

Biochemical and physiological responses of peach rootstocks against drought stress

Naveed Hamayat, Ishfaq Ahmed Hafiz*, Touqeer Ahmad, Irfan Ali and Abdul Ahad Qureshi

Department of Horticulture, Faculty of Crop & Food Sciences, PMAS, Arid Agriculture University, Rawalpindi, Pakistan

*Corresponding author: Ishfaq Ahmed Hafiz (decenthafiz@gmail.com)

Received: 8 January 2020; Accepted: 16 May 2020; Published online: 20 May 2020

Key Message: This study was performed to evaluate the drought resistant peach rootstock for arid and semi-arid areas of Pakistan. It was confirmed that out of three rootstocks used, exotic rootstock (GF-677) proved to be superior against all the levels of drought.

Abstract: Climate change is affecting the peach growing areas of Pakistan, especially semi-arid areas of Pothwar plateau which has great potential for peach production. Drought is one of the major effects of climate change. Therefore, the present study was conducted to evaluate the response of peach rootstocks (GF-677, Peshawar Local and Swat Local) under *in-vitro* drought stress condition, provided by polyethylene glycol (PEG) at various osmotic potential levels. Least leaf osmotic potential (-11.25 bars) was obtained in GF-677 at stress level of -6 bars, while Peshawar Local and Swat Local exhibited higher values i.e. -2.73 and -2.17 bars, respectively. Relative water

contents at osmotic potential -10 bar was lower in Swat Local (77.33%) followed by GF-677 (81.55 %) and Peshawar Local (80.22%). Chlorophyll contents, K contents and membrane stability index (MSI) of the rootstocks were found to have inverse relation with the levels of stress. On contrary, proline contents were found to be positively correlated with the increasing level of drought stress. The interaction between stress levels and rootstocks is elaborated in the present article. From the results, it is clear that GF-677 has greater potential to withstand the water shortage as compared to the local rootstocks being used in the peach industry. So, it can be concluded that GF-677 can be a better rootstock for peach especially in the arid to semi-arid areas of Pakistan which have water shortage as a major problem for fruit production. © 2020 Department of Agricultural Sciences, AIOU

Keywords: Drought, *In-vitro*, Peach, Polyethylene glycol, Rootstocks

To cite this article: Hamayat, N., Hafiz, I. A., Ahmad, T., Ali, I., & Qureshi, A. A. (2020). Biochemical and physiological responses of peach rootstocks against drought stress. *Journal of Pure and Applied Agriculture*, 5(1), 82-89.

Introduction

Peach (*Prunus persica*), owing to its high medicinal and nutritional value, is considered as one of important stone fruit crops of subtropical to temperate regions worldwide (Khushk & Laghari, 2007). Increasing demand of the quality peach fruit throughout the world needs innovative and modern approaches in peach fruit industry. Keeping in view, the selection of proper rootstock is one of the most significant approaches as far as adaptability to soil, vigor of the plants, production life and stresses are concerned (Younas et al., 2008). Swat Local (SL) and Peshawar Local (PL) are the rootstocks being used by the peach industry in Pakistan (Rahman et al., 2000). Due to better anchorage and adaption to local soils and environment, PL is a popular rootstock in Baluchistan and KPK provinces of Pakistan (Ullah et al., 2000). Due to better girth at early stages, it is considered as a better option for vegetative propagation i.e. budding (Khan et al., 1996). SL is a potential rootstock to get higher yield, vigor, and scion-stock compatibility (Ahad et al., 1987). Though well adapted to local conditions, these rootstocks have certain limitations and cannot meet the demand of international fruit market. Another rootstock i.e. GF-677 (almond and

peach hybrid) is also under the use of a few progressive farmers. It is considered as a vigorous rootstock and is considered as a better choice for normal density orchards. Furthermore, it can withstand the problems related to nutrient deficiencies especially iron chlorosis and is considered as a better option for particularly poor and calcareous soils (Monticelli et al., 2000).

Peach cultivation is gaining momentum in subtropical areas of the country due to improved and low chilling peach cultivars. Yet there are certain issues which are creating hindrance to developing peach industry in the region. Deficiency of irrigation water is one of the major issues for peach industry in the region (Wu & Cosgrove, 2000), which is considered as the striking features of the arid areas of the country. Due to erratic and uneven seasonal rainfall, the crop yield varies year by year (Specht et al., 2001). Furthermore, shortage of water for irrigation is increasing day by day due to urbanization and population rise (Boretti & Rosa, 2019). Hence, any knowledge regarding plant drought relationship will help to elucidate the harmful effect of drought stress during water shortage (Nadeem et al., 2019). Furthermore, this knowledge may be helpful for the research to develop new drought resistant rootstocks and to screen the existing rootstocks for drought resistance (Nehra et al., 2005). Plants

response to various biotic and abiotic stresses by adopting the alteration in physiological processes i.e. root elongation, reduction in leaf area, leaf drop, decrease in the photosynthesis and closure of stomata (Galovic et al., 2005). So keeping in view the present climate change scenario, the screening of the available root-stocks for drought resistance is a dire need to exploit the genetic potential of the germplasm (Sivritepe et al., 2008).

Plants response to various abiotic stresses (salinity and drought) has been studied in the numerous studies. These trials were based on field study such as deficit irrigation practices and osmotica use in soil (Kaya et al., 2006). These methods have certain limitations such as environmental effects and time intensiveness. Furthermore, plants also response very slow towards these methods. On the other hand, tissue culture method has a great advantage over the traditional approaches of screening by reducing environmental effects. Moreover, this is a rapid method of screening in which large number of samples can be handled in a smaller area (Gomez et al., 2005). *In-vitro* trials for the evaluation of drought tolerance by the use of various osmotica have also been carried out with great success for many plants (Molassiotis et al., 2006). The selection varies for osmotic agent owing to its advantage for specific plant species and certain benefits over one another. Polyethylene glycol (PEG) is reported to be a suitable chemical for *in vitro* drought-stress trial by lowering water potential of media without being taken up, hence being safe (Sakthivelu et al., 2008). Keeping in view, the present research was carried out to evaluate the performance of various rootstocks (SL, PL and GF-677) *in vitro* against drought stress. This study will be helpful for rising fruit industry of arid and semi arid areas with better economic returns to the farming community.

Materials and Methods

Shoot tips from the plants (PL, SL and GF-677) were used as explants, established already in PTC (Plant Tissue Culture Laboratory) Laboratory of Hort. Deptt. PMASAAUR. Explants were set in culture jars in which medium of growth was MS (Murashige & Skoog, 1962), having pH 5.8, modified according to Ahmad et al. (2003). Media were autoclaved (15 minutes at 121 °C). Incubation of cultures was carried out at 25 °C ± 1 °C under 16/8 hour light (2000 lux). After every 4 weeks, the sub-culturing was done for plant material propagation. To study the level of tolerance of given rootstocks following parameters were noted.

Osmotic potential

Frozen leaves in liquid nitrogen were crushed using mortar and pestle. This crushed sample was centrifuged @ 5000 rpm for ten minutes. Osmometer was used to measure the osmotic potential.

Relative water contents (RWC)

Relative water contents of fully expended leaves were recorded by the method followed by Barrs & Weatherley (1962).

Chlorophyll contents

Leaf chlorophyll contents (LCC) were noted by the method described by Makeen et al. (2007) and the calculation was done by the formula provided by Arnon (1949).

Potassium contents

One gram ground dried sample was taken in hundred milliliter flask. The flask was rinsed with 1 normal hydrochloric acid (HCl). Twenty-five milliliter of 1 normal hydrochloric acid was poured in flask having samples, further it was left for 24 hours. Filtration of the sample was done using filter paper (Whatman No.1) and the potassium contents were estimated by using filtrate on flame photometer by the method explained by Yoshida et al. (1972).

Membrane stability index and proline contents

Membrane stability index (MSI) was analyzed by the method adopted by Chandrasekar et al. (2000) with slight modification. While, proline contents were calculated following the method explained by Bates et al. (1973).

Statistical analysis

The trial was laid out according to two factor factorial (Drought × Rootstock) CRD (completely randomized design). The data were analyzed statistically by ANOVA and means were compared by LSD method at p level 5% (Steel et al., 1997).

Results and Discussion

Osmotic potential

Results pertaining to osmotic potential (OP) reveal that different PEG concentrations significantly interacted with the rootstocks (PL, SL and GF-677) (Fig. 1). Least OP values were exhibited by GF-677 at stress level -6 bar, whereas PL and SL produced greater values of OP at the same level of stress. Decrease in OP values (negative) is a common response of drought-resistant plant species/cultivars which proved to be very helpful in mitigating the negative effects of drought (Guarnaschelli et al., 2003). Lower water potential and changes in turgidity under drought stimulate certain physiological mechanisms which help plants to adapt stressful conditions and continuation of growth to some extent (Chaitanya et al., 2009). In order to lower OP, osmotic adjustment is a key mechanism helping plants to withstand drought (Merchant et al., 2007). The osmolytic compounds like aspartic acid, glutamic acid, proline alanine, glycine betaine, sucrose, fructans and mannitol are noted to be built up inside the plant cells hence lowering

the water potential (Sakamoto & Murata, 2002). Decrease in OP due to accumulation of such compounds helps to maintain turgidity of cells by absorption of water. This phenomenon also protects metabolic apparatus of the cell in lack of moisture conditions (Chaves et al. 2003). Lower OP allows leaf cells to endure higher evaporative conditions without losing turgidity (Dichio et al., 2006). On contrary to that rise in OP values were observed for SL at -6 and -10 bar drought stress levels. An observation of the means at different stress levels shows a rise in OP values with increased PEG suggesting a negative effect on peach rootstocks by drought. This rise in OP might be ascribed because membrane leakage resulting in loss of osmolytes hence lowering osmotic adjustments (Bajji et al., 2001). On the other hand, rootstock means as affected by stress levels indicate that GF-677 had least OP value (-9.8375 bar) with increase in drought followed by Peshawar Local and Swat Local (Fig. 1). Hence, GF-677 could be a better option under drought conditions.

Relative water contents (%)

The values of relative water contents (RWC) (%) for all the used rootstocks were noted to be inversely proportional to PEG levels as evidenced in Fig. 2. Least value was shown for Swat Local under stress level where 77% decrease in water contents was observed when stress level at -10 bar was applied. Contrary to that GF-677 and Peshawar Local had maximum water contents (81 and 80%, respectively). In order to assess the plant drought response, RWC is considered as one of the basic indices related to other physiological processes i.e. transpiration, stomatal conductance, photosynthesis and turgidity (Kramer & Boyer, 1995), reflecting balance between supply and loss of water through evapotranspiration (Dhanda & Sethi, 2002). This allows stress recovery consequently, affecting the yield (Jones & Ritchie, 1990). Significantly variable behavior of rootstocks was observed against varying stress levels (Fig. 2). Significantly higher values for RWC were exhibited by GF-677 (88.613 %), while least was recorded in SL i.e. 81.223 %. Intermediate result was observed for PL (84.78 %). The finding of the present work is in accordance with the report of Alarcon et al. (2002) who concluded GF-677 to be more resistant against drought stress when compared to other rootstocks (seedlings), as it avoids water loss due to transpiration, hence maintaining higher water potential of the leaves under drought. It is obvious from the results that the level of moderate drought (-6 bar) did not affect RWC, yet higher level of stress (-10 bar) significantly decreased RWC. Lopez et al. (2009) reported a continued decline in RWC in *Pinus* with increased water stress under hydroponic conditions. Polyethylene glycol with higher molecular weight could be ascribed to hinder the water transport system of the root, hence lowering RWC caused dehydration (Fan & Blake, 1997).

Chlorophyll contents

The data presented in Fig. 3 revealed that interaction of rootstock with drought levels significantly affected leaf chlorophyll contents. Greater values for chlorophyll contents were calculated in all the three rootstocks in all control treatments (without PEC i.e. T_0). Nevertheless, increasing levels of stress significantly decreased chlorophyll contents of the leaves. GF-677 at -10 bar stress level had least value for the chlorophyll contents. Decrease in leaf chlorophyll contents occurs predominantly under drought because of decline in chlorophyll production or enhanced chlorophyll destruction due to instability of cell membrane (Ashraf & Foolad, 2007). Chlorophyll degradation under drought is influenced in many ways. Closing of stomata in this regard is the major factor causing chlorophyll reduction (Kawasaki et al., 2001). Furthermore, stress conditions inhibit the assimilation and translocation of photosynthates which could be ascribed as the second most important factor in this regard (Demural et al., 2005). Likewise, an imbalance between light utilization and capture causes losses in photosynthetic apparatus (Chaves et al., 2003). Loss in viability and leaf necrosis was also noted in explants due to drought as the level of stress increased. Alike results were reported by Molassiotis et al. (2006) where progressive increase in the drought caused a decrease in chlorophyll contents in many plants during *in vitro* studies. Higher chlorophyll contents at initial stress levels could be ascribed as coupling with photo-respiration and moderate stress does not damage chlorophyll contents (Brestic et al., 1995). On the other hand, rise in the drought levels, where OP falls below threshold level, chlorophyll gets damaged hence affects the photosynthetic activity negatively (Brodribb, 1996).

Though, chlorophyll contents were affected by the drought conditions in all the rootstocks yet the comparison among them was found to be statistically non-significant (Fig. 3). Photosynthesis is a physiologically ideal activity to predict vitality and health of plant, hence, knowledge about chlorophyll contents can be a key marker to assess the drought effects (Taiz & Zieger, 2002).

Potassium contents

K contents of all the studied rootstocks were significantly influenced by raising stress level *in vitro*. Both, PL and GF-677, achieved the highest K contents (18.50 mg g^{-1}) at T_0 . Statistically a significant reduction for K was noted for the rootstocks used, Swat Local producing least K values i.e. 13.70 mg g^{-1} when provided the stress level -10 bar (Fig. 4). This could be explained because of negative influences of drought on transmission of potassium into plant cells. K concentration in the cells is one of main attributes by which plant stress tolerance can be judged (El-Hadi et al., 1997). Moreover, raised levels of K in plants during drought is reported to have a protective function against the damage caused by photo-oxidation. Furthermore, higher K contents play a key role in timely closing and opening of stomata, hence counteracting the drought stress by regulating the stomatal movements (Egilla et al., 2001). Loss of water from chloroplasts during drought

makes the release of large amounts of K that could be another reason of making the K contents to be counted less during the rising levels of drought stress (Gupta et al., 1989). From the comparison of rootstocks, it was noted that GF-677 produced significantly higher K contents i.e. 16.60 mg g^{-1} , afterward Peshawar Local (15.80 mg g^{-1}) and SL (15.20 mg g^{-1}), proving the exotic rootstock (GF-677) better to tolerate drought as compared to other rootstocks used in the study. Significant amount of K contents in plants impacts cellular solute contents ultimately affects osmoregulation of the cells (Verslues & Bray, 2004), and hence it is considered as a facilitator in reducing OP by maintaining cellular turgidity (Majid et al., 2007). K reduction during drought has been reported already in many plants (Sivritepe et al., 2008).

Membrane stability index

Interactive effects of rootstocks with drought show significant differences ($p < 0.05$) regarding membrane stability index (MSI) as evident from Fig. 5. Under control

conditions i.e. T_0 , both SL and PL rootstocks produced significantly higher values for MSI i.e. 56 and 55.6, respectively, being statistically at par with each other. Yet, increase in drought levels markedly reduced in MSI increasing membrane leakage in all rootstocks, SL producing least values at -6 and -10 bar i.e. 35.5 and 35.42, respectively. Maintaining cellular integrity is a key mechanism of drought tolerant species (Bajji et al., 2001). Drought causes tonoplast to be loosened consequently leaking the vacuolar material, resulting in bursting of the chloroplast. These distractions in cellular chambers release hydrolases (lipases) that digest the membrane. Moreover, oxidative stress caused by drought can be attributed as the second most important factor causing membrane rupture. Variation in behavior of rootstocks at different levels of stress might be because of variation in the commencement of mechanism responsible for drought-resistance which might be an uplift superoxide dismutase (SOD) activity and scavenging free radicals (Joyce et al., 2003).

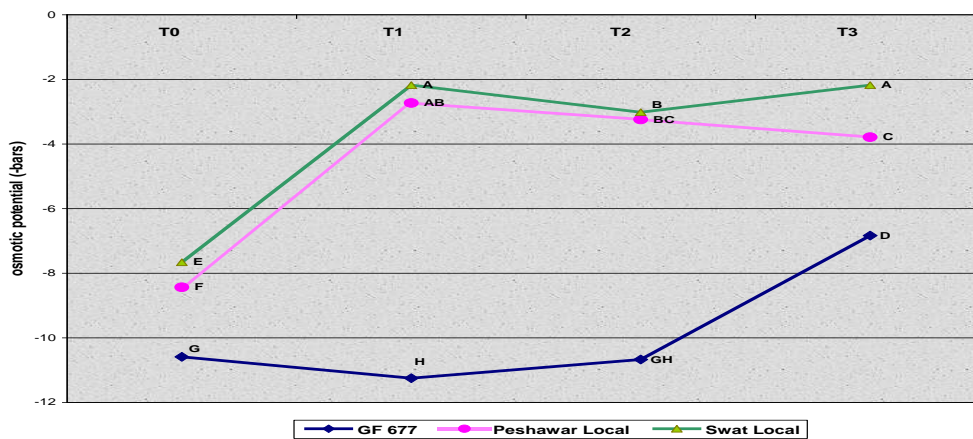


Fig. 1 Osmotic potential of peach rootstocks at different drought levels

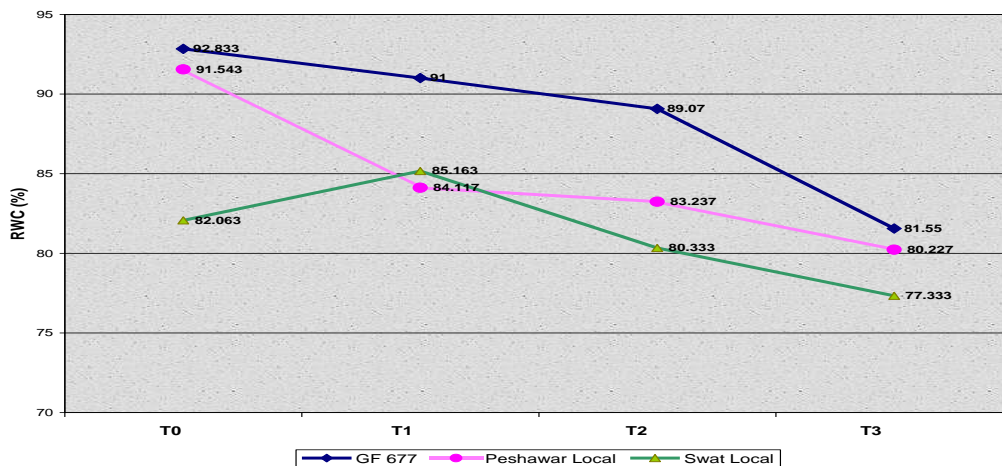


Fig. 2 RWC of peach rootstocks at different drought levels

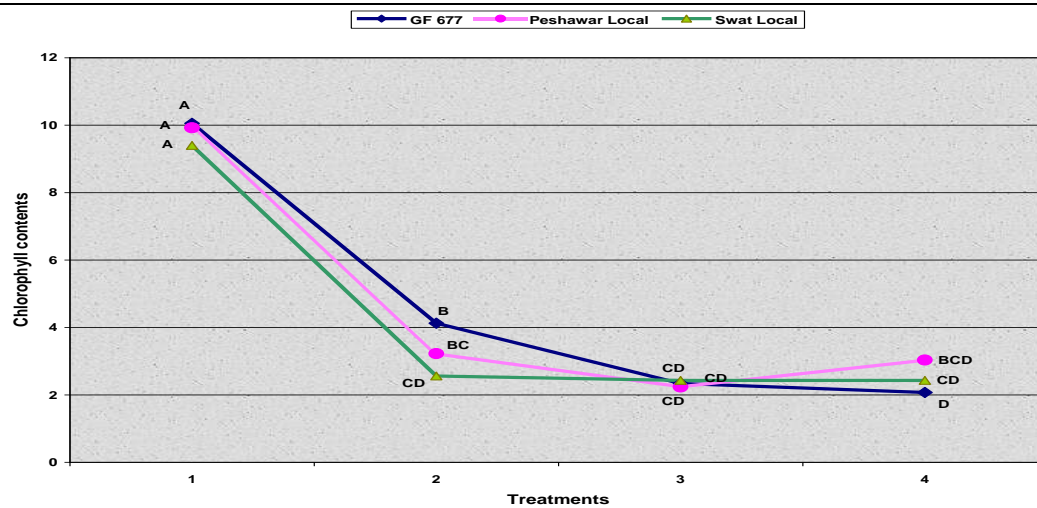


Fig. 3 Chlorophyll contents of peach rootstocks at different drought levels

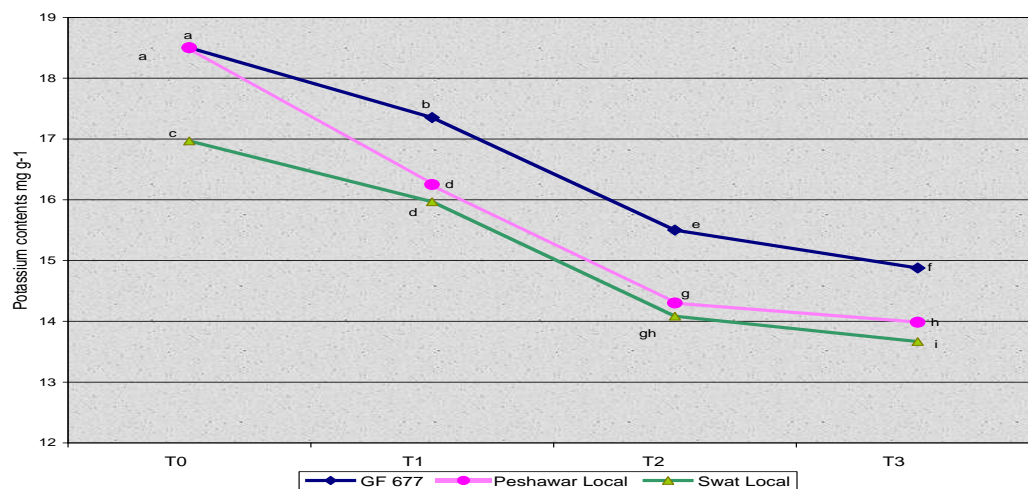


Fig. 4 Potassium contents of peach rootstocks at different drought levels

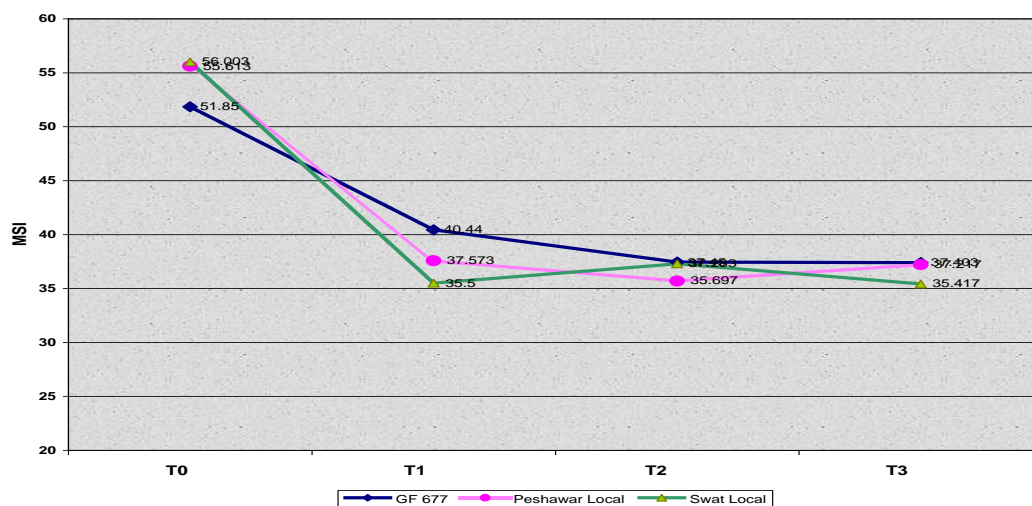


Fig. 5 MSI of peach rootstocks at different drought levels

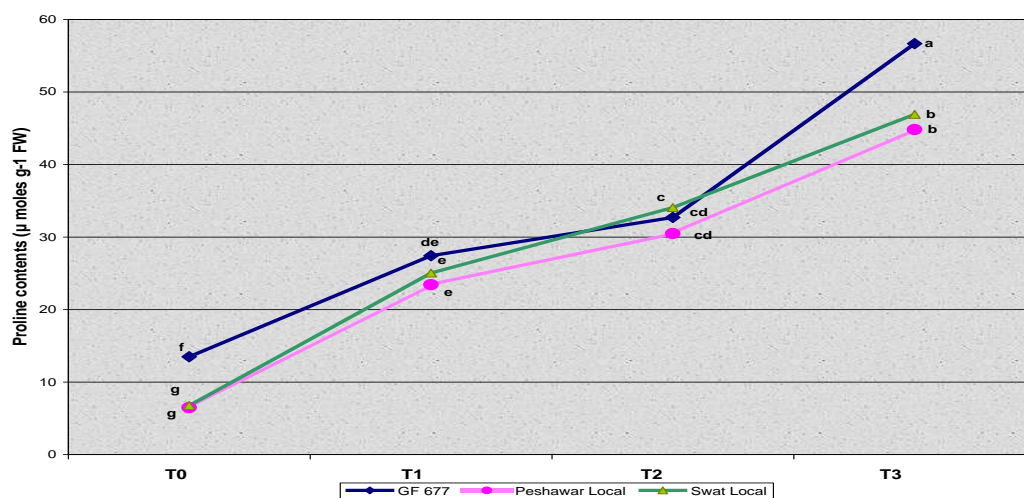


Fig. 6 Proline contents of peach rootstocks at different drought levels

Proline contents

It is clear from the analyzed data that interaction between rootstocks and drought was found to be significant regarding proline contents (Fig. 6). It was revealed that GF-677 achieved maximum levels of proline contents i.e. $56.70 \mu\text{moles g}^{-1}$ when applied stress treatment of -10 bar. On the contrary, SL and PL proved to be statistically inferior to GF-677 with 46.9 and $44.8 \mu\text{moles g}^{-1}$ of proline values, respectively. Least values were noted in PL and SL (6.50 and $6.60 \mu\text{moles g}^{-1}$, respectively) when no PEG was applied i.e. T_0 . While GF-677 produced significantly higher levels of proline i.e. $13.50 \mu\text{moles g}^{-1}$ in the same treatment. Abiotic stress like drought affects plant's physiological processes in numerous ways. Proline is considered to have a key role in mitigating the abiotic stress and it is thought to be accumulated during stressful conditions in plants. Higher proline concentrations is linked with plant organs which usually have lower osmotic potential i.e. inflorescence and seeds (Khan et al., 2009) suggesting its role in mitigating abiotic stress. Accumulation of proline is one of the initial responses of plants facing stress to reduce the cellular injury (Ashraf & Foolad, 2007). It one of the most commonly present osmolytes having many roles in plants other than osmotic adjustments within the cells including scavenging of free radicals, regulation of cytosolic pH, osmolyte balancing and stabilizing proteins, maintenance of membrane integrity, working as sink of energy and source for nitrogen and carbon to the plant (Matysik et al., 2002). Observations regarding proline contents exhibited a direct relationship of proline contents with drought levels (Fig. 6). Proline accumulation in plant cells during drought is generally thought to be due to its enhanced biosynthesis and reduction degradation of this biomolecule. Furthermore, equilibrium between biosynthesis and degradation of this compound is also necessary for functioning as osmo-protectant. Osmoregulation by the proline buildup has been identified as one of the possible means for mitigating water stress in plants (Al-Khayri & Al-Bahrany, 2004). Data

regarding mean comparison of rootstock regarding proline contents suggest that GF-677 had significantly better levels of proline i.e. $32.60 \mu\text{mole g}^{-1}$ followed by SL ($28.20 \mu\text{moles g}^{-1}$) and PL $26.30 \mu\text{moles g}^{-1}$, later two were statistically at par. The genotype effect on accumulation of proline is well documented which could be due to growth habit of the genotypes. At higher growth, the intensity may be higher at a given growth stage in plants. Moreover, genotypes with longer biological cycles exhibited higher accumulation of proline (Karamanos, 1995). Similarly, genotypes that were found to be more tolerant to drought had more proline accumulation (Verbruggen & Herman, 2008), which was even confirmed by the transgenic approaches (Roosens et al., 2002).

Conclusion

Climate change is becoming a serious threat to agriculture in the future. Shortage of water is one of the major features of climate change and is a major constraint to plant productivity. Hence, drought tolerant rootstock for fruit production will be of utmost importance. Peach is an emerging fruit crop which is economically very important especially for arid and semi arid areas of Pakistan. Suitable rootstock selection for these areas is essential for better adaptability and successful peach orchard management. From the results, it was noted that rootstocks responded significantly to different drought stress levels. GF-677 proved to be superior as compared to the local rootstocks used and can be used as a successful alternative as peach rootstock instead of Swat Local and Peshawar Local.

Author Contribution Statement: Naveed Hamayat conducted the experiments and collected the data. Ishfaq Ahmed Hafiz and Touqeer Ahmad supervised the research study. Irfan Ali wrote the manuscript. Abdul Ahad Qureshi analyzed the data.

Conflict of Interest: The authors certify that they have no conflict of interest.

Acknowledgements: The authors are grateful to Dr. Nadeem Akhtar Abbasi for his kind cooperation and guidance for this research study.

References

- Ahad, F. S., Shah, M., Khan, J., & Khan, M. (1987). The effect of rootstock on survival, vigor, fruit drop and yield of peach (*Prunus persica*) Batch cultivar 6-A peach for the Malakand division. *Sarhad Journal of Agriculture*, 3, 471-476.
- Ahmad, T., Ur-Rehman, H., Ahmed, C. M. S., & Leghari, M. H. (2003). Effect of culture media and growth regulators on micropropagation of peach rootstock GF 677. *Pakistan Journal of Botany*, 35(3), 331-338.
- Alarcon, J. J., Sanchez-Blanco, M. J., Nicolas, E., Egea, J., & Dicenta, F. (2002). Effects of drought on shoot water relations in 'Antoneta' and 'Marta' almond cultivars grafted onto 'Garrigues' and 'GF677' rootstocks. *Acta Horticultureae*, 591, 345-349.
- Al-Khayri, J. M., & Al-Bahrany, A. M. (2004). Growth, water content, and proline accumulation in drought-stressed callus of date palm. *Biologia Plantarum*, 48(1), 105-108.
- Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*, 24, 1-15.
- Ashraf, M., & Foolad, M. R. (2007). Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environmental and Experimental Botany*, 59, 206-216.
- Bajji, M., Kinet, J. M., & Lutts, S. (2001). The use of the electrolyte leakage method for assessing cell membrane stability as a water stress tolerance test in durum wheat. *Plant Growth Regulation*, 00: 1-10.
- Barrs, H. D., & Weatherley, P. E. (1962). A re-examination of the relative turgidity technique for estimating water deficit in leaves. *Australian Journal of Biological Sciences*, 15, 413-428.
- Bates, L. S., Waldorn, R. P., & Teare, I. D. (1973). Rapid determination of free proline for water stress studies. *Plant Soil*, 39, 205-208.
- Boretti, A., & Rosa, L. (2019). Reassessing the projections of the world water development report. *NPJ Clean Water*, 2(1), 1-6.
- Brestic, M., Cornic, G., Fryer, M. J., & Baker, N. R. (1995). Does photorespiration protect the photosynthetic apparatus in French bean leaves from photoinhibition during drought stress? *Planta*, 196, 450-457.
- Brodribb, T. (1996). Dynamics of changing intercellular CO₂ concentration (Ci) during drought and determination of minimum functional Ci. *Plant Physiology*, 111, 179-185.
- Chaitanya, K. V., Rasineni, G. K., & Reddy, A. R. (2009). Biochemical responses to drought stress in mulberry (*Morus alba* L.): Evaluation of proline, glycine betaine and abscisic acid accumulation in five cultivars. *Acta Physiology Plantarum*, 31, 437-443.
- Chandrasekar, V., Sairam, R. K., & Srivastava, G. C. (2000). Physiological and biochemical responses of hexaploid and tetraploid wheat to drought stress. *Journal of Agronomy and Crop Sciences*, 185, 219-227.
- Chaves, M. M., Maroco, J. P., & Pereira, J. S. (2003). Review: Understanding plant response to drought- from genes to the whole plant. *Functional Plant Biology*, 30, 239-264.
- Demural, M. A., Aydin, M., & Yorulmaz, A. (2005). Effect of salinity on growth chemical composition and antioxidative enzyme activity of two malting Barley (*Hordeum vulgare* L.) cultivars. *Turkish Journal of Biology*, 29, 117-123.
- Dhanda, S. S., & Sethi, G. S. (2002). Tolerance to drought stress among selected Indian wheat cultivars. *Journal of Agriculture Science*, 139, 319-326.
- Dichio, B., Xiloyannis, C., Sofo, A., & Montanaro, G. (2006). Osmotic regulation in leaves and roots of olive trees during a water deficit and rewatering. *Tree Physiology*, 26, 179-185.
- Egilla, J. N., Davies, F. T., & Drew, M. C. (2001). Effect of potassium on drought resistance of *Hibiscus rosa-sinensis* cv. Leprechaun: Plant growth, leaf macro and micronutrient content and root longevity. *Plant and Soil*, 229(2), 213-224.
- El-Hadi, A. H. A., Ismail, K. M., & El-Akabawy, M. A. (1997). Effect of potassium on the drought resistance of crops in Egyptian conditions. In *Proceeding of regional workshop of IPI* (pp. 328-336). Izmir, Turkey: Bornova.
- Fan, S., & Blake, T. J. (1997). Comparison of polyethylene glycol 3350 induced osmotic stress and soil drying for drought simulation in three woody species. *Trees*, 11, 342-348.
- Galovic, V., Kotaranin, Z., & Dencic, S. (2005). *In vitro* assessment of wheat tolerance to drought. *Genetika*, 37(2), 165-171.
- Gomez, P. M., Perez, R. S., Rubio, M., Dicenta, F., Gradziel, T. M., & Sozzi, G. O. (2005). Application of recent biotechnologies to Prunus tree crop genetic improvement. *Ciencia e Investigacion Agraria*, 32(2), 73-96.
- Guarnaschelli, A. B., Lemcoff, J. H., Prystupa, P., & Basci, S. O. (2003). Responses to drought preconditioning in *Eucalyptus globulus* Labill. provenances. *Trees Structure and Function*, 17, 501-509.
- Gupta, A., Berkowitz, G. A., & Pier, P. A. (1989). Maintenance of photosynthesis at low leaf water potential in wheat. *Plant Physiology*, 89, 1358-1365.
- Jones, J. W., & Ritchie, J. T. (1990). Crop growth models. In G. J. Hoffman, T. A. Howel, & K. H. Solomon (Eds.), *Management of farm irrigation systems* (pp. 63-89). St. Joseph, MN: ASAE.
- Joyce, S. M., Cassells, A. C., & Jain, S. M. (2003). Stress and aberrant phenotypes in *in vitro* culture. *Plant Cell Tissue and Organ Culture*, 74, 103-121.
- Karamanos, A. J. (1995). The involvement of proline and some metabolites in water stress and their importance as drought resistance indicators. *Bulgarian Journal of Plant Physiology*, 21(2-3), 98-110.
- Kawasaki, S., Borchert, C., & Deyholos, M. (2001). Gene expression profiles during the initial phase of salt stress in rice. *Plant Cell*, 13, 889-905.

- Kaya, M. D., Okcu, G., Atak, M., Cikili, Y., & Kolsarici, O. (2006). Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). *European Journal of Agronomy*, 24(4), 291-295.
- Khan, J., Hussain, S. A., & Nabi, G. (1996). Performance of peach stones under field conditions of Peshawar. *Sarhad Journal of Agriculture*, 12(3), 269-271.
- Khan, M. A., Shirazi, M. U., Khan, M. A., Mujtaba, S. M., Islam, E., Mumtaz, S., Shereen, A., Ansari, R. U., & Ashraf, M. Y. (2009). Role of proline, K/Na ratio and chlorophyll content in salt tolerance of wheat (*Triticum aestivum* L.). *Pakistan Journal of Botany*, 41(2), 633-638.
- Khushk, A. M., & Laghari, N. (2007, August 13). Peach production technology. *Dawn*. Retrieved from <http://www.dawn.com>
- Kramer, P. J., & Boyer, J. S. (1995). *Water relations of plants and soils*. San Diego, CA: Academic Press.
- Lopez, R., Aranda, I., & Gil, L. (2009). Osmotic adjustment is a significant mechanism of drought resistance in *Pinus pinaster* and *Pinus canariensis*. *Forest System*, 18, 159-166.
- Majid, S. A., Asghar, R., & Murtaza, G. (2007). Potassium-calcium interrelationship linked to drought tolerance in wheat (*Triticum aestivum* L.). *Pakistan Journal of Botany*, 39(5), 1609-1621.
- Makeen, K., Babu, G. S., Lavanya, G. R., & Abraham, G. (2007). Studies of chlorophyll content by different methods in black gram (*Vigna mungo* L.). *International Journal of Agriculture Research*, 2(7), 651-654.
- Matysik, J., Alia, Bhalu, B., & Mohanty, P. (2002). Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Current Science*, 82, 525-532.
- Merchant, A., Callister, A., Arndt, S., Tausz, M., & Adams, M. (2007). Contrasting physiological responses of six *Eucalyptus* Species to water deficit. *Annals of Botany*, 100, 1507-1515.
- Molassiotis, A. N., Sotiropoulos, T., Tanou, G., Kofidis, G., Diamantidis, G., & Therios, I. (2006). Antioxidant and anatomical responses in shoot culture of the apple rootstock MM 106 treated with NaCl, KCl, mannitol or sorbitol. *Biologia Plantarum*, 50, 61-68.
- Monticelli, S., Puppi, G., & Damiano, C. (2000). Effects of *in vivo* mycorrhization on micro propagated fruit tree rootstocks. *Applied Soil Ecology*, 15, 105-111.
- Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum*, 15, 473-497.
- Nadeem, M., Li, J., Yahya, M., Sher, A., Ma, C., Wang, X., & Qiu, L. (2019). Research progress and perspective on drought stress in legume: A review. *International Journal of Molecular Science*, 20(10), 25-41.
- Nehra, N. S., Becwar, M. R., Rottmann, W. H., Pearson, L., Chowdhury, K., Chang, S., Wilde, H. D., Kodrzycki, R. J., Zhang, C., Gause, K. C., Parks, D. W., & Hinchee, M. A. (2005). Forest biotechnology: Innovative methods, emerging opportunities. *In Vitro Cellular & Developmental Biology - Plant*, 41, 701-717.
- Rahman, N., Nabi, G., Khan, J., & Shafqatullah. (2000). Vegetative growth performance of different plum rootstocks. *Pakistan Journal of Biological Sciences*, 3(10), 1630-1631.
- Roosens, N., Bitar, F. H., Loenders, K., Angenon, G., & Jacobs, M. (2002). Overexpression of ornithine- δ -aminotransferase increases proline biosynthesis and confers osmo-tolerance in transgenic plants. *Molecular Breeding*, 9, 73-80.
- Sakamoto, A., & Murata, N. (2002). The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant, Cell and Environment*, 25, 163-171.
- Sakthivelu, G., Devi, M. K. A., Giridhar, P., Rajasekaran, T., Ravishankar, G. A., Nedev, T., & Kosturkova, G. (2008). Drought-induced alterations in growth, osmotic potential and *in vitro* regeneration of soybean cultivars. *General and Applied Plant Physiology*, 34(1-2), 103-112.
- Sivritepe, N., Erturk, U., Yerlikaya, C., Turkan, I., Bor, M., & Ozdemir, F. (2008) Response of the cherry rootstock to water stress induced *in vitro*. *Biologia Plantarum*, 52(3), 573-576.
- Specht, J. E., Chase, K., Macrander, M., Graef, G. L., Chung, J., Markwell, J. P., Germann, M., Orf, J. H., & Lark, K. G. (2001). Soybean response to water: A QTL analysis of drought tolerance. *Crop Science*, 41(2), 493-509.
- Steel, R. G., Torrie, J. H., & Dickey, D. A. (1997). *Principles and procedures of statistics: A biological approach*. McGraw-Hill.
- Taiz, H., & Zieger, E. (2002). *Plant physiology*, 3rd ed., Sunderland (MA): Sinauer Associates Inc.
- Ullah, T., Muhammad, W., Nabi, G., Rehman, N., Arshad, M., & Naeem, N. (2000). Bud take success of different almond varieties on peach rootstock. *Pakistan Journal of Biological Sciences*, 3(11), 1805-1806.
- Verbruggen, N., & Hermans, C. (2008). Proline accumulation in plants: A review. *Amino Acids*, 35, 753-759.
- Verslues, P. E., & Bray, E. A. (2004). LWR1 and LWR2 are required for osmoregulation and osmotic adjustment in *Arabidopsis*. *Plant Physiology*, 136, 2831-2842.
- Wu, Y., & Cosgrove, D. J. (2000). Adaptation of roots to low water potentials by changes in cell wall extensibility and cell wall proteins. *Journal of Experimental Botany*, 51(350), 1543-1553.
- Yoshida, S., Forno, D. A., Cock, J. H., & Gomez, K. A. (1972). *Laboratory manual for physiological studies of rice*. 2nd Ed. Laguna, Philippines: IRRI Press.
- Younas, M., Rahman, H. U., Siddiqui, S. U., & Chaudhary, M. F. (2008). Effect of different carbon sources on *in vitro* shoot proliferation and rooting of peach rootstock GF 677. *Pakistan Journal of Botany*, 40(3), 1129-1134.