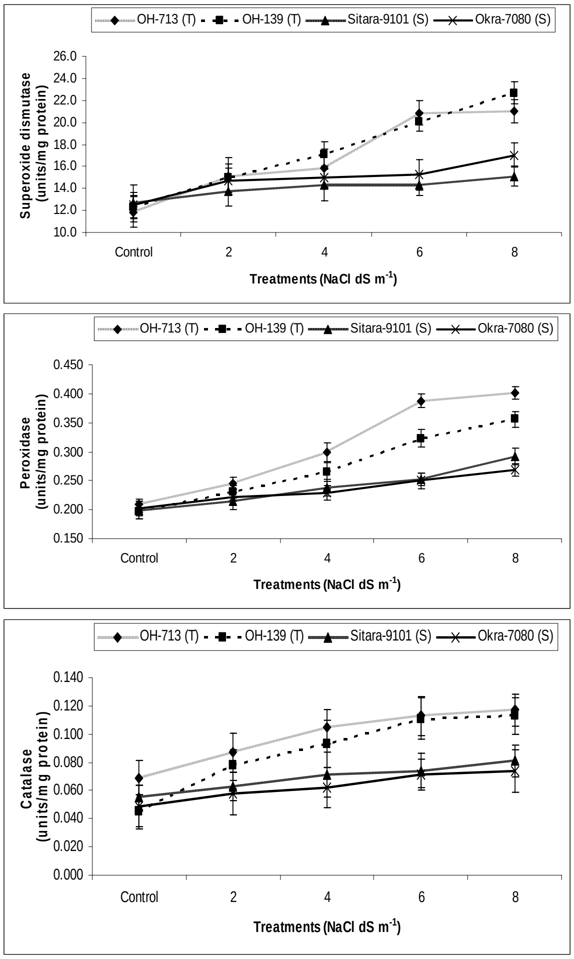

Fig. 3: Effect of salt stress on antioxidant enzymatic attributes of okra cultivars under salt stress

However, salt sensitive cultivars presented minimum percentage increase in CAT activity under salt stress.

Ionic attributes

Sodium (Na) concentration in leaves and roots of all the tested okra cultivars was increased with the increasing salt stress (Figure 4). It was noted that roots of salt tolerant OH-713 and OH-139 accumulated higher Na concentration as compared to salt sensitive Sitara-9101 and Okra-7080. The root and leaf Na contents were found to be enhanced with the increment in salt stress, however the tolerant cultivars exhibited high ratios of Na in their roots as compared to the leaves. On the basis of Na contents, it can be concluded that OH-713 and OH-139 created a hindrance for the transport of high amount of Na to shoot/leaves therefore, they accumulated high concentration of Na in their roots but that kind of mechanism was not observed in salt sensitive cultivars.

Results regarding chloride contents showed that roots of salt tolerant cultivars (OH-713 and OH-139) accumulated high Cl contents as compared to salt

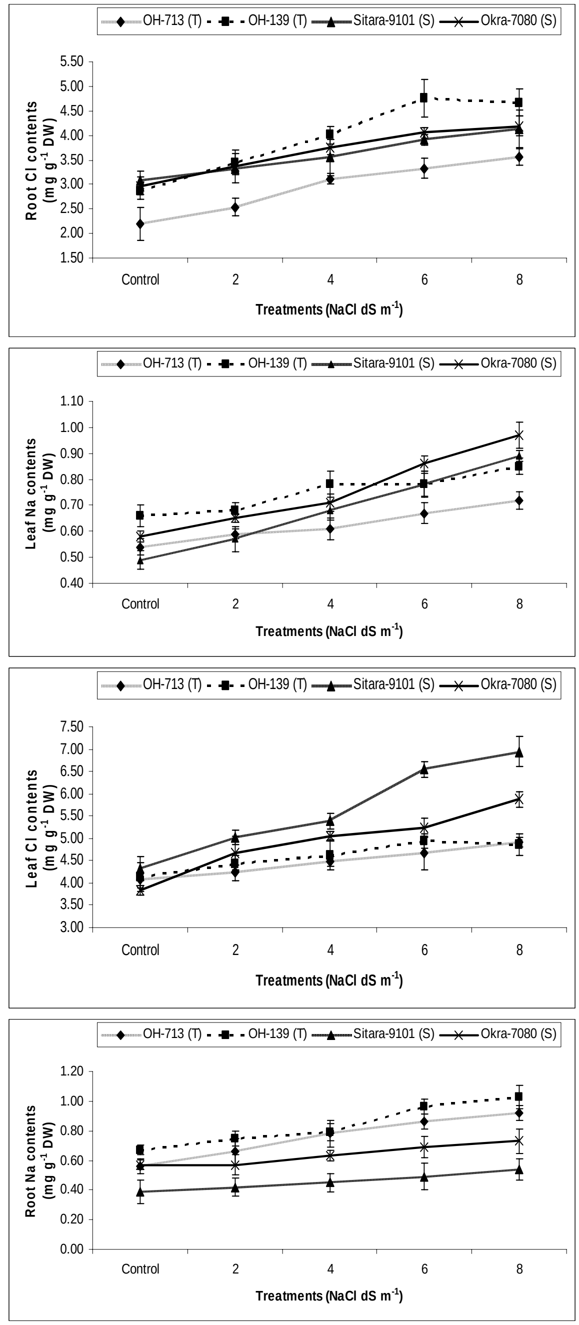

Fig. 4: Effect of salt stress on ionic attributes of okra cultivars under salt stress

sensitive cultivars (Sitara-9101 and Okra-7080). On the other hand, leaves of salt sensitive cultivars (Sitara-9101 and Okra-7080) had maximum Cl accumulation as compared to salt tolerant cultivars (OH-713 and OH-139). On the basis of Cl contents, it can be concluded that OH-713 and OH-139 did not transport high amounts of Cl in upper plant parts due to this reason these cultivars showed high ratios of Cl in their roots and low in their leaves.

DISCUSSION

Plant growth is negatively correlated with the amount of salts in root zone. In present study, salt stress significantly reduced both fresh and dry plant biomass in salt-tolerant and non-tolerant okra genotypes. This reduction in plant biomass could be attributed to nutritional imbalance, ion toxicity, reduction in cell turgidity and disturbance in photosynthetic apparatus. Salt stress creates a nutritional imbalance within the plant by limiting entry of beneficial nutrients involved in various plant metabolisms which consequently cause reduction in plant biomass production. Furthermore, salt stress also reduces the photosynthetic activity by reducing chlorophyll contents, number of stomata and stomatal conductance (Balal et al., 2012), so it could also be a reason of reduced fresh and dry plant biomass in tested salt-tolerant and non-tolerant okra cultivars exposed to saline conditions. In short it can also be claimed that tolerant cultivars (OH-713 and OH-139) showed less reduction in plant biomass because they deposited less ratios of toxic ions in their tissues, while non-tolerant cultivars (Sitara-9101 and Okra-7080) could not stop the entry of these toxic ions in their shoot and leaf tissues, so they had maximum reduction in plant biomass in response to salinity stress. Like other morphological attributes salt stress also reduced leaf area of both salt-tolerant and non-tolerant okra cultivars. Although, leaf area is regulated by specific genes but abiotic stresses, especially salt stress have negative correlation with this morphological attribute because okra plants of both tolerant and non-tolerant cultivars grown under saline and non-saline conditions had significant variations in their leaf area. The results are confirmed with the findings of Latef and Chaoxing (2011) and Oraby and Ahmad (2012) who found a significant reduction in leaf area of tomato and oat plants, respectively submitted to saline conditions. The green pigment chlorophyll is vital for photosynthesis and absorbs the light. The excessive amount of salts/ions (Na and Cl) within the leaf tissue may disturb the cellular metabolisms and cause degeneration of cell organelles and it leads to the destruction of green pigments. In present study, senescence may be the possible reason for reduction in chlorophyll contents because a significant number of yellow leaves were observed in salt-stressed plants of tested okra cultivars (OH-713, OH-139, Sitara-9101 and Okra-7080). The reactive oxygen species (ROS) destroy membranous structures of chloroplasts by oxidizing their phospholipids, which leads to degradation in chlorophyll contents (Hassine and Lutts, 2010). Minimum decline in chlorophyll contents of OH-713 and OH-139 could have been due to less generation of ROS species, ultimately reduced lipid peroxidation of their membranes. Whereas, Sitara-9101 and Okra-7080 had high ratio ROS species so exhibited maximum decline in green pigments by the process of lipid peroxidation. Excessive amounts of toxic ions in the leaf tissues of tested okra cultivars may also be another chlorophyll degrading agents. These toxic ions (Na and Cl) displace the Mg in the structure of chlorophyll thus causes its degradation. Various researchers studied brassica, maize and wheat plants under salt-stressed conditions and recorded a significant reduction in chlorophyll contents (Qu et al., 2012; Zheng et al., 2012).

Under stressed conditions, plants accumulate various compatible solutes i.e. proline and glycinebetaine which enhance the osmotic adjustment potential of plants. Since, in present study salt stress accelerated the accumulation of proline and glycinebetaine both in salt-tolerant (OH-713 and OH-139) and salt-sensitive (Sitara-9101 and Okra-7080) cultivars, so it can be regarded as an adaptation to withstand saline conditions. OH-713 and OH-139 had maximum accumulation of proline and glycinebetaine, which indicated that these cultivars had more efficient osmotic adjustment capacity so they well overcame the drastic effects of ion-toxicity and osmotic stress. Our results confirmed the findings of Nounjan et al. (2012) and Yiu et al. (2012) who studied the various plant species under saline condition sand significant elevation in these compatible solutes were recorded with increasing salinity levels.

The formation of reactive oxygen species (ROS) is a very drastic effect of salt stress (Devasagayam et al., 2004). The elevation in the concentration of ROS can cause a severe damage to cell structures by the process of oxidation of cell membranes, which is termed as oxidative stress (Apel and Hirt, 2004; Hussain et al., 2013). Under stressed conditions, a defensive system called as antioxidant system becomes activated. This system consists of various antioxidant enzymes like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), these enzymes scavenge the ROX species. Although, in this study the salt-stressed plants of tested okra cultivars exhibited a significant elevation in their antioxidant activities as compared to those grown under non-saline conditions, but maximum enhancement was recorded in salt-tolerant (OH-713 and OH-139) cultivars in comparison to salt-sensitive (Sitara-9101 and Okra-7080) cultivars. The high antioxidant activity of OH-713 and OH-139 showed that these cultivars had well withstood the saline conditions by significantly eliminating the ROS, because of high antioxidant activity of SOD, POD and CAT. From the results regarding the antioxidant activities it is depicted that a strong correlation exists between the salt tolerance potential and antioxidant activities. The results of present investigation are in accordance with the findings of Nounjan et al. (2012)

and Petridis et al. (2012) who studied the *Oryza sativa* and *Olea europaea* plant species.

Salinity stress has significantly caused a nutritional imbalance in tested okra cultivars. The accumulation of toxic ions in roots and leaves is a significant tool for evaluating salt tolerance potential of plant species. The low concentration of accumulation of toxic ions in the leaves of OH-713 and OH-139 showed that their leaves had faced less salt-injury due to ion-toxicity. A significant adoption was observed in case of tolerant cultivars because they accumulated high ratios of toxic ions (Na and Cl) in their roots. However, such mechanisms were not observed in case of salt-sensitive cultivars, so high ratios of Na and Cl were transported to upper plant parts (stems and leaves), where they negatively influenced various morpho-physiological and biochemical process.

So it can be concluded that salt stress had produced drastic effects on all tested okra cultivars but the tolerant cultivars (OH-713 and OH-139) were better adapted to stressed conditions as compared to sensitive cultivars (Sitara-9101 and okra-7080).

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