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Physiological and Yield Characteristics of Barley (*Hordeum vulgare*) Genotypes Subjected to Drought Stress

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ABSTRACT

Understanding agronomic features under water deficit is crucial for maximizing crop productivity potential and harvest. To further understand how drought stress affected barley's chlorophyll content and stability index, stem reserve mobilization, canopy temperature depression, plant height, number of spikelets per spike, spike length, number of productive tillers per plant and biomass, a pot experiment was performed in Department of Botany, Baba Mastnath University, in complete randomized design. Genotype varied from 29.0 to 38.0% (SRM), 0.09 to 1.08 °C (CTD), 38.9 to 28.0 mg/g FW (chl. 'a'), 15.2 to 14.6 mg/g FW (chl. 'b'), 11.80 to 8.97% (CSI), 89.23 to 76.30 cm (plant height), 9.00 to 7.13 cm (spike length), 12.71 to 11.60 g (biomass), 11.71 to 10.87 (number of spikelet/spike) and 73.42 to 49.88 (number of productive tillers/plant). In light of recent results, the barley genotypes BH-393, BH-855, DWRB-828 and RD-57 were identified as promising among all studied genotypes which can be utilized for cultivation in areas subjected to drought and for further physiological studies.

Key words: Barley, drought stress, physiological traits, yield attributes

INTRODUCTION

Barley (*Hordeum vulgare* L.) is one of the most important and first domesticated cereal crops of old world agriculture (Gurel *et al.*, 2016). Barley is the world's fourth most important cereal crop after rice, maize and wheat in terms of its quantity of production and areas of cultivation (Abdulhamed *et al.*, 2021). Due to its superior nutritional and therapeutic qualities, it has also surpassed wheat as a preferred cereal (Kaur *et al.*, 2016). It has been cultivated in many developed countries and can be grown in a wide range of climatic environments in the world such as at the high altitudes of the Himalayas and near the Arctic Circle (Zhu, 2017), where it is frequently exposed to drought stress which influences its growth, production and grain yield (Wenzel *et al.*, 2015). Barley is a natural stress tolerant crop and is highly adaptive to drought stress, salinity stress and fungal diseases and therefore can also survive on marginal lands (Gurel *et al.*, 2016). According to Munns *et al.* (2016), when faced by stress it responds by fastening its growth and phenological development, resulting in an early maturity.

Drought stress is undoubtedly the most damaging environmental factor that drastically limits the plant growth and crop productivity worldwide in the course of global climate change (Alghabari *et al.*, 2015). Global warming is expected to increase the frequency and intensity of drought in the twenty-first century. It has been predicted that high temperatures and scarce rainfall resulting in prolonged drought from 1 to 3% of the land for the present day to 30% by the 2090s (Adhikari *et al.*, 2015). Prolonged drought periods are not only restricted to arid and semi-arid regions, it profoundly affects the agriculture globally (Templer *et al.*, 2017). This is especially challenging given the requirement to increase the crop yields up to 70% to feed over 9.7 billion people by 2050. The damage caused by it is incalculable and to cope up with the severe effects of drought stress, crop shows certain changes in its developmental, morphological and physiological processes (Fahad *et al.*, 2017). It results in growth inhibition; accumulation of ABA, proline, mannitol and sorbitol; scavenging of reactive oxygen species; formation of radical compounds such as ascorbate, glutathione, α-tocopherol etc.; stomatal closure leading to reduced transpiration rates; decrease in water potential and reduction in photosynthetic rate (Fahad *et al.*, 2017). Sharma *et al.* (2019) hypothesized that this accumulation of compounds aided the stressed cells in two ways: by acting as

cytoplasmic osmolytes facilitating water uptake and retention, and by protecting and stabilizing macromolecules (i.e. proteins, liposomes, chloroplasts and membranes). Drought stress interferes with the process of photosynthesis of the crop by altering the ultrastructure of organelles and concentrations of various enzymes, pigments and metabolites (Seleiman *et al.*, 2021). Water deficit can occur at any growth stage whether at vegetative or reproductive. Some genotypes may tolerate drought at germination or seedling stage, but these may be susceptible to drought at the flowering stage or vice-versa (Sallam *et al.*, 2019).

Many breeding programmes aim at improving the drought tolerance of the crop with intensive studies on identifying the key morphological, physiological and molecular traits which can be used as criteria to understand the mechanism of plant resistance to drought stress (Fang and Xiong, 2015). Furthermore, improvement of drought tolerance and yield should be in parallelism because farmers need to produce their agricultural products profitably under drought stress (Sallam *et al.*, 2019). In this context, an investigation was conducted to evaluate barley genotypes for physiological parameters and yield attributes under limited water application.

MATERIALS AND METHODS

A total of 14 barley genotypes (AMBER, BH-902, BH-946, BH-393, BH-855, BH-959, C-164, DWRB-171, DWRB-172, DWRB-828, DWRB-92, RD-2907, RD-57 and SONU) were collected from the CCS Haryana Agricultural University, Hisar, India and used in the current study to assess their performance under irrigated as well as drought conditions at the research area of Department of Botany, Baba Mastnath University, Rohtak (HRY).

0.2% mercuric chloride ($HgCl_2$) was used for surface sterilization of the seeds for 5 min to prevent any contamination during the experiment. Five sterilized seeds were planted in earthen pots with 10 kg of fertile agricultural soil keeping only three healthy plants after pruning. In every two days, 50 ml of sterile tap water was used to maintain the field capacity of pot soil until emergence.

Pots under complete randomized design (CRD) were kept both under irrigated and drought environment. Drought stress was maintained by withholding irrigation after first visible date of heading and sprinkling water just once every seven days and control plants were watered to the point of saturation by sterile tap water every two days. The plants were treated for drought stress. Data were recorded in three replications. Thus, the total numbers of treatment were two and, numbers of genotypes were 14 and numbers of experimental pots were 84.

For estimation of stem reserve mobilization at anthesis and maturity, five stems were randomly chosen from each pot and were divided into penultimate and peduncle stems, which were then dried for 72 h at 80°C in an oven. The stem reserve mobilization was determined using the following formula after the weight of the stem pieces was measured using an analytical balance:

$$\text{SRM (\%)} = \frac{\text{DMSHT (Ant)} - \text{DMSHT (Mat)}}{\text{DMSHT (Ant)}} \times 100$$

Where, SRM stands for stem reserve mobilization (g/plant); DMSHT (Ant) for above ground dry matter of stem parts at anthesis stage (g); DMSHT (Mat) for above ground dry matter of stem parts at maturity stage (g). Penultimate and peduncle SRM from the stem section were calculated individually.

Portable infrared thermometer was used to measure the temperature of the canopy. A canopy view of 10 x 25 cm was obtained by taking readings with the equipment held at an angle of 30° to the horizontal plane, 1 m from the plot's edge, and around 50 cm above the crop. Measurements were made in full daylight i.e. 0.5 h before and 2 h after noon time. The temperature of the canopy was subtracted from the ambient temperature to calculate the CTD.

Chlorophyll was estimated as:

Chlorophyll 'a' (mg/g FW) = (12.7 × A663) × (2.69 × A645) × (V/1000 × W)
Chlorophyll 'b' (mg/g FW) = (22.9 × A645) × (4.68 × A663) × (V/1000 × W)
Total chlorophylls = (20.08 × A645 + 8,02 × A663) × (V/1000 × W)

Where, V = Extract volume (ml) and W = Fresh weight of sample (g)

CSI was determined as: CSI% = (Total chlorophyll under stress/Total chlorophyll under irrigated condition as control) × 100.
Plant height (cm) was measured when the plant reached its physiological maturity by using a metric ruler. The height of three plants from each genotype in a replication was measured as the average length in cm from the base to the tip of the plant, omitting the awns. Five primary spikes from each genotype for every replication were enumerated to determine the number of spikelets per spike at maturity. To measure the spike length (cm) of the plant, five independently selected plants at maturation phase from every genotype for each replication were measured in centimeters with the help of the ruler, and an average was derived. The number of fully grown spikes carrying tillers per plant was used to average for evaluating the number of productive tillers per plant. Biomass (g) per meter square was calculated by clipping stem of the plants at the base and the weight of the plants in grams was assessed using a spring balance and then the average was determined.
OPSTAT software (accessible on www. http// hau.ernet.in.) was used to analyze the data using analysis of variance (ANOVA) for the complete randomized design (CRD) and CD at 5% was determined.

RESULTS AND DISCUSSION

The onset of water stress in the tested genotype showed an increase in stem reserve mobilization as compared to irrigated condition. Mean stem reserve mobilization fluctuated between 29.60 (BH-946) to 38.23% (BH-393). Overall increment in genotype was 33.46% as compared to irrigated condition (Table 1). It was due to the availability of carbohydrates from three distinct suppliers determining grain maturation: post-anthesis carbohydrates that were newly synthesized and directly shifted to the grains, post-anthesis carbohydrates that were transiently loaded in the stem before being remobilized to the grains, and pre-anthesis carbohydrates that were chiefly reserved in the stem but were mobilised towards the grains at the kernel-filling juncture (Sallam *et al.* 2015). The onset of drought caused photosynthesis to rapidly diminish after anthesis, which limited the amount of available assimilates that can be added to the grain, drastically cutting the proportion of dry matter in the kernels (Abid *et al.* 2017). The content of carbohydrates in the various parts amplified despite the fact that photosynthesis diminished and in order to adequately offset this loss, pre-anthesis reserves must provide a good share of the carbohydrates needed for grain filling (Pozo *et*

Table 1. Effect of drought on stem reserve mobilization and canopy temperature depression in barley genotypes

Genotypes	SRM (%)			CTD (°C)		
	IR	DR	Mean (G)	IR	DR	Mean (G)
AMBER	29.6	38.5	34.1	0.34	1.81	1.08
BH-902	26.3	35.3	30.8	-0.27	0.65	0.19
BH-946	25.6	33.6	29.6	-0.31	0.43	0.06
BH-393	33.5	43.0	38.2	0.64	1.81	1.23
BH-855	32.4	41.8	37.1	0.58	1.82	1.20
BH-959	28.2	37.4	32.8	0.15	0.94	0.55
C-164	31.3	40.7	36.0	0.54	1.76	1.15
DWRB-171	29.3	37.4	33.4	0.16	0.93	0.55
DWRB-72	27.1	35.6	31.4	-0.05	0.47	0.21
DWRB-828	30.8	38.8	34.8	0.44	1.66	1.05
DWRB-92	27.3	39.8	33.6	-0.32	0.50	0.09
RD-2907	26.7	35.4	31.1	-0.57	0.36	-0.11
RD-57	27.0	35.4	31.2	-0.52	0.08	-0.22
SONU	30.5	38.7	34.6	0.48	1.83	1.16
Mean (T)	29.0	38.0		0.09	1.08	
Statistical factors	C. D.	S. E(d)	S. E(m)	C. D.	S. E(d)	S. E(m)
Treatment (T)	0.440	0.219	0.155	0.010	0.005	0.004
Genotypes (G)	1.163	0.579	0.410	0.027	0.014	0.010
T × G	1.645	0.819	0.579	0.039	0.019	0.014

SRM – Stem reserve mobilization and CTD – Canopy temperature depression.

al., 2019). In present study, remobilization efficiency was recorded to be a little less than 10% higher when water was withheld, the result was also supported by the investigations of Poureisa *et al.* (2019) and Firoozabadi *et al.* (2022). All the genotypes showed statistical significance with respective drought treatment as well as significant interaction between genotype and stress environment.

Canopy temperature depression showed increase in the pattern of all tested genotypes under stress as well as control condition (Table 1). Mean CTD extended from -0.22 (RD-57) to 1.23°C (BH-393). Average rise in CTD in restricted environment was 1.08°C as compared to the control (0.09°C). Under drought stress, genotypes with lower canopy temperatures or high CTD were discovered to absorb more moisture from the soil, causing cooling effect, and minimizing extreme dehydration (Sofi *et al.*, 2021). CTD under both control and drought contexts was correlated linearly to the yield potential of the genotype and might be operated as a reference characteristic to determine the sensitivity of genotype to drought (Purushothaman *et al.*, 2017). According to the research, CTD can serve as a reliable predictor of crop performance in both irrigated and water-stressed environments with the maximum potential in genotypes RD-57 and RD-2907. Results from preset investigation were supported by Elbasyoni *et al.* (2022).

In light of the damage that drought stress-induced ROS causing to chloroplasts, drought stress limited the production of chloropyll pigment and reduced the fraction of chlorophyll-binding proteins (Zhanassova *et al.,* 2021). The carbon reduction cycle, stomatal regulation of CO_2, thylakoid electron transport and other crucial processes were all disrupted as a result of water deficiency. Results of chlorophyll content of the tested genotypes showed a remarkable reduction on the onset of drought stress as compared to the control (Table 2). Mean reduction in treatments ranged from 38.9 to 28.0 mg/g FW for chlorophyll 'a' and 15.2 to 14.6 mg/g FW for chlorophyll 'b'. Mean decrement in genotype ranged from 32.1 to 24.0 mg/g FW for chlorophyll 'a' and 19.7 to 11.2 mg/g FW for chlorophyll 'b'. Genotype BH-393 recorded maximum in chlorophyll 'a' content closely followed by genotypes BH-855 and BH–393 showing highest chlorophyll 'b' content in irrigated and drought condition. These results are according to Mahmood *et al.* (2019) who also conducted their experiments on barley crop. All examined genotypes showed significant reduction under stress condition and the interaction effect between genotypes and treatment was also significant compared to controlled environment.

Table 2. Effect of drought on the chlorophyll content in barley genotypes

Genotypes	Chl. 'a'			Chl. 'b'		
	IR	DR	Mean (G)	IR	DR	Mean (G)
AMBER	39.0	17.6	28.3	12.1	10.2	11.2
BH-902	38.8	18.0	28.4	13.9	11.1	12.5
BH-946	36.8	15.6	26.2	13.5	11.7	12.6
BH-393	43.1	21.2	32.1	20.3	19.1	19.7
BH-855	43.0	20.5	31.8	19.5	17.7	18.6
BH-959	38.0	17.4	27.7	14.0	11.9	13.0
C-164	37.5	19.7	28.6	15.0	14.5	14.8
DWRB-171	41.6	19.5	30.6	17.5	17.4	17.5
DWRB-172	35.7	13.5	24.6	13.9	13.4	13.7
DWRB-828	40.4	19.1	29.8	17.5	16.6	17.0
DWRB-92	37.9	14.0	26.0	12.5	11.3	11.9
RD-2907	36.9	13.0	25.0	11.9	12.6	12.2
RD-57	35.3	12.7	24.0	15.6	15.2	15.4
SONU	40.2	19.0	29.6	16.0	13.0	14.5
Mean (T)	38.9	17.2	28.0	15.2	14.0	14.6
Statistical factors	C. D.	S. E(d)	S. E(m)	C. D.	S. E(d)	S. E(m)
Treatment (T)	0.399	0.199	0.140	0.187	0.093	0.066
Genotypes (G)	1.055	0.525	0.372	0.495	0.246	0.174
T × G	1.492	0.743	0.525	0.700	0.349	0.246

Chl. 'a' – Chlorophyll a and Chl. 'b' – Chlorophyll b.

Chlorophyll stability index was strongly affected by the imposition of drought stress. A decline in the chlorophyll stability index was observed in all the examined genotypes during water deficit condition as compared to the irrigated environment (Table 3). The CSI is temperature-dependent and it illustrates the degree of sensitivity of the chlorophyll molecules to stress (Hassan *et al.,* 2021). It allows higher yield varieties to preserve their photosynthetic rate in drought-stressed conditions (Mishra *et al.*, 2016) and therefore, photosynthetic efficacy and drought tolerance may be directly correlated with higher value of CSI (Goswami *et al.*, 2020). Present research revealed that DWRB-92 and BH-393 had the highest percentages of CSI among all the investigated genotypes, and this characteristic helped these varieties to endure drought effectively. Mean chlorophyll stability index for drought treatment shifted from 11.80 to 8.97%. Average chlorophyll stability index for all genotypes ranged from 13.52 to 8.45%. Results with respect to tested genotypes studies and stress were statistically significant.

Drought treatment resulted in the drop of the height of plant from 89.2 (RD-57) to 76.3 (BH-855) cm and the mean decrement in genotype ranged between 85.37 and 78.24 cm as compared to irrigated conditions. Plant height is a crucial adaptability characteristic in drought prone environments, since a dry period during the growth season causes a severe drop in stem elongation with a loss in crop productivity and this fall renders harvesting challenging or impracticable (Table 3).

Length of the spike decreased as a result of the drought treatment from 9.00 to 7.13 cm, whereas the mean decrement in genotype for spike length varied between 8.26 and 7.68 cm (Table 4). DWRB-828 exhibited the highest value and BH-946 presented the lowest measurement under both the drought and the control condition. The number of spikelets per spike was found to decrease in all the tested genotypes during water deficit condition. Mean number of spikelet per spike for all genotypes ranged from 11.71 to 10.87. Minimum decrease in number of spikelets per spike was recorded in C-164 followed by SONU.

Drought treatment led to the reduction in the number of productive tillers per plant from 73.42 to 49.88 and the average reduction in genotype fluctuated between 67.73 and 61.21 (Table 5). A downfall of mean biomass per plant in all genotypes was also observed ranging from 12.71 to 11.60 g after application of the stress. Genotype AMBER showed maximum biomass per plant measuring 13.23 g followed by BH-393 at 5% CD level. A statistically significant interaction between genotype and stress environment as well as each genotype's response to its specific drought treatment,

Table 3. Effect of drought on the chlorophyll stability index and plant height in barley genotypes

Genotypes	CSI (%)			Plant height (cm)		
	IR	DR	Mean (G)	IR	DR	Mean (G)
AMBER	10.63	8.57	9.60	84.0	77.3	80.7
BH-902	11.27	8.57	9.92	90.1	82.4	86.3
BH-946	10.37	8.57	9.47	84.3	78.7	81.5
BH-393	15.17	11.20	13.18	90.1	81.7	85.9
BH-855	11.53	9.30	10.42	81.3	71.3	76.3
BH-959	10.33	8.90	9.62	82.8	75.4	79.1
C-164	12.13	8.53	10.33	87.7	75.1	81.4
DWRB-171	10.00	6.90	8.45	78.7	75.0	76.8
DWRB-172	11.57	7.73	9.65	85.2	83.4	84.3
DWRB-828	10.77	8.00	9.38	89.4	81.0	85.2
DWRB-92	15.20	11.83	13.52	82.5	74.5	78.5
RD-2907	11.13	8.60	9.87	82.0	75.8	78.9
RD-57	11.60	9.43	10.52	94.5	84.0	89.2
SONU	13.50	9.50	11.50	82.5	79.8	81.1
Mean (T)	11.80	8.97		85.4	78.2	
Statistical factors	C. D.	S. E(d)	S. E(m)	C. D.	S. E(d)	S. E(m)
Treatment (T)	0.12	0.060	0.043	0.911	0.454	0.321
Genotypes (G)	0.32	0.160	0.113	2.411	1.200	0.849
T × G	0.45	0.226	0.160	3.410	1.698	1.200

CSI – Chlorophyll stability index.

Table 4. Effect of drought on spike length and number of spikelets per spike in barley genotypes

Genotypes	Spike length (cm)			No. of spikelets/spike		
	IR	DR	Mean (G)	IR	DR	Mean (G)
AMBER	8.20	7.87	8.03	12.0	10.1	11.1
BH-902	7.77	7.07	7.42	10.9	10.5	10.7
BH-946	7.20	7.07	7.13	11.4	10.2	10.8
BH-393	7.73	6.93	7.33	10.8	9.0	9.9
BH-855	8.60	8.30	8.45	12.8	12.5	12.7
BH-959	7.97	7.53	7.75	10.5	9.6	10.0
C-164	8.23	7.27	7.75	9.8	8.9	9.3
DWRB-171	8.07	7.33	7.70	11.1	9.1	10.1
DWRB-172	8.63	8.33	8.48	13.7	13.4	13.5
DWRB-828	9.20	8.80	9.00	15.1	14.7	14.9
DWRB-92	8.43	7.53	7.98	12.0	10.9	11.5
RD-2907	8.90	8.33	8.62	13.9	13.0	13.5
RD-57	8.17	8.13	8.15	11.5	10.5	11.0
SONU	8.60	7.03	7.82	10.1	9.2	9.7
Mean (T)	8.26	7.68		11.8	10.8	
Statistical factors	C. D.	S. E(d)	S. E(m)	C. D.	S. E(d)	S. E(m)
Treatment (T)	0.091	0.045	0.032	0.127	0.063	0.045
Genotypes (G)	0.241	0.120	0.085	0.336	0.167	0.118
T × G	0.340	0.169	0.120	0.475	0.236	0.167

Table 5. Effect of drought on number of productive tillers per plant and biomass per plant in barley genotypes

Genotypes	No. of productive tillers/plant			Biomass (g)/plant		
	IR	DR	Mean (G)	IR	DR	Mean (G)
AMBER	65.7	60.5	63.1	13.9	12.6	13.2
BH-902	66.8	59.2	63.0	12.8	11.5	12.2
BH-946	55.2	44.6	49.9	11.7	10.7	11.2
BH-393	60.8	53.5	57.1	13.6	12.7	13.1
BH-855	71.7	67.9	69.8	12.7	12.0	12.4
BH-959	68.1	62.6	65.4	11.9	9.6	10.7
C-164	66.2	59.7	62.9	12.9	11.5	12.2
DWRB-171	69.9	57.9	63.9	13.0	12.0	12.5
DWRB-172	69.1	68.5	68.8	12.7	12.2	12.4
DWRB-828	73.4	71.0	72.2	12.4	11.3	11.9
DWRB-92	72.0	64.6	68.3	12.4	11.9	12.1
RD-2907	76.0	70.8	73.4	12.5	11.5	12.0
RD-57	67.7	63.2	65.4	13.1	12.6	12.9
SONU	65.8	53.1	59.5	12.4	10.2	11.3
Mean (T)	67.7	61.2		12.7	11.6	
Statistical factors	C. D.	S. E(d)	S. E(m)	C. D.	S. E(d)	S. E(m)
Treatment (T)	0.724	0.361	0.255	0.137	0.068	0.048
Genotypes (G)	1.916	0.954	0.674	0.361	0.180	0.127
T × G	2.709	1.349	0.954	0.511	0.254	0.180

were shown for all the genotypes. Present results indicated that drought stress severely impeded plant development and reduced biomass, plant height, spike length, spikelet number and number of productive tillers per plant in all 14 accessions evaluated. These parameters are the prospective targets in the yield stability of the crop which is a crucial feature in the breeding objective for subsistence agriculture (Verma *et al.*, 2021; Saed-Moucheshi *et al.*, 2022).

The mean sum of square for the genotypes (G) and drought treatments (T) for stem reserve mobilization, chlorophyll content, chlorophyll stability index and canopy temperature depression are shown in Table 6. On the physiological measures examined, interactions between genotypes and drought were also shown to be significant at 1% level of significance.

Table 7 represents the mean sum of square for treatments and genotypes for plant height, spike length, number of productive tillers per plant, number of spikelets per spike and

Table 6. Mean sum of square of barley genotypes for stem reserve mobilization, chlorophyll content, chlorophyll stability index and canopy temperature depression under drought and irrigated condition

Source of variation	d. f.	SRM (%)	Chl. 'a' (mg/g FW)	Chl. 'b' (mg/g FW)	CSI (%)	CTD (°C)
Treatment (T)	1	1692.911**	9862.611**	34.333**	167.736**	6.762**
Genotypes (G)	13	38.415**	40.583**	43.092**	12.336**	0.584**
T × G	13	2.01**	3.382**	1.609**	1.052**	0.908**

**Significant at 1% level of significance.

Table 7. Mean sum of square of barley genotypes for plant height, spike length, number of productive tillers per plant, number of spikelets per spike and biomass per plant under drought and irrigated condition

Source of variation	d. f.	Plant height (cm)	Spike length (cm)	No. of productive tillers/plant	No. of spikelets/spike	Biomass (g)/plant
Treatment (T)	1	1067.784**	7.143**	891.774**	21.097**	25.723**
Genotypes (G)	13	89.146**	1.668**	231.498**	17.121**	3.108**
T × G	13	13.61**	0.24**	17.904**	0.501**	0.459**

**Significant at 1% level of significance.

biomass per plant interpreting a significant interaction at 1% level of significance between genotypes and treatment. This demonstrated that genotypes responded differently to drought conditions.

CONCLUSION

The present study's implications showed that the optimal approach of genotype selection for drought conditions was based on the physiology of the barley genotype. Genotypes BH-393, BH-855, DWRB-828 and RD-57 exhibited good outcomes when drought stress was applied to barley genotypes. These genotypes can be further employed to produce drought resistant varieties, offering support in particular research fields and breeding programmes for future prospects.

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