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Effect of drought stress on physio-biochemical parameters in *Festuca* and *Lolium* genotypes

Anjali Kumari◆**, V.K. Sood and Arushi Arora***
Department of Genetics and Plant Breeding, CSK HPKV Palampur-176061, Himachal Pradesh, India
**Department of Plant Breeding and Genetics, PAU, Ludhiana-141001, Punjab, India*

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Abstract

A polyhouse study was conducted to study the effect of drought stress on physio-biochemical parameters in 10 *Festuca* and four *Lolium* genotypes at CSKHPKV, Palampur, Himachal Pradesh, India. Physio-biochemical parameters, *viz*., root length, shoot length, root weight, shoot weight, root:shoot ratio, leaf relative water content (LRWC), chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Tchl), ratio of chlorophyll a to chlorophyll b (Chl a/b), proline content (PC) and two selection indices, stress tolerance index (STI) and drought susceptibility index (DSI) were studied. Drought stress caused reduction in shoot traits, LRWC and Chl b. Based on the STI, among *Festuca genotypes*, Hima-3 was identified as the tolerant genotype in stress condition while among *Lolium* genotypes, Palam rye grass was identified as the superior genotype. Drought stress increased the root traits, Chl a, Tchl, Chl a/b and PC. Results showed that *Festuca* genotypes are more tolerant towards drought condition as compared to *Lolium* genotypes; still there is scope for improvement in *Lolium* genotypes through breeding programmes. Changes in chlorophyll and proline content can be documented as crucial components affecting drought tolerance in *Festuca* and *Lolium* genotypes.

1. Introduction

Livestock plays a vital role in sustaining the livelihood of people in north western Himalayas, but the limited forage resources are hardly enough to meet the forage requirement of the existing livestock population. To meet out this demand, planting of improved forage grasses which are ecologically adaptable can assure a promising increase in the forage production and its availability in the region (Kumar *et al*., 2015).

Festuca, generally known as fecues is a large and diverse genus which belongs to the family Poaceae. It comprises of approximately 450 species (Clayton and Renovoiz, 1986; Qiu *et al*., 2019) which vary in chromosome number from diploid (2n=2x=14) to dodecaploid (2n=12x=84) (Smarda *et al*., 2008). Genus *Lolium*, generally known as ryegrass comprised of eight species (Terrell, 1968; Clayton and Renovoiz, 1986) which have diploid (2n=2x=14) chromosome number. *Festuca* species are highly persistent to hard abiotic stresses such as drought, freezing, *etc*., but have poor nutritive value, low in palatability and digestibility. On the other hand, *Lolium* species are more palatable, digestible and highly nutritious, but lacks the tolerance towards abiotic stresses. In view of the expected climate change, researchers and grass breeders are interested in grasses performing better during drought periods.

Corresponding author: Ms. Anjali Kumari
Department of Genetics and Plant Breeding, CSK HPKV Palampur-176061, Himachal Pradesh, India
E-mail: katochanjali01@gmail.com
Tel.: +91-8988036205

Drought is among the key abiotic stresses limiting the survival and growth of plants in arid and semi-arid areas of the world. It is a period of sub-optimal water supply to plants that reduces water potential, turgor pressure and subsequently inhibits normal plant functions (Jaleel *et al*., 2009). Various mechanisms like avoidance, escape and tolerance are used by the plants in response to drought stress conditions which ultimately helps in improvement of water uptake efficiency, its use and loss (Wang and Huang, 2004). Drought tolerance characteristics include root penetration into deeper portions of the soil profile, higher accumulation of proline content, low drought susceptibility index, *etc*. Bonos *et al*. (2004) suggested selection for longer root length in tall fescue which is related to drought tolerance. Karcher *et al*. (2008) proposed the selection of genotypes on the basis of high root to shoot ratio as a feasible method for improving drought tolerance of turf grass in the field. The photosynthetic apparatus damages and chlorophyll content diminishes under drought stress conditions (Fu and Huang, 2001). Chlorophyll content has been considered as a reliable indicator in screening genotypes for drought tolerance (Rong-Hua *et al*., 2006). Bajji *et al*. (2001) reported that proline content is related with osmotic regulations during drought and other stresses among the solutes in annual and perennial grasses. Plant species can differ considerably in their ability to accumulate proline upon stress (Maggio *et al*., 2002). Keles and Oncel (2004) concluded that genotypes with high leaf relative water content under stress conditions to be closely related to drought tolerance.

Selection indices such as stress tolerance index (STI) and drought susceptibility index (DSI) have been suggested for identification of genotypes which produce high yield under both stress and non-stress conditions. Ebrahimiyan *et al*. (2012) evaluated drought tolerance of 75 tall fescue genotypes using two selection indices *viz.*, susceptibility and drought tolerance indices. Keeping all the above factors in view, the present study was undertaken to study the comparative response and performance of *Festuca* and *Lolium* genotypes under drought stress conditions.

2. Materials and Methods

2.1 Plant material and growth conditions

The study was conducted at the fodder farm and Molecular Cytogenetics and Tissue Culture Lab of Department of Genetics and Plant Breeding of CSK HPKV, Palampur, India (32°6' N latitude, 76°3' E longitude, 1290 m amsl). The soil is acidic in nature with pH ranging from 5.0 to 5.6 and soil texture is silty clay loam. The material for present study comprised of 10 genotypes of *Festuca* species (Sel-88, Hima-1, EC178184, Sel-63, Hima-15, Sel-71, Hima-4, EC1942, Hima-3 and EC178182) and four genotypes of *Lolium* species (Punjab ryegrass-1, Kashmir ryegrass, Palam ryegrass and Makhhan ryegrass). Polyvinyl chloride (PVC) pots filled with sterilized silt-loam soil, which was collected from the fodder farm, were used for the experimental study. A drought stress to 45 days old plants was given by with-holding the irrigation for 10 days. A comparison was then made between the drought stressed plants and well watered plants for physio-biochemical parameters.

2.2 Measurements and statistical analysis

The characteristics such as root and shoot length were recorded in centimetres with the help of scale while root and shoot weight was measured with the help of weighing balance and expressed in grams. Root: shoot ratio was calculated by dividing the root weight by shoot weight. Leaf relative water content (LRWC) was determined according to the method developed by Ghoulam *et al.* (2002) using the following equation:

$$\text{LRWC (\%)} = (FW - DW)/(TW - DW) \times 100$$

where FW is fresh weight, DW is dry weight and TW is turgid weight. Spectrophotometry was used to measure total chlorophyll (Tchl), chlorophyll a, chlorophyll b and Chl a/b (Arnon, 1949). Moreover, proline content (PC) was determined based on the method described by Bates *et al.* (1973). The two selection indices, *viz.*, stress tolerance index (STI) (Fernandez, 1992) and drought susceptibility index (DSI) (Dencic *et al.*, 2000) for each genotype were calculated based on the green forage yield (GFY) of control and drought stressed plants according to the following formulae:

$$STI = Y_s \times Y_p / Y_{mp}$$

$$DSI = [1 - (Y_s/Y_p)/1 - (Y_{ms}/Y_{mp})]$$

where Y_s is the yield of the ith genotype in the stress condition, Y_p is the yield of the ith genotype in the normal condition, Y_{ms} is the mean yield of all genotypes in the stress condition and Y_{mp} is the mean yield of all genotypes in the normal condition.

The data on various physiobiochemical parameters were replicated three times and analyzed by complete randomised design (CRD). Mean values were calculated for all the parameters studied and used for statistical analysis.

3. Results

3.1 Root and shoot length (cm)

The data presented in Figure 1 and showed an increase in root length in all the genotypes studied under drought condition in comparison to the controlled condition. The per cent variation in root length during stress condition varied from 16-108 per cent. Among all *Festuca* genotypes, maximum increase in LRWC was observed for Hima-15 with an increase of 108 per cent whereas among *Lolium* genotypes, it was observed for Makhhan ryegrass with an increase of 107 per cent. A general decrease in shoot length was observed in all the genotypes under drought condition in comparison to the controlled condition (Figures 2). The per cent variation in shoot length during stress condition varied from 11-24 per cent. Among all *Festuca* genotypes, minimum decrease in shoot length was observed for Hima-15 with a decrease of 11 per cent whereas among *Lolium* genotypes, it was observed for Kashmir ryegrass with a decrease of 6 per cent.

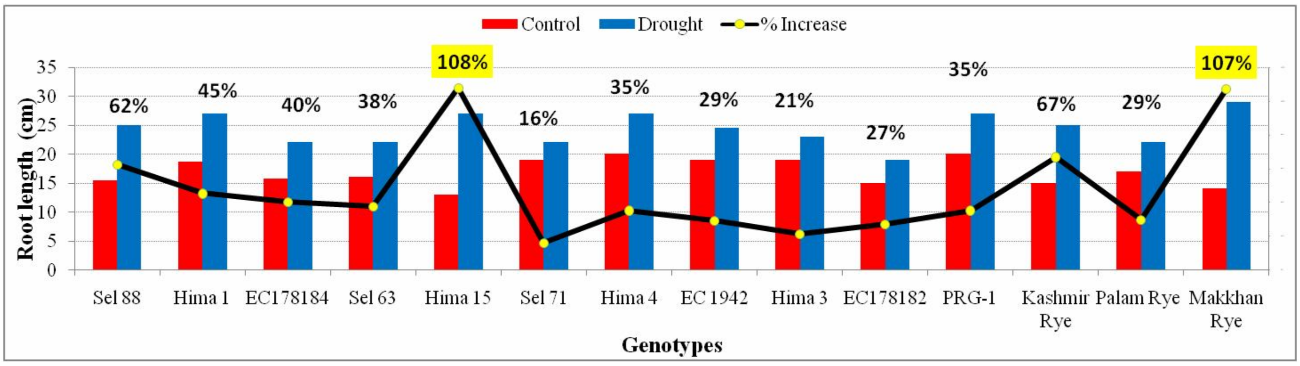

Figure 1: Per cent variation in root length of *Festuca* and *Lolium* genotypes under controlled and drought conditions (Sel: Selection; PRG-1: Punjab ryegrass-1).

3.2 Root and shoot weight (g)

An appraisal of data given in Figure 3 revealed that root weight of the genotypes increased under drought condition in comparison to the controlled condition. Among all *Festuca* genotypes, maximum increase in root weight was observed for EC178184 with an increase of 28 per cent whereas among *Lolium* genotypes, it was observed for Makhhan ryegrass with an increase of 35 per cent. Shoot weight of the genotypes decreased under drought condition in comparison to the controlled condition (Figure 4). Among all *Festuca* genotypes, minimum decrease in shoot weight was observed for Hima-4 with a decrease of 7 per cent whereas among *Lolium* genotypes, it was observed for Punjab ryegrass-1 with a decrease of 8 per cent.

3.3 Root: shoot ratio

A general increase in root to shoot ratio (Figure 5) of the genotypes was observed under drought condition in comparison to the controlled condition. Among all *Festuca* genotypes, maximum increase in root to shoot ratio was observed for Sel-88 with an increase of 48 per cent whereas among *Lolium* genotypes, it was observed for Makhhan ryegrass with an increase of 55 per cent.

3.4 Leaf relative water content

Perusal of the data given in Figure 6 revealed a decrease in leaf relative water content of the genotypes under drought condition in comparison to the controlled condition. Among all *Festuca* genotypes, minimum decrease in root length was observed for Sel-63 with a decrease of 3 per cent whereas among *Lolium* genotypes, it was observed for Kashmir ryegrass with a decrease of 17 per cent.

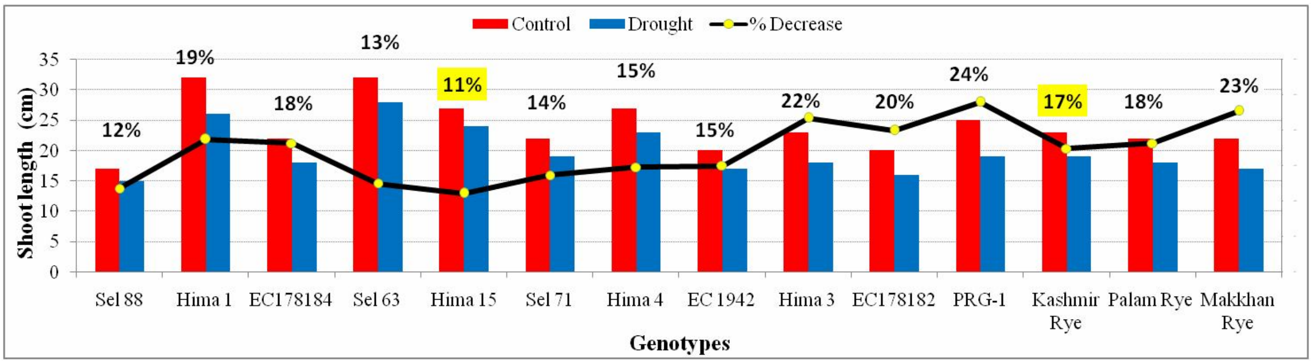

Figure 2: Per cent variation in shoot length of *Festuca* and *Lolium* genotypes under controlled and drought conditions (Sel: Selection; PRG-1: Punjab ryegrass-1).

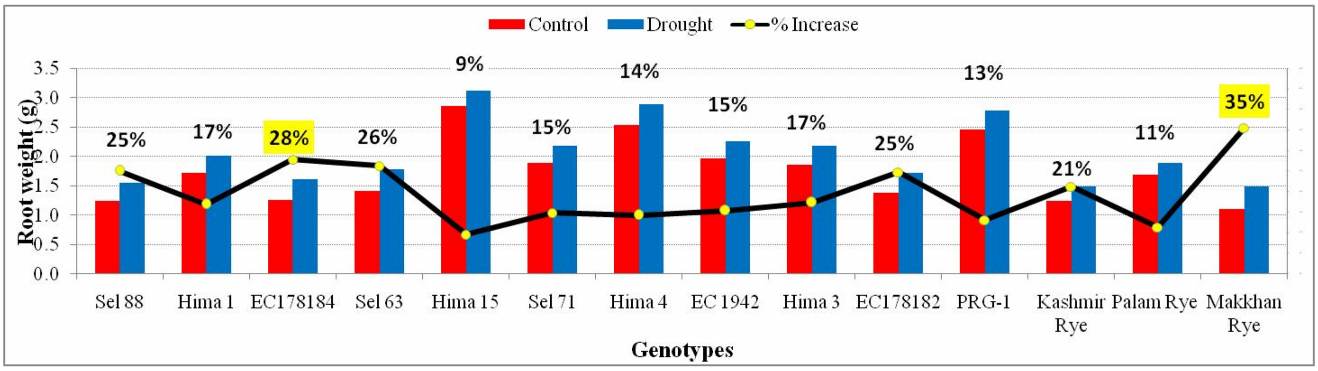

Figure 3: Per cent variation in root weight of *Festuca* and *Lolium* genotypes under controlled and drought conditions (Sel: Selection; PRG-1: Punjab ryegrass-1).

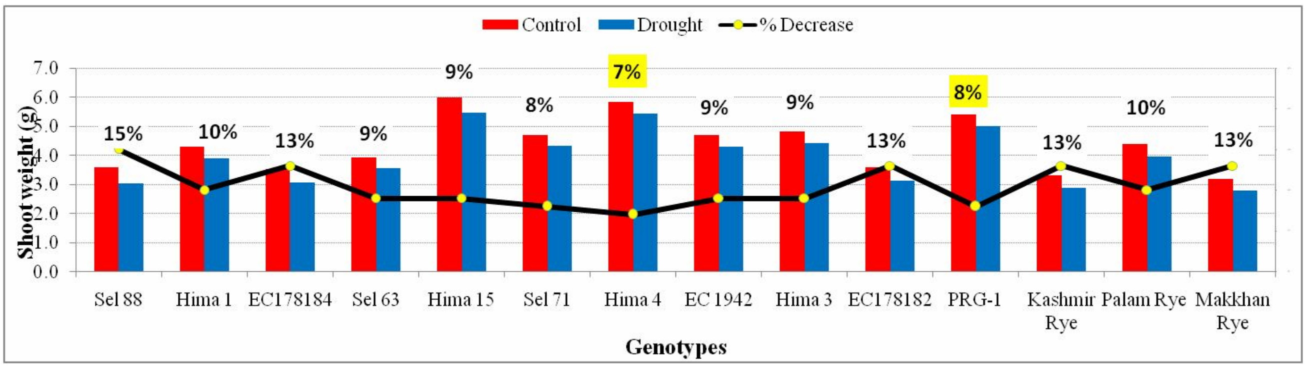

Figure 4: Per cent variation in shoot weight of *Festuca* and *Lolium* genotypes under controlled and drought conditions (Sel: Selection; PRG-1: Punjab ryegrass-1).

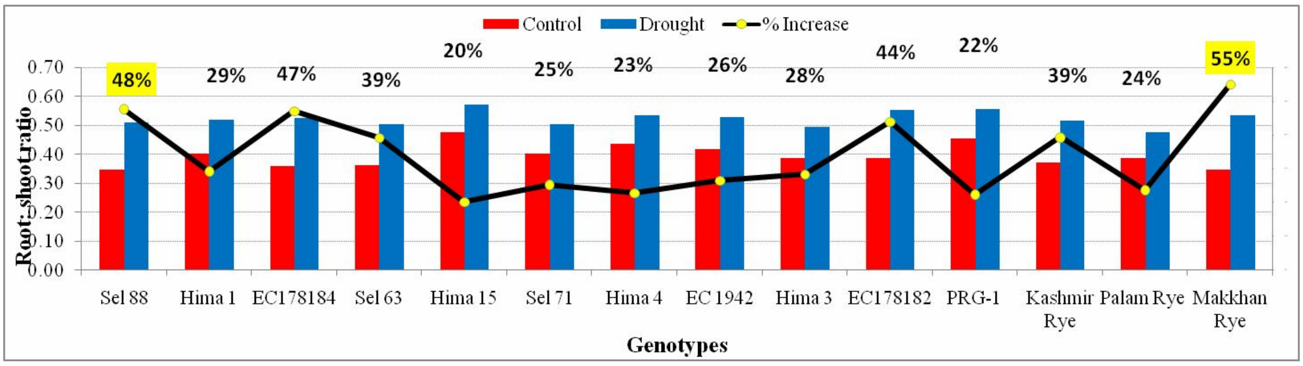

Figure 5: Per cent variation in root: shoot ratio of *Festuca* and *Lolium* genotypes under controlled and drought conditions (Sel: Selection; PRG-1: Punjab ryegrass-1).

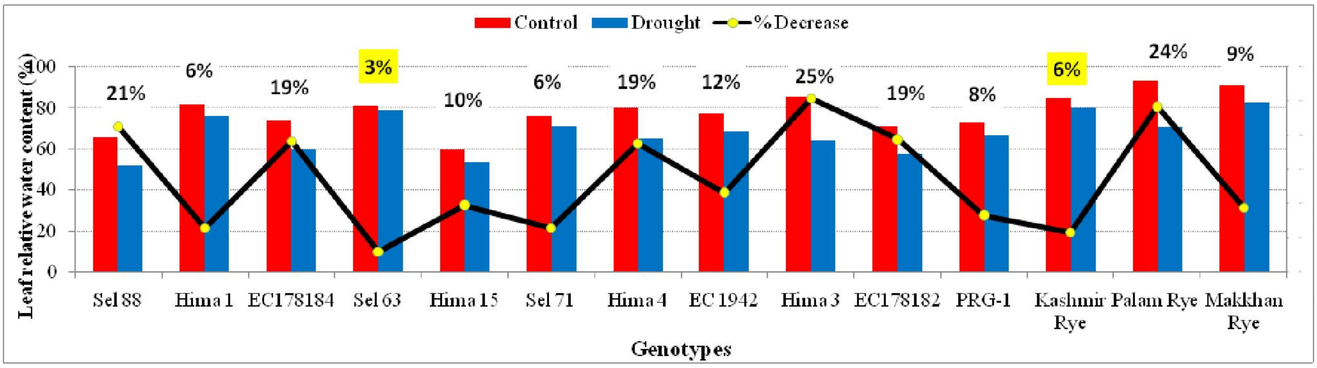

Figure 6: Per cent variation in leaf relative water content of *Festuca* and *Lolium* genotypes under controlled and drought conditions (Sel: Selection; PRG-1: Punjab ryegrass-1).

3.5 Chlorophyll content (mg/g of leaf)

Data pertaining to chlorophyll a, chlorophyll b, total chlorophyll and chlorophyll a/b of *Festuca* and *Lolium* genotypes under controlled and drought conditions have been presented in Figure 7-10. Chlorophyll a content of the genotypes ranged from 0.37-1.19 mg/g leaf under controlled conditions and from 0.55-1.23 mg/g leaf under stress conditions. A general increase in chl a was observed in all the genotypes under drought condition in comparison to the controlled condition. Among all *Festuca* genotypes, maximum increase in chl a content was observed for EC178182 with an increase of 95 per cent whereas among *Lolium* genotypes, it was observed for Palam ryegrass with an increase of 157 per cent. Chl b of the genotypes decreased except for EC1942 (no change in chl b) under drought condition in comparison to the controlled condition. Among all *Festuca* genotypes, minimum decrease in chl b content was observed for Hima-3 with a decrease of 4 per cent whereas among *Lolium* genotypes, it was observed for Punjab ryegrass-1 with a decrease of 28 per cent.

A general increase in total chl content was observed in all the genotypes under drought condition in comparison to the controlled condition. Among all *Festuca* genotypes, maximum increase in total chl content was observed for Sel-63 with an increase of 137 per cent whereas among *Lolium* genotypes, it was observed for Palam ryegrass with an increase of 241 per cent. Chlorophyll a/b ratio of the genotypes showed a mixed response as in some genotypes ratio was increased whereas in some, it was decreased. Maximum increase of 150 per cent was observed in *Festuca* genotype Hima-1 whereas among *Lolium* genotypes, maximum increase of 1 per cent in Punjab ryegrass-1 was observed. Minimum decrease was observed in Hima-3 (1%) and Kashmir ryegrass (20 %).

3.6 Proline content (µmol/g of leaf)

The proline content (Figure 11) of the genotypes increased under stress condition in all the genotypes as compared to controlled conditions. Among all *Festuca* genotypes, maximum per cent increase was observed in Hima-3 (2019 % whereas among *Lolium* genotypes, maximum per cent increase in proline content was observed in Palam ryegrass (849 %).

3.7 Drought susceptibility index (DSI) and stress tolerance index (STI)

An appraisal of data given in Figure 12 revealed that DSI of the genotypes ranged from 0.35-1.93 under stress conditions. Among all *Festuca* genotypes, minimum DSI was observed for Hima-1 with a DSI of 0.35 whereas among *Lolium* genotypes, minimum DSI was observed in Palam ryegrass with DSI 0.89. STI of the genotypes ranged from 0.15-2.30. Among all *Festuca* genotypes, maximum STI was observed for EC178182 with STI of 2.30 whereas among *Lolium* genotypes, maximum STI was observed in Palam ryegrass with STI of 1.33.

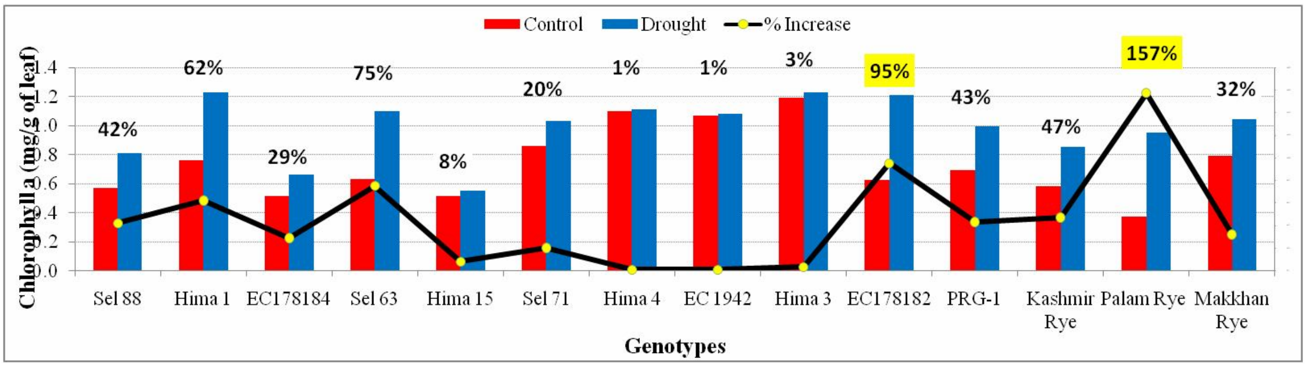

Figure 7: Per cent variation in chlorophyll a content of *Festuca* and *Lolium* genotypes under controlled and drought conditions (Sel: Selection; PRG-1: Punjab ryegrass-1).

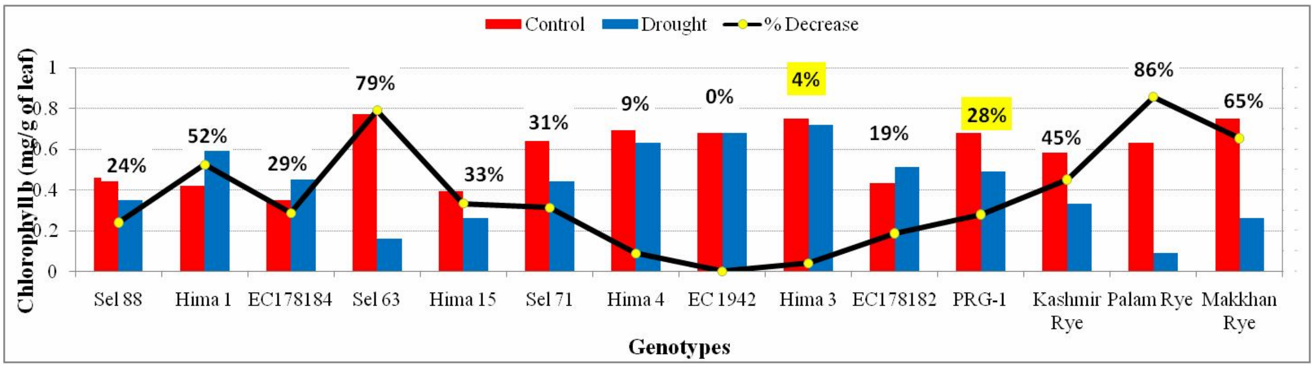

Figure 8: Per cent variation in chlorophyll b content of *Festuca* and *Lolium* genotypes under controlled and drought conditions (Sel: Selection; PRG-1: Punjab ryegrass-1).

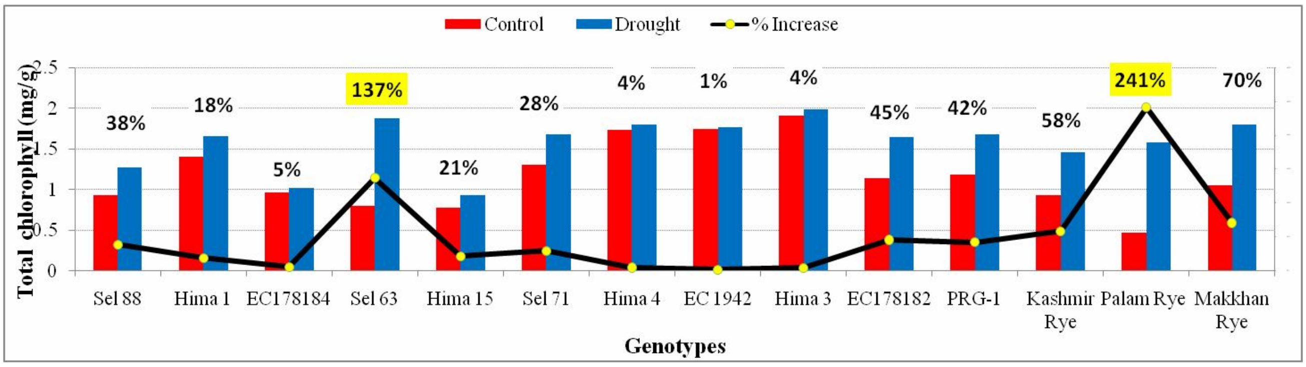

Figure 9: Per cent variation in total chlorophyll content of *Festuca* and *Lolium* genotypes under controlled and drought conditions (Sel: Selection; PRG-1: Punjab ryegrass-1).

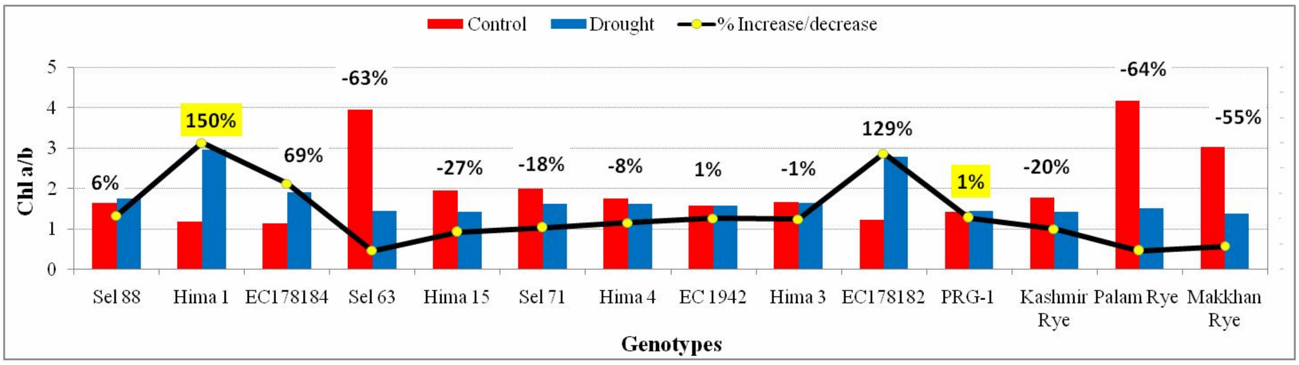

Figure 10: Per cent variation in chlorophyll a/b of *Festuca* and *Lolium* genotypes under controlled and drought conditions (Sel: Selection; PRG-1: Punjab ryegrass-1).

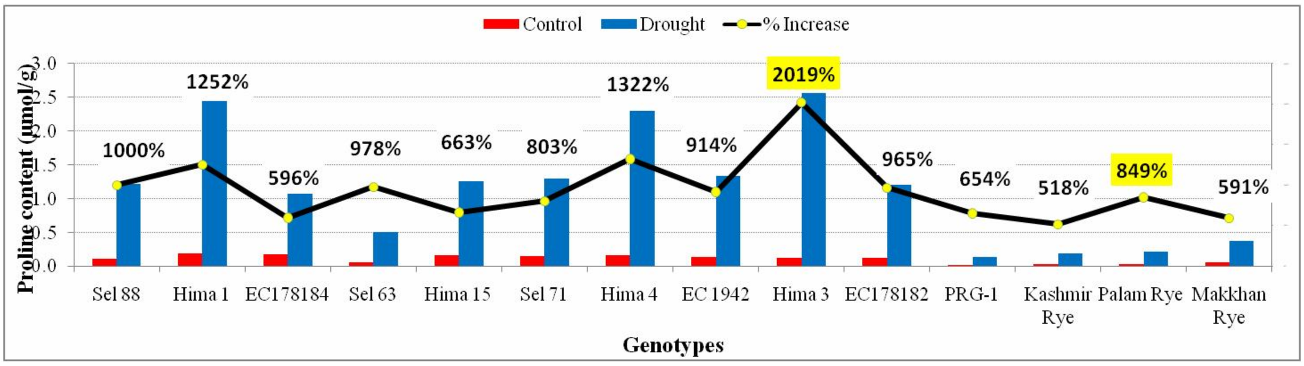

Figure 11: Per cent variation in proline content of *Festuca* and *Lolium* genotypes under controlled and drought conditions (Sel: Selection; PRG-1: Punjab ryegrass-1).

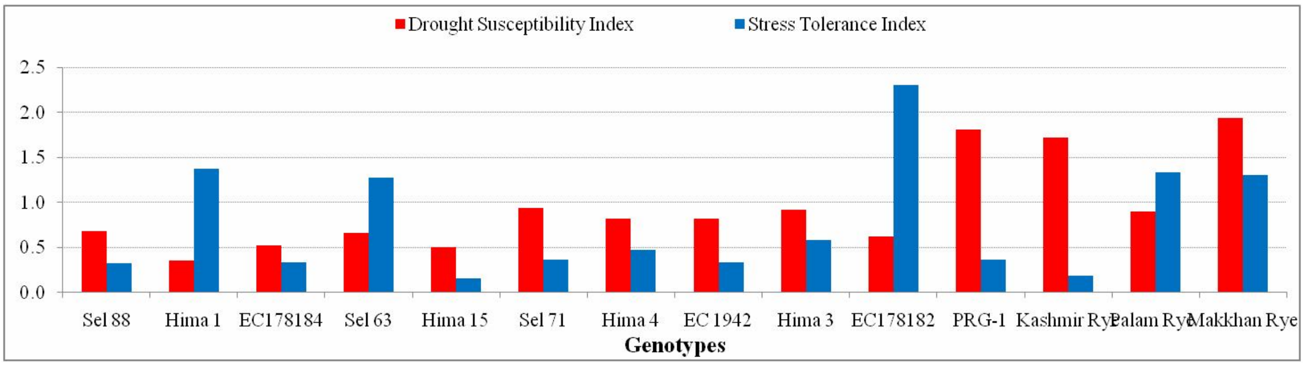

Figure 12: Drought susceptibility and stress tolerance index of *Festuca* and *Lolium* genotypes under drought conditions (Sel: Selection; PRG-1: Punjab ryegrass-1)

4. Discussion

In the present study, drought stress significantly influenced the root and shoot parameters in stressed plants. In both *Festuca* and *Lolium* genotypes drought stress increased the root length (RL), root weight (RW) and root: shoot ratio (R:S). This indicates that the elevation in root parameters during stress conditions boosts the root system distribution at deeper layers of soil which causes better water and nutrient uptake (Serraj *et al*., 2004; Farre and Faci, 2009). Pirnajmedin *et al.* (2015) reported that tall fescue genotypes with deeper root system had greater volume which enhanced the water absorption capacity and were tolerant to drought stress condition. Consistent with our findings, some studies have documented elevated root length and root weight at deeper soil layers under drought stress condition (Asseng *et al*., 1998; Huang and Gao, 2000; Wang and Huang, 2004; Wang *et al*. 2009). A decrease in shoot length and weight was observed in both *Festuca* and *Lolium* genotypes which may be due to dehydration of the protoplast which is correlated with turgor loss and reduced cell expansion. Khodarahpur (2011) reported a decrease in shoot length through PEG induced osmotic stress. An increase in root to shoot ratio (R/S) was observed in both *Festuca* and *Lolium* genotpyes which was in confirmation with the findings of Karcher *et al.* (2008); Merewitz *et al.* (2010); Pirnajmedin *et al.* (2016).

Drought stress decreased the leaf relative water content (LRWC) and chlorophyll b (Chl b). On the other hand, stress conditions increased chlorophyll a (Chla), total cholorophyll (Tchl) chlorophyll a/b (Chla/b) and proline content (PC) in both *Festuca* and *Lolium* genotypes. This is in accordance with previously reported results for *Festuca arundinacea* (Ebrahimiyan *et al*., 2013; Pirnajmedin *et al*., 2015). Under drought stress, chlorophyll content is the most indispensible compound and also considered as a major determinant of photosynthetic capacity (Sravanthi and Rao, 2014). Hence, selection of genotypes on the basis of increased or stable chlorophyll content may prevent yield loss under drought stress and ultimately increases drought tolerance. Ebrahimiyan *et al.* (2013) also observed an increase in chlorophyll a/b in tall fescue that might be due to faster damage of chlorophyll b as compared to chlorophyll a under drought stress condition. Under drought stress conditions, a decline in chlorophyll content has been considered as a generally observed phenomenon (Bayat *et al*., 2009; Ebrahimiyan *et al*., 2012), but according to some reports, increase in chlorophyll content under drought stress has also been observed which is similar to our findings (Jiang and Huang, 2001; Garcýa-Valenzuela *et al*., 2005). This study has indicated that in different plant species, the effect of drought stress on chlorophyll content varies differently. The Chla/Chlb ratio increased in some genotypes while decreased in others under drought stress conditions. This is presumably due to faster damage of Chla compared to Chlb in some genotypes. El-Tayeb (2006) showed that decrease in the Chla/Chlb ratio is faster in drought sensitive than in drought tolerant genotypes.

In this study drought stress increased proline content under stress conditions in all genotypes. Some reports suggest that proline accumulation is a reaction to stress (De-Lacerda *et al*., 2003). Accummultion of proline has been advocated as a selection parameter for drought tolerance (Verbruggen and Hermans, 2008).

Increase in proline during stress is in agreement with Hsu *et al.* (2003), Gunes *et al.* (2008), Bayoumi *et al.* (2008), Din *et al.* (2011), Ebrahimiyan *et al.* (2012) and Sepehri and Golparvar (2012).

The results indicated that breeders should work on root and physiological traits to improve forage yield and selection of the cultivars with higher LRWC, chlorophyll and proline content may improve *Festuca* and *Lolium* growth and quality under drought stress conditions.

5. Conclusion

In conclusion, the results of above study suggest that drought stress greatly influences physio-biochemical parameters that affect the growth and production of *Festuca and Lolium* genotypes. The results indicated that root and shoot traits were associated with drought tolerance in fescue grass and are efficient indirect selection tools to improve forage yield and identifying superior genotypes. In summary, drought stress reduced SL, SW, LWC and Chl b, whereas it increased the RL, RW, R:S, Chl a, TChl and proline content. The tolerance of *Festuca* spp. towards drought stress conditions is through changes in root and shoot morphology and through osmotic adjustment to maintain sufficient turgor pressure. The minimum decrease in SL, SW, Chl b, LWC for Hima-3 fescue during the drought-stress period suggested its greater drought tolerance as compared to Palam ryegrass which has a potential for improvement in these parameters through breeding program.

Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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