

MORPHOLOGICAL, PHYSIOLOGICAL AND BIOLOGICAL OVERVIEW ON FOUR TROPICAL FOREST TREE SPECIES UNDER DIFFERENT DROUGHT AND SALINITY INTERACTION STAGES

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Abstract

Plants suffered majorly when encountered the combined drought and salinity stresses. In current study, different levels of combined drought and salinity stresses impact on growth characteristics, some physiological and biochemical parameters of *Tectona grandis*, *Gmelina arborea*, *Dalbergia latifolia* and *Pongamia pinnata* seedlings have been examined. The pot culture method adopted in the CRBD manner for one year. Stress conditions were set according to CPE values, PWP and saline water respectively. Five treatments *viz*: control, 4 dS/m + moderate drought, 4 dS/m + severe drought, 8 dS/m + moderate drought, and 8 dS/m + severe drought, were considered to perform the experiment. The height, collar diameter, total biomass and leaf size reduced with the increasing combined drought and salinity levels in each species seedlings. This also includes reduction of photosynthetic rate, stomatal conductance, transpiration rate, total chlorophyll content, protein and ascorbic acid (*T. grandis*). The ascorbic acid in the rest of the species and proline content come up with the reverse trend. *D. latifolia* seedlings exceptionally showed higher growth, physiological and biochemical performances under severe drought conditions at the same salinity level. Furthermore, evaluation of tree species for drought and salinity stresses can help in raising plantations in such areas.

Keywords: Drought, Salinity, Pot culture, Growth, and Parameters.

Introduction

The changing climatic conditions are affecting humankind majorly over the last three decades. Consequently, the fundamental sources of our daily resources, that is, plants, are going through

significant yield and productivity losses which are still in continuation. For instance, the global production of wheat has decreased by 5.5% (Lobell *et al.*, 2011). These global environmental changes continue to increase the severity of the various abiotic stress forms (Tester and Langridge 2010). The abiotic environmental factors such as drought and salinity are the significant plant stressors which mainly influence the plant development and originate serious productivity losses [Tester and Langridge 2010; Agarwal *et al.*, 2013; Kosova *et al.*, 2018]. Apparently, several countries are facing water deficiency and salt accumulation conditions, creating salinization of groundwater. Therefore forcing to develop of additional water resources for agricultural purposes (Dawalibi *et al.*, 2015).

According to the UN body, 12 million hectares of arable land are lost annually due to drought and desertification (Bafana 2017). Moreover, the recent data studies from 1980 to 2015 for wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.) reported 21 and 40% yield reductions on a global scale due to water deficiency conditions (Daryanto *et al.*, 2016). Similarly, salinization has influenced approximately 25% of the total global land (Luo *et al.*, 2017) including 20% of total and 33% of irrigated agricultural lands (Pitman and Lauchli 2002). Salinity is accountable for wheat's 60% yield loss, thus, insuring food insecurity. The forest trees also face challenges under drought and salinity stresses. These species and related genotypes are mostly capable to withstand such stresses which are further used for forest management purposes (Lukac *et al.*, 2010).

Salinity and drought stress show a high degree of similarity with respect to physiological, biochemical, molecular and genetic effects (Sairam and Tyagi 2004). Salt or drought stress impairs the mineral nutrient uptake and metabolic activities related to protein synthesis in plants (Alrashedi *et al.*, 2018). The salt-stressed plants face toxic damage in addition to osmotic imbalances which become the function of nutritional disequilibrium and high salt levels uptake for plants (Munns and Tester 2008). Under drought conditions, water deficiency becomes the main limiting factors for plant growth causing various morphological and eco-physiological changes like the decline of water content, turgor, total water potential, wilting point, stomatal conductance, photosynthesis, disturbance in metabolism, and cell enlargement and growth reduction (González *et al.*, 2012; Shao *et al.*, 2008; Amarjit *et al.*, 2005).

The combination of salinity and drought stress affects the functioning of plant's antioxidant enzymes thereby decreasing physiological mechanisms (Umar and Siddiqui 2018) and cellular energy and altering their cellular architecture (Miller *et al.*, 2010; Zhu *et al.*, 2010). Even an external supply of nutrients is not sufficient to improve plant growth if these stresses are severe (Hu and Schmidhalter 2005). The combination stresses effects are very complex and result in adaptive changes or deleterious effects. Both the stresses are responsible for the decrease in the water potential, reduction in cell growth, root growth, and shoot growth and also cause inhibition of cell expansion and reduction in cell wall synthesis (Chaitanya *et al.*, 2003). Tavousiet *et al.*, (2015) reported a wide impact on plant metabolism resulting in an impaired growth under salinity and drought stress.

In arid and semi-arid regions, seed screening for salinity stress has become important for different growth stages of the plant (Mohammadizad *et al.*, 2013). Therefore, breeding crops for stress tolerance plays a significant role in agriculture. However, the process requires a proper understanding of physiological characteristics and natural variations. In the present study, seedling growth of four commercially important tropical forest tree species was examined under different salinity and drought combination stresses for one year. Further, the study compares the tolerance strategy on the basis of relationship among different morphological, physiological, and biochemical parameters.

1. Material and Methods

1.1. Experimental setup

Seedlings of *Tectona grandis*, *Gmelina arborea*, *Dalbergia latifolia*, and *Pongamia pinnata* were raised by sowing seeds in nursery beds (10 x 1 m) using sand as a sowing medium. Further, the raised seedlings with 2-3 leaves are then transferred to polythene bags (15 x 23 cm) filled with soil, sand, and farmyard manure (2:1:1) such that the weight of each bag remains constant (2 kg). The pot culture experiment was established in a factorial RBD manner and subjected to drought conditions (Moderate and Severe) under two salinity levels (4 and 8 dS/m). This includes total 5 treatments *viz*: C - control, 4MD - 4 dS/m + moderate drought, 4SD - 4 dS/m + severe drought, 8MD - 8 dS/m + moderate drought, and 8SD - 8 dS/m + severe drought.

The drought condition was created by maintaining water deficiency through Cumulative Pan Evaporation (CPE) readings calculated to Permanent Wilting Point (PWP) in each species. This was considered as the establishment of severe drought condition while CPE counted till half of the days of attained PWP was considered as moderate drought. The PWP was recorded according to Savva and Frenken (2002) procedure in each species *viz*: 6.42% in *Tectona grandis*, 8.61% in *Gmelina arborea*, 10.89% in *Dalbergia latifolia*, and 3.41% in *Pongamia pinnata* attained in 50 (half - 25), 14 (half - 7), 20 (half - 10), and 31 days (half - 15) respectively. The seedlings were then irrigated with saline water (doses equal to 4 and 8 dS/m) equal to the species specific field capacity calculated by following Tyree *et al.* (2002) procedure one day before attaining respective fully and semi both PWP. The control condition in each species was maintained by simply irrigating the seedlings equal to field capacity in 2-3 days. Each treatment of particular species consists of nine seedlings and is replicated thrice. The experiment was carried out for one year and observations began after one month of seedling establishment. The experimental site was covered with polythene shed on rainy days.

1.2. Growth measurements

The height (cm) and collar diameter (cm) of each species seedlings were recorded after every three months including the pre-treatment stage using scale and vernier caliper. Similarly, the mature leaves per treatment were plucked after every three months and considered for leaf size (cm²) measurement using Systronics make Leaf Area Meter. The selected species shown strong mortality behavior after crossing 6 months duration of the experiment, therefore, the data presented here is the average data of first six months. The mortality rate of each species was measured for second

half year. Total biomass (dried at 50 ± 5 °C) of seedlings was recorded by harvesting three seedlings from each treatment after one year duration.

1.3. Physiological measurements

The photosynthetic rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration rate and stomatal conductance ($\text{mmol m}^{-2} \text{s}^{-1}$) of the seedlings in each species were estimated from mature leaves at three months interval for half year using CID – 340 make Photosynthesis System, analyzed in the morning (8:00 AM to 10:00 AM).

1.4. Biochemical analysis

Following biochemical parameters were analyzed after six months of experiment through mature fresh (total chlorophyll) and dried (proline, ascorbic acid and protein) leaves.

Arnon's method (1949) used for quantification of total chlorophyll (mg/g f.wt.) content. Proline content (mg/g d.wt.) in the leaves was analyzed according to the Bates *et al.*, (1973) procedure. Ascorbic acid (Vitamin C) content (mg/g d.wt.) was determined using spectrophotometric method suggested by Malik and Singh (1980). Total soluble protein content (mg/g d.wt.) was estimated following Bradford's (1976) method by UV-VIS Spectrophotometer.

1.5. Statistical analysis

The data were analyzed using ANOVA (Analysis of Variance) table and CD (critical difference) like statistical tools by SX software at 5% significance levels among the various treatments.

2. Results

2.1. Growth characteristics

The significant ($P < 0.05$) difference was observed between the treatments in each species regarding growth characteristics. Figure 1-3 illustrates that the average values of height, collar diameter and total biomass decreased with the increasing severity of drought and salinity levels in all species except in *D. latifolia* species. Here, severe drought values were found higher than moderate one at the same salinity level. In addition, the *T. grandis* and *P. pinnata* seedlings shown little higher height, collar diameter and total biomass readings at 8 dS/m (under both SD and MD respectively) salinity level. In fact, the above species still followed the decreasing trend and the measurements were lower than control seedlings. The highest height and collar diameter under combined stress treatments was observed in *G. arborea* (22.19% and 23.35%) at 4MD while total biomass was observed in *D. latifolia* (26.76%) at 4SD whereas *G. arborea* showed least height (13.07%) and collar diameter (14.01%) and *P. pinnata* seedlings showed least biomass (5.85%) at 8SD. Furthermore, the average height, and collar diameter found rising with the increasing age of seedlings by 7.16% & 1.33 times (*T. grandis*), 6.10 & 50.18% (*G. arborea*), 69.99% & 1.24 times (*D. latifolia*), and 10.41% & 1.02 times (*P. pinnata*) on an average of all treatments respectively, for half year. After six months as per treatment effects the seedlings started dying and recorded 100% mortality rate recorded (Figure 2) at the end of year/experiment, leaving alive only control seedlings of each species.

The number of leaves were continue to decline with the increasing age of seedlings followed by complete leaf senescence when reached to 6th month in *D. latifolia* and 8th month in rest of the species except control seedlings. The average leaf size (Figure 4) was observed continuously reducing with the growing age and increasing stress levels as compare to control with maximum average value at 4MD and minimum value at 8SD in *T. grandis*. (39.79% and 4.75%) under combination stress treatment.

The root/shoot ratio was observed higher than control under combination stress treatments in all the species and showed values <1 in *T. grandis* and *P. pinnata* seedlings (Table 1). In *G. arborea* and *D. latifolia* seedlings root/shoot ratio recorded highest at 4 dS/m level under moderate drought while in *T. grandis* and *P. pinnata* recorded under severe drought at the same salinity level as compared to control.

2.2. Physiological changes

The physiological performances (photosynthetic rate, transpiration rate and stomatal conductance) observed maximum in control and then decreased with the increase of combination stress severity (Figure 5-7). All the three selected physiological parameters found increasing with the progressive age in control seedlings for six months. The transpiration rate and stomatal conductance followed the same trend in stress prone seedlings as control but the photosynthetic rate recorded reducing with the increasing age. In *P. pinnata* and *G. arborea* seedlings highest photosynthetic rate, and transpiration rate and stomatal conductance were observed while *D. latifolia* and *T. grandis* showed lowest in case of control seedlings. Under combination stresses, maximum photosynthetic rate, transpiration rate and stomatal conductance was observed at 4MD (24.00%) and SD (26.00%) by *D. latifolia*, at 4MD (25.11%) by *T. grandis*, and at 4SD (28.84%) by *D. latifolia* seedlings respectively while minimum was observed at 8SD by *P. pinnata* (5.42%) and *G. arborea* (8.02% and 12.11%) seedlings.

2.3. Biochemical performances

The selected biochemical parameters found significantly ($P < 0.05$) differed among the different treatments in each species (Figure 8-11). The total chlorophyll observed maximum in control and followed decreasing trend with the increase of combination stress levels except *D. latifolia* seedlings where the amount increased with the increasing severity of drought at 8 dS/m salt concentration. The highest amount was found at 4MD (29.80%) in *P. pinnata* seedlings while lowest was observed at 8SD (8.50%) in *G. arborea*. The protein analysis showed variable results in the selected species. *T. grandis* and *G. arborea* seedlings followed decreasing trend with the rising combined stresses except at 4SD where the values raised a little. In *D. latifolia* seedlings protein showed firstly decreasing trend then increased at 8 dS/m salinity level. *P. pinnata* seedlings showed continuously decreased in protein with the increasing combination stresses. The highest and lowest amount was found at C (48.85%) and 8SD (12.66%) in *P. pinnata* seedlings respectively.

The ascorbic acid content also found reducing with the increasing combination stresses in *T. grandis*, *G. arborea* (except at 4SD) and *P. pinnata* seedlings while *D. latifolia* followed reverse

trend. The maximum and minimum values obtained at 8SD (49.84%) in *D. latifolia* and at C (11.27%) in *G. arborea* seedlings respectively. The proline content found higher in stress seedlings (highest at 8SD in *P. pinnata*– 57.52%) than control increased with the increasing combination stresses.

3. Discussion

3.1. Growth characteristics

Water availability under combined drought and salinity conditions become major constraint as compared to individual stresses for the plant growth as increasing salinity levels makes soil water potential and plant osmotic potential low (Lee *et al.*, 2004). This indicates that the effects of combined drought and salinity stress on plants are more devastating and complex than the individual one which makes the plants either tolerant or sensitive (Ma *et al.*, 2020). In the present investigation, the species showed decline in the growth performances (height, collar diameter and total biomass) under the effect of increasing combination stresses. Studies on several species like *Nerium oleander* (Kumar *et al.*, 2017), *Brassica oleracea* (Sahin *et al.*, 2018) *Larix decidua* (Plesa *et al.*, 2018) and *Punica granatum* (Tavousi *et al.*, 2015) supports the above trend and showed reduction in plant growth characteristics under combination of drought and salinity stress. This stress combination causes reduction in cell, cell wall synthesis, root and shoot growth and inhibition of cell expansion (Chaitanya *et al.*, 2003) due to low water potential which inhibits the differentiation of plant tissue (Yang *et al.*, 2006). The additive effect of drought and salinity creates osmotic tension which together with the ionic toxicity (due to uptake of excessive Na⁺ Cl⁻ ions) disturbs the normal physiological process of plant (Munns, 1992). Similar results reported by Li *et al.*, (2018) during his studies in birch (*Betula platyphylla*) who observed germination rate, germination potential and germination index reduction with increasing salt concentrations (200, 400 and 800 mM). The adverse effects of combined salt and drought stress experiment on *Brassica oleracea* were observed on fresh and dry weights of shoot and root than individual stresses (Sahin *et al.*, 2018). The pomegranate yield also found decreasing with increasing water deficiency and salinity levels in combination by Tavousi *et al.*, (2015).

On the contrary, the reverse trend in *Dalbergia latifolia* species at same salinity level might be the case where ionic toxicity perhaps rise in MD as compared to SD as frequency of saline irrigation is more in MD than the SD. Alternatively, the salt and water movement is comparatively higher in MD than SD. Also, one of the reasons is that, at higher salt concentration there might be the occurrence of ions channelization process (Munns, 1992). However, the above discussed facts like maximum ionic toxicity in MD and ion channelization perhaps supports the sudden slight growth at 8 dS/m salinity level in *T. grandis* and *P. pinnata* species.

The growth parameters (height and collar diameter) of selected species seedlings showed increasing pattern with the age inspite being under the pressure of different combined drought and salinity treatments for six months and then started showing rapid mortality with the 100% mortality rate till the end of year. This implies the tolerance ability of the plant species to withstand against the water deficiency and ionic toxicity level as an adaptive feature for six months (Munns, 1992).

The uptake of ionic concentration is higher when subjected to stress treatment for shorter duration initially. This helps the plant in escalating the physiological processes which further improve the plant growth. Further, *D. latifolia* species showed growth after defoliation probably due to shoot photosynthesis. In fact, the woody stem and shoots are also responsible for photosynthesis in the absence of plant leaves (Bossard *et al.*, 1992; Tikhonova *et al.*, 2017). During absence of leaves, plant utilizes its maximum energy into growing taller to maximize its exposure to light with the help of auxin hormone which is present in the shoot tip inhibiting the growth of axillary buds (Moore *et al.*, 1995). The combined effect of osmotic disturbances and ionic stress is the cause of rapid increase of mortality rate. The presence of higher ionic concentration (Na^+ and Cl^-) for longer duration disturbs the plant physiology mainly by lowering down the photosynthetic activity (energy source) with the strong inability to recover even through effort of little watering. In this way in the absence of required energy, accumulation of ions in the leaves continue to takes place causing ionic toxicity, leaf senescence and then leading to plant death. The study on high salinity associated with drought in the *Spartina alterniflora* halophyte demonstrated reduction in survival to 71% (Brown *et al.*, 2006).

The leaf size in the selected species was found to decrease with increase in salt and drought stress. Similar trend was reported by Munns (1992) who claimed that specific effect appears as accumulated salt injury in leaves causes its die off because of a further rapid ionic movement in the cell walls or cytoplasm when the vacuoles can no longer sequester incoming salts. Niu *et al.*, (2013) studied comparative effect of salinity and drought stress on growth in *Jatropha curcas* seedlings which showed higher restrictions in leaf growth when compared to the salt-stressed ones. Salinity and drought in combination reduces leaf, shoot and root dry/fresh weights in transgenic tobacco (Yang *et al.*, 2005) and in Tibetan wild & cultivated barleys (Ahmed *et al.*, 2013) which become more pronounced as the severity increases.

All the stressed seedlings of our experiment showed increase in root/shoot ratio as compared to control, which is in agreement with Dawalibi (2015), who reported increase in the root/shoot ratio under combined stress conditions and concluded that this is due to adaptive mechanism to reduce transpirational surface and encourage water absorption from the soil (Joly *et al.*, 1989). Abdallah *et al.*, (2018) also recorded growth reduction of the above ground plant organs than the underground organs in Olive plant (*Olea europaea* L.).

3.2. Physiological changes

The physiological process of plants alters as a response to existing environmental stresses. The physiological parameters exhibited a declining trend when subjected to various concentrations of salinity and water deficit conditions. The effects of drought and salt stresses on photosynthesis can be in two ways, firstly, direct (retarding diffusion through the stomata or changes in photosynthetic metabolism) and secondly, secondary (the oxidative stress) response. The alteration of gene expression and the down-regulation of some photosynthetic genes also responsible for reduction in photosynthetic activity. Compared to drought, salt stress affect more genes and their combined

effects lead to dehydration, osmotic stress and ionic toxicity in plants (Chaves *et al.*, 2009). The saline conditions are also responsible for low soil water potential which makes plants to adapt and manage the low osmotic potential strategy, thus, preventing loss of turgor and develop lower plant water potential. This further helps the plants to access little water from the soil solution for their growth (Taiz and Zeiger, 2006). González *et al.*, (2012) reported marked reduction of photosynthetic rate and stomatal conductance under water deficit and salinity conditions in *Nicotiana glauca*. The species adapted the osmotic adjustment strategy by stomatal closure.

3.3. Biochemical performances

The biochemical components came up with the remarkable and significant changes when imposed to different level of salt and drought stresses. With the increase in combination stresses, total chlorophyll generally decreased. Kalaji *et al.*, (2018) reported similar results due to mechanism of action on the light-dependent photosynthesis phase causing damage to the oxygen-evolving complex and reaction centers with a simultaneous increase in dissipated energy under salinity and drought conditions. Kumar *et al.*, (2017) found that drought and salinity stress combination are responsible for reduction in photosynthetic pigments, slightly increasing of specific osmolytes of leaves and induction of oxidative stress by the accumulation of malondialdehyde (MDA). Likewise, in Olive (*Olea europaea* L.) crop the reduction of all photosynthetic parameters (the integrity of photosystem II and leaf nitrogen content, and corresponding representation of photosynthetic apparatus proteins, Calvin-Benson cycle and nitrogen metabolism) took place under combination stresses (Abdallah *et al.*, 2018). Plesa *et al.*, (2018) observed degradation of photosynthetic pigments in European larch tree (*Larix decidua*) and concluded that salinity and drought combined conditions are responsible for such changes.

Other estimated biochemicals in our experiment like proline showed increasing trend in selected species seedlings. Ascorbic acid and protein also showed decrease with increase in salt concentration. These findings corroborate with other authors, who also reported increase in some biochemicals and antioxidants with stressed intensities in *Tamarix chinensis* (Liu *et al.*, 2014) and European larch tree (*Larix decidua*) (Plesa *et al.*, 2018). Sahin *et al.*, (2018) found that the proline content and other such activities increase under both salinity and drought stress as well as under their combination. Jang *et al.*, (2014) reported that the drought stress damages the aquaporins, inhibiting the incorporation of amino acids into proteins, causing decrease in the protein content of the tissues.

Conclusion

The overall growth reduction in each selected species increased with the increasing severity of combination of drought and salinity stresses for initial six months and after it shown complete mortality. This indicates the species inability to withstand under such stresses for longer duration. However, *T. grandis* and *P. pinnata* species showed the mortality sign much later than other two species which is a struggle to maintain tolerance ability. Therefore, such species plantations can be raised for reclamation of the moderate type of drought and salinity combination stress prone area. Furthermore, the reduction in attributes like total chlorophyll content, protein, photosynthetic

rate, transpiration rate and stomatal conductance followed by early leaf fall are the reasons for overall growth reduction in the selected species. The growth of plants, no doubt, is the result of their biochemical and physiological processes upholding. On the contrary, the increased biochemicals like ascorbic acid and prolin including with reduced physiological mechanisms is the sign of activation of internal defense mechanism in the species which might have supportive role in their growth for initial six months. However, there is need to incorporate more studies associated to molecular work in this context for further cultivation of tolerant species and related tree breeding programmes. In addition, there is need to screen the tree species against combined drought and salinity stress conditions which may help to raise plantation programs on such affected areas.

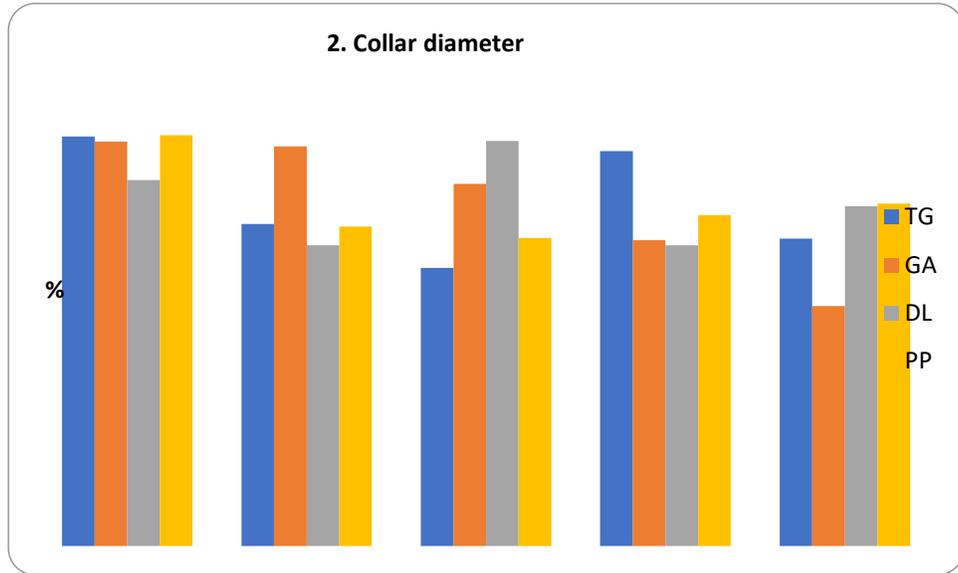
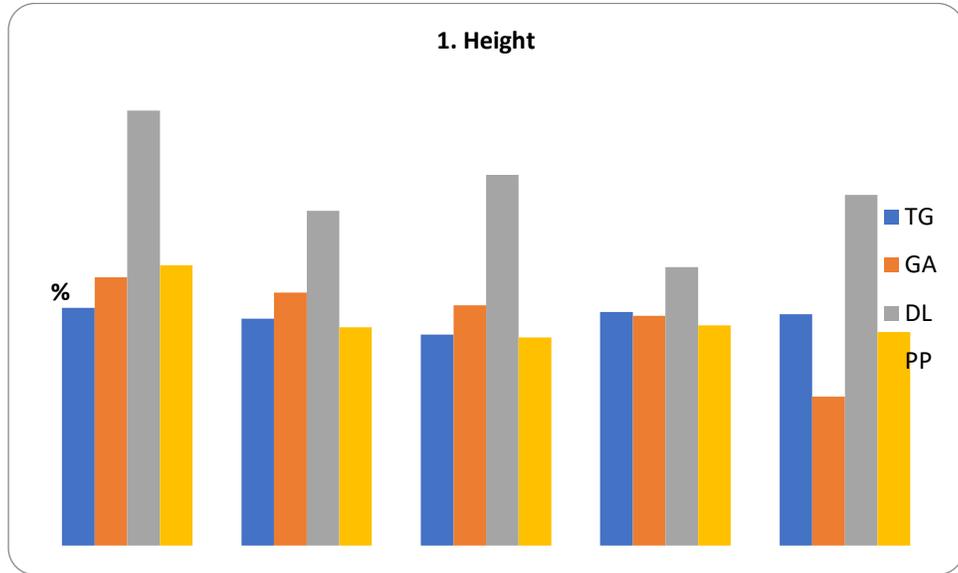
Literature cited

- Abdallah, M. Ben, D. Trupiano, A. Polzella, E. De Zio, M. Sassi, A. Scaloni, M. Zarrouk, B.N. Youssef, and G.S. Scippa. 2018. Unraveling physiological, biochemical and molecular mechanisms involved in olive (*Olea europaea* L. cv. Chetoui) tolerance to drought and salt stresses. *Journal of Plant Physiology* 220:83-95.
- Agarwal, P.K., P.S. Shukla, K. Gupta, and B. Jha. 2013. Bioengineering for salinity tolerance in plants: State of the art. *Molecular Biotechnology* 54:102–123.
- Ahmed, I.M., F. Cao, M. Zhang, X. Chen, G. Zhang, and F. Wu. 2013. Difference in yield and physiological features in response to drought and salinity combined stress during anthesis in Tibetan wild and cultivated barleys. *PLoS ONE* 8(10):e77869.
- Alrashedi, A., S.O. Bafeel, A.A.A. Toukhy, Y.A. Zahrani, and H. Alsamadany. 2018. Effect of drought and salinity stresses on mineral and total protein contents of Moringa. *International Journal of Biosciences* 12(5):161-168.
- Amarjit. K.N., S. Kumari, and D.R. Sharma. 2005. In vitro selection and characterization of water-stress tolerant cultures of bell pepper. *Indian Journal of Plant Physiology* 10(1):14-19.
- Arnon, D.I. 1949. *Plant Physiology* 24:1.
- Bafana, B. 2017. *The High Price of Desertification: 23 Hectares of Land a Minute*. Inter Press Service, BULAWAYO, Zimbabwe.
- Bates, L.S., R.P. Waldeen, and I.D. Teare, 1973. *Plant Soil* 39:205.
- Bossard, C.C. and M. Rejmanek. 1992. Why have green stems? *Functional Ecology* 6:197–205.
- Bradford, M.M. 1976. *Analytical Biochemistry* 72.
- Brown, C.E., S.R. Pezeshki, and R.D. Delaune. 2006. The effects of salinity and soil drying on nutrient uptake and growth of *Spartina alterniflora* in a simulated tidal system. *Environmental and Experimental Botany* 58(1-3):140-148.
- Chaitanya, K.V., D. Sundar, P.P. Jatur, and R.A. Ramachandra. 2003. Water stress effects on photosynthesis in different mulberry cultivars. *Plant Growth Regulation* 40:75–80.
- Chaves, M.M., J. Flexas, and C. Pinheiro. 2009. Photosynthesis under drought and salt stress: Regulation mechanisms from whole plant to cell. *Annals of Botany* 103(4):551–560.
- Daryanto, S., L. Wang, and P.A. Jacinthe. 2016. Global synthesis of drought effects on maize and wheat production. *PLoS ONE* 11:e0156362.

- Dawalibi, V., M.C. Monteverdi, S. Moscatello, A. Battistelli, and R. Valentini. 2015. Effect of salt and drought on growth, physiological and biochemical responses of two *Tamarix* species. *iForest* 8:772-779.
- González, A., W. Tezara, E. Rengifo, and A. Herrera. 2012. Ecophysiological responses to drought and salinity in the cosmopolitan invader *Nicotianaglauca*. *Brazilian Journal of Plant Physiology* 24(3):213-222.
- González, A., W. Tezara, E. Rengifo, and A. Herrera. 2012. Ecophysiological responses to drought and salinity in the cosmopolitan invader *Nicotianaglauca*. *Brazilian Journal of Plant Physiology* 24(3):213-22.
- Hu, Y., and U. Schmidhalter. 2005. Drought and salinity: A comparison of their effects on mineral nutrition of plants. *Journal of Plant Nutrition and Soil Science* 168:541-549.
- Jang, H.Y., J., Rhee, J.E. Carlson, and S.J. Ahn. 2014. The Camelina aquaporin CsPIP2; 1 is regulated by phosphorylation at Ser273, but not at Ser277, of the C-terminus and is involved in salt and drought stress responses. *Journal of Plant Physiology* 171(15):1401-1412.
- Joly, R.J., W.T. Adams, and S.G. Stafford. 1989. Phenological and morphological responses of mesic and dry site sources of coastal Douglas-fir to water deficit. *Forestry Science* 35:987-1005.
- Kalaji, M. Hazem, L. Rackova, V. Paganova, T. Swoczyna, S. Rusinowski, and K. Sitko. 2018. Can chlorophyll-a fluorescence parameters be used as bio-indicators to distinguish between drought and salinity stress in *Tiliacordata* Mill? *Environmental and Experimental Botany* 152:149-157.
- Kosova, K., P. Vitamvas, M.O. Urban, I.T. Prasil, and J. Renaut. 2018. Plant abiotic stress proteomics: The major factors determining alterations in cellular proteome. *Frontier of Plant Science* 9:122.
- Kumar, D., M. Al Hassan, M.A. Naranjo, V. Agrawal, M. Boscaiu, and O. Vicente. 2017. Effects of salinity and drought on growth, ionic relations, compatible solutes and activation of antioxidant systems in oleander (*Nerium oleander* L.). *PLoS ONE* 12(9):e0185017.
- Kumar, D., M. Al Hassan, M.A. Naranjo, V. Agrawal, M. Boscaiu, and O. Vicente. 2017. Effects of salinity and drought on growth, ionic relations, compatible solutes and activation of antioxidant systems in oleander (*Nerium oleander* L.). *PLoS ONE*, 12(9):e0185017.
- Lee, G., R.R. Duncan, and R.N. Carrow. 2004. Salinity tolerance of seashore paspalum ecotypes: Shoot growth responses and criteria. *Horticultural Science* 39:1138-42.
- Li, Z., X. Pei, S. Yin, X. Lang, X. Zhao, and G.-Z. J. Qu. 2018. Plant hormone treatments to alleviate the effects of salt stress on germination of *Betula platyphylla* seeds. *Journal of Forestry Research* 30(3):779-787.

- Liu, J., J. Xia, Y. Fang, T. Li, and J. Liu. 2014. Effects of salt-drought stress on growth and physiobiochemical characteristics of *Tamarix chinensis* seedlings. *The Scientific World Journal* 7.
- Lobell, D.B., W. Schlenker, and J. Costa-Roberts. 2011. Climate trends and global crop production since 1980. *Science* 333:616-620.
- Lukac, M., M. Pensa, and G. Schiller. 2010. Tree Species' Tolerance to Water Stress, Salinity and Fire. Vol. 212 in *Forest Management and the Water Cycle. Ecological Studies (Analysis and Synthesis)*, Bredemeier, M., S. Cohen, D. Godbold, E. Lode, V. Pichler, and P. Schleppei (eds.). Springer, Dordrecht.
- Luo, J.-Y., S. Zhang, J. Peng, X.-Z. Zhu, L.-M. Lv, C.-Y. Wang, et al. 2017. Effects of soil salinity on the expression of Bt toxin (Cry1Ac) and the control efficiency of *Helicoverpa armigera* in field-grown transgenic Bt cotton. *PLoS one* 12(1):e0170379.
- Ma, Y., M.C. Dias, and H. Freitas. 2020. Drought and Salinity Stress Responses and Microbe-Induced Tolerance in Plants. *Front. Plant Sci.* 11:591911.
- Malik, E.P., and M.B. Singh. 1980. *Plant Enzymology and Histochemistry* (1st edn.). Kalyani Publishers, New Delhi. 286 p.
- Miller, G., N. Suzuki, S. Ciftci-Yilmaz, and R. Mittler, 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant Cell Environment* 33:453–467.
- Mohammadizad, H.A., I. Khazaei, M. Ghafari, M.F.F. Sinehsar, and R. Barzegar. 2013. Effect of salt and drought stresses on seed germination and early seedling growth of *Nepeta persica*. *International Journal of Farming and Allied Sciences* 2(21):895-899.
- Moore, R., W.D. Clark, and K.R. Stern. 1995. *Atlas of Plant Anatomy: Botany*. William, C. Brown Publishers, Boston.
- Munns, R. 1992. A leaf elongation assay detects an unknown growth inhibitor in xylem sap from wheat and barley. *Australian Journal of Plant Physiology* 19:127–135.
- Munns, R., and M. Tester. 2008. Mechanisms of salinity tolerance. *Annual review of Plant Biology* 59(1):651-681.
- Niu, G., D. Rodriguez, M. Mendoza, J. Jifon, and G. Ganjegunte. 2013. Responses of *Jatropha curcas* to salt and drought stresses. *International Journal of Agronomy* 7.
- Pitman, M.G., and A. Lauchli. 2002. Global impact of salinity and agricultural ecosystems. P. 3-20 in *Salinity: environment – plants – molecules*, Lauchli, A., and U. Luttge (eds.). Kluwer, Dordrecht.
- Plesa, I.M., S. González-Orenga, M. Al Hassan, A.F. Sestras, O. Vicente, J. Prohens, R.E. Sestras, and M. Boscaiu. 2018. Effects of drought and salinity on European larch (*Larix decidua* Mill.) seedlings. *Forests* 9(6):320.
- Sahin, U., M. Ekinici, S. Ors, M. Turan, S. Yildiz, and E. Yildirim. 2018. Effects of individual and combined effects of salinity and drought on physiological, nutritional and biochemical

- properties of cabbage (*Brassica oleraceavar. capitata*). *Scientia Horticulturae* 240:196-204.
- Sairam, R.K., and A. Tyagi. 2004. Physiology and molecular biology of salinity stress tolerance in plants. *Current Science* 86:407-421.
- Savva, A.P., and K. Frenken. 2002. *Crop water requirements and irrigation scheduling irrigation manual module 4*, FAO, Harare.
- Shao, H.B., L.Y. Chu, C.A. Jaleel, and C.X. Zhao. 2008. Water-deficit stress-induced anatomical changes in higher plants. *Comptes Rendus Biologies* 54(3):215–225.
- Taiz, L. and E. Zeiger. 2006. *Plant physiology* (Fourth Edition). Sinauer Associates, Inc., Publishers, Sunderland, USA, 764 p.
- Tavousi, M., F. Kaveh, A. Alizadeh, H. Babazadeh, and A. Tehranifar. 2015. Effects of drought and salinity on yield and water use efficiency in pomegranate tree. *Journal of Materials and Environmental Sciences* 6(7):1975-1980.
- Tester, M., and P. Langridge. 2010. Breeding technologies to increase crop production in a changing world. *Science* 327:818-822.
- Tikhonova, K.G., M.S. Khristina, V.V. Klimova, M.A. Sundirevab, V.D. Kreslavskia, R.A. Sidorovc, V.D. Tsidendambayevc, and T.V. Savchenkoa. 2017. Structural and functional characteristics of photosynthetic apparatus of chlorophyll-containing grape vine tissue. *Russian Journal of Plant Physiology* 64(1):73–82.
- Tyree, M.T., G. Vargas, B.M.J. Engelbrecht, and T.A. Kursar. 2002. Drought until death do us part: A case study of the desiccation-tolerance of a tropical moist forest seedling-tree, *Licania platypus* (Hemsl.) Fritsch. *Journal of Experimental Botany* 53(378):2239-2247.
- Umar, M., and Z.S. Siddiqui. 2018. Physiological performance of sunflower genotypes under combined salt and drought stress environment. *Acta Botanica Croatica* 77(1):36–44.
- Yang, X., Z. Liang, and C. Lu. 2005. Genetic engineering of the biosynthesis of glycinebetaine enhances photosynthesis against high temperature stress in transgenic tobacco plants. *Plant Physiology* 138:2299–2309.
- Zhu, J., B.H. Lee, M. Dellinger, X. Cui, C. Zhang, S. Wu, E.A. Nothnagel, and J.K. Zhu. 2010. A cellulose synthase-like protein is required for osmotic stress tolerance in *Arabidopsis*. *The Plant Journal* 63:128–140.



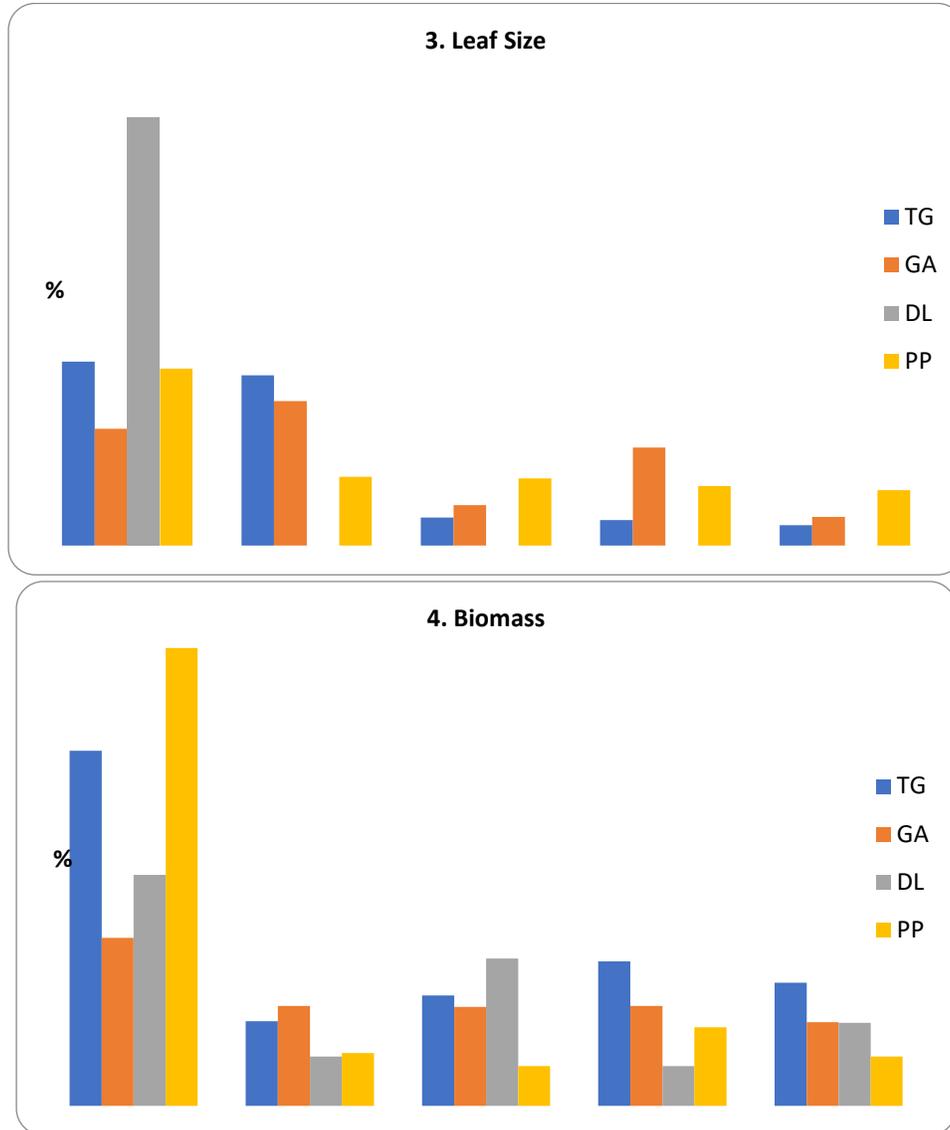
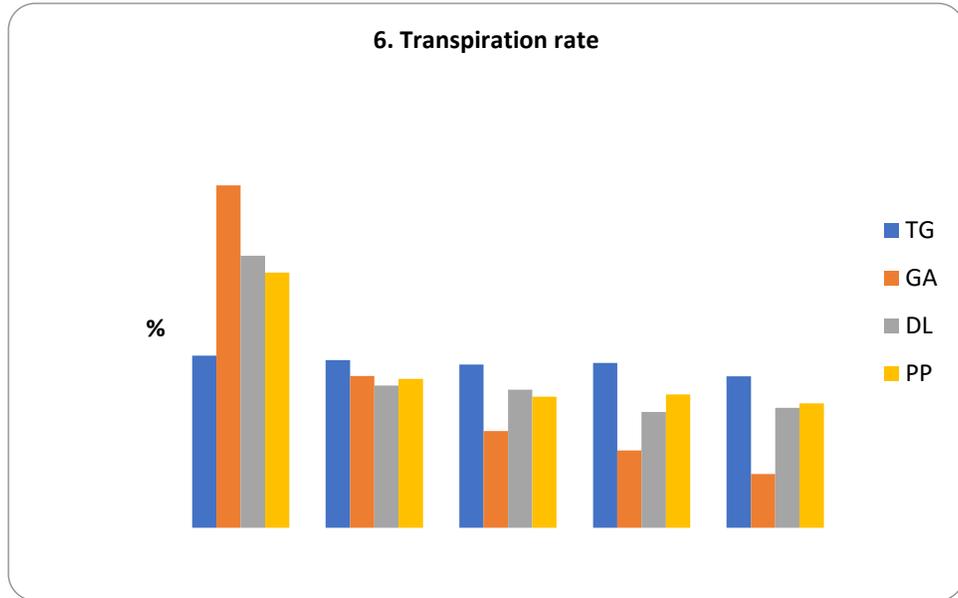
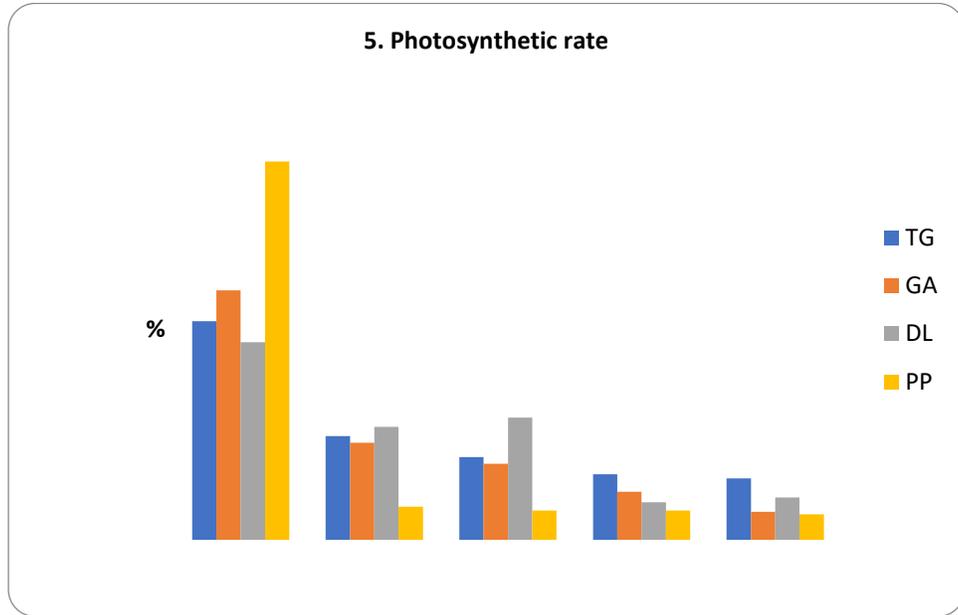


Figure 1-4: 1. Height 2.Collar diameter 3.Leaf size 4.Total biomass under combined drought and salinity stress.



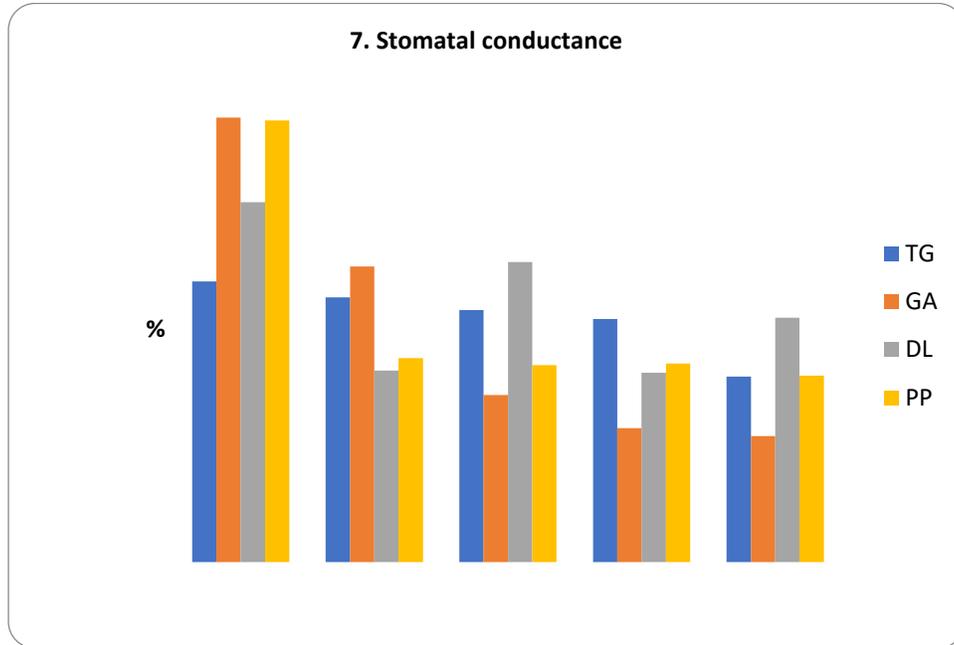
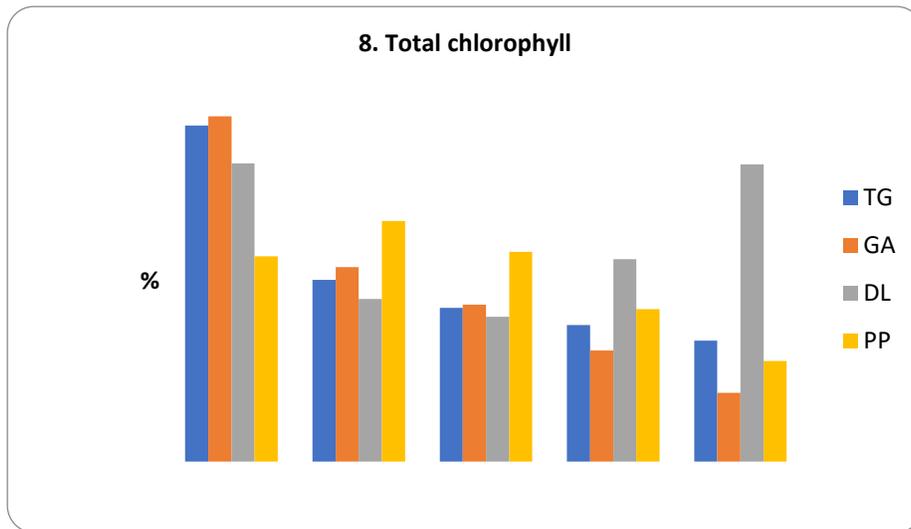


Figure 5-7: 5. Photosynthetic rate 6. Transpiration rate 7. Stomatal Conductance under combined drought and salinity stress.



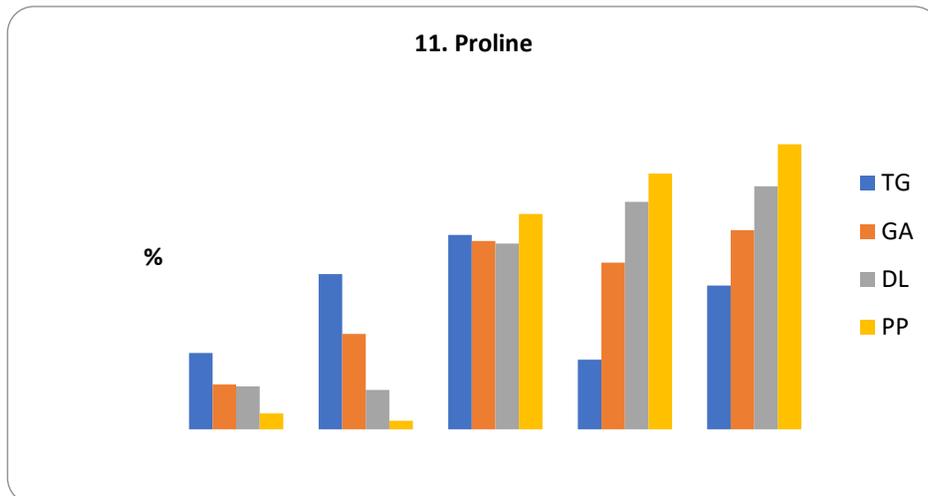
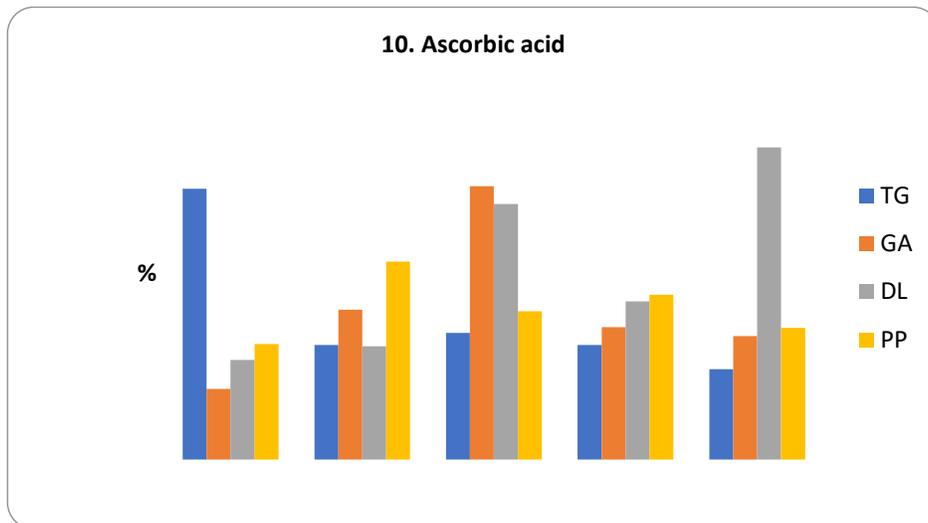
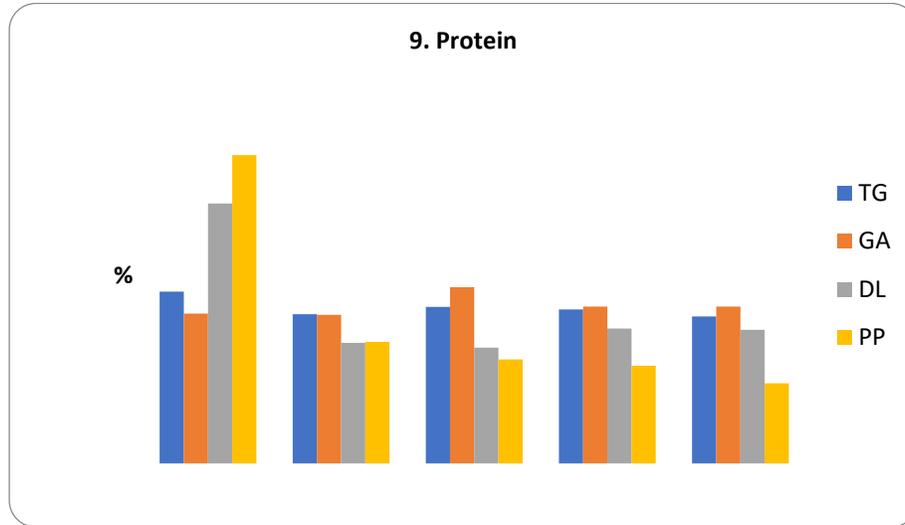


Figure 8-11: 8. Total chlorophyll 9. Protein 10. Ascorbic acid 11. Proline under combined drought and salinity stress.

Table 1: Root/shoot ratio under combined drought and salinity stress.

	TG	GA	DL	PP
Control	1.32	0.94	0.45	1.26
4MD	1.41	1.04	1.84	1.37
4SD	1.68	0.92	0.46	1.95
8MD	1.39	0.95	0.57	1.92
8SD	1.38	1.02	1.25	1.33