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Assessment of maize (*Zea mays* L.) genotypes on the basis of biochemical contents in respect to drought

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Abstract
Corn is a food crop that has an important role both in industries and as a staple food in many countries throughout the world. Its growth, development and productivity seriously affected by drought. An experiment was carried out to investigate the effect of drought stress on sugar and proline content of eighty maize genotypes raised by half diallel crosses under irrigated and partial irrigated conditions in randomized block design (RBD) with two replications. Proline and sugar content was analyzed to assess the performance of genotypes in respect to drought tolerance. Increase in the level of sugar and proline content among genotypes was found highly significant at 1% probability level. Among eighty maize genotypes, genotypes IL1XIL4, IL3XIL5, IL2XIL5, IL2XIL11, IL7XIL11, IL9XIL10, IL8, IL5XIL11, IL1XIL7 and IL1XIL8 displayed increased values of sugar and proline content. Hence may be considered as drought tolerant and may be employed in crop improvement programme in future.

Keywords: Maize, drought, sugar and proline content

Introduction
Corn is a food that has an important role both in industries and as a staple food in many countries throughout the world (Khayatnezhad *et al*., 2011; Naghavi *et al*., 2013; Yadav *et al*., 2022) [18, 33, 47]. It is well known that one of the environmental factors that could influence growth, development and production of many crop plants including corn is drought (Bahar and Yildirim, 2010; Batlang, 2010; Krasensky and Jonak, 2012; Lipiec *et al*., 2013; Choudhary *et al*., 2021a; Choudhary *et al*., 2021b; Mishra *et al*., 2021 a; Mishra *et al*., 2021b; Mishra *et al*., 2021c; Mishra *et al*., 2021d; Mishra *et al*., 2022a; Mishra *et al*., 2022b; Sharma *et al*., 2021) [2, 5, 20, 21, 9, 10, 24, 25, 26, 27, 30, 31, 39]. This is understandable because water is the main constituent components of living organisms, including corn. When faced with certain environmental conditions such as drought, some plants have the ability to adapt to the environment where they are grown (Lisar *et al*., 2012) [22].
Drought is one of the most important abiotic stresses affecting plant growth, development and productivity. In the future, global warming and the growth of human population can lead to reduction of water resources and an increase in arid and semiarid areas. Therefore, the study of mechanisms of plant adaptation and tolerance to drought, as well as of the ability to recover after water deficit is an important task for modern research (Sinay *et al*., 2014) [44].
To elucidate plant ability to survive under drought, it is of importance to study the different morpho-physiological (Mishra *et al*., 2021`a; Yadav *et al*., 2022) [24, 47] and biochemical adaptation (Shyam *et al*., 2019; Shahu *et al*., 2020a; Shahu *et al*., 2020b; Gupta *et al*., 2021; Mishra *et al*., 2021e; Mishra *et al*., 2021f; Sharma *et al*., 2021; Shyam *et al*., 2021) [42, 40, 41, 13, 28, 29, 39, 43] and tolerance as well as the mechanisms of recovery under rehydration. Plant tolerance to water deficit requires the aptitude to maintain functions under unfavorable water conditions and to recover water status and functions rapidly after rewatering. Recent studies showed that recovery phase is as important as the stress treatment since the efficient recovery affects further plant growth and development (Chen *et al*., 2016; Kosová *et al*., 2018) [8, 19].
Evaluation of plant tolerance to drought stress can be done by identifying the physiological characteristics necessary tolerance (Nio Song and Banyo, 2011) [35]. This is an indicator use often in attempt to select resistant varieties or tolerance to drought (Chaves *et al*., 2003; Bartles and Chandler, 2007; McCann and Huang, 2008; Nio Song and Banyo, 2011) [6, 3, 23, 35]. Physiological responses that can be employed is organic matter accumulation in the cytosol, like osmolyte and to maintain turgor pressure inside the cell.

The changes in carbohydrate metabolism under drought conditions are closely related to photosynthesis and transpiration and are of great importance for stabilization of water balance of plants (Hare *et al*.,1998; Tarchevsky, 2001; Reddy *et al*., 2004, Chaves *et al*., 2009; Sinay *et al.*, 2014) [14, 45, 36, 7, 44]. Previously, a sharp increase in the content of reducing sugars and proline simultaneously with significant reduction in the rate of photosynthesis and transpiration during the adaptation of maize seedlings to drought (Nikolaeva *et al*., 2017) [34] has been documented. Accumulation of osmolytes in the cells is known to lead to the formation of concentration gradient between the inside and outside cell compartments. This concentration gradient might create favorable conditions for the transfer of osmolytes from the photosynthesizing cells into apoplast.

The aim of present investigation was to qualify a hypothesis that the accumulation of soluble sugars and proline under drought probably leads to a decrease in ψwa (water potential of mesophyll cells' apoplast in substomatal cavity) and, on the contrary, the reduction of osmolyte content after rewatering results in an increase in ψwa alongside with restoration of the leaf water status. Understanding physiological behavior of corn cultivars under drought conditions may results in predicting drought tolerant cultivars of corn. Keeping these facts in mind, this research was designed to evaluate the response of corn genotypes such as proline and total soluble sugars contents in the corn leaves, at the vegetative growth phase under irrigated and partially irrigated conditions.

Materials and Methods

Plant material and growth conditions

The seeds of 12 maize inbred lines were acquired from Sam Higgonbottom Agriculture Science and Technology University, Prayagraj, U.P., India and a crossing programme was initiated following half diallel analysis as per method suggested by Jinks and Hayman (1953) [16] and 66 F_1 hybrids were raised. These hybrids along with two checks *viz*., drought tolerant HKI1105 and drought susceptible HKI1128. At final a total of 80 genotypes including 12 parents, 66 F_1 hybrids and two checks were grown under irrigated and partial irrigated conditions in a randomized block design (RBD) with two replications. Each genotype was sown in 2 rows of 4 meters with a spacing of 60 cm between rows and 20 cm between plant to plant. Drought stress was imposed from 10 days before flowering by with-holding irrigation. The irrigation was resumed when soil moisture reaches temporary wilting point. The collection of corn leaf for sugar and proline content analysis was done on reproductive stage after planting. The leaves that were used for sugar and proline analyses were the second leaf from the top leaf.

Analysis of sugar content

Total sugar content was analyzed employing Anthrone method as suggested by Irigoyen *et al.* (1992) [15]. Zero-point five gram of the fresh leaf was crushed in a mortar and pestle and 5.0 ml of 80% hot alcohol was added to it. The mixture was centrifuged at 6000 rpm for 15 min. The obtained supernatant was separated into another test tube and 12.5 ml of 80% alcohol was added to it. 1.0 ml of the solution was taken and added with1.0 ml of 0.2% anthrone. The mixture was heated in a water bath at 100°C for 10 min. The reaction was terminated by incubating the mixture on ice for 5 min. Total soluble sugar content was determined using a spectrophotometer at 620 nm wavelength. Calculation of the total soluble sugar content was done by creating a standard curve using a standard glucose and was expressed in μgg^{-1} fresh weight (μgg^{-1} FW).

Proline content analysis

Proline content was analyzed following the Bates method (Bates *et al*., 1973; Mohammadkhani and Heidari, 2008) [4, 32]. One gram of the fresh leaf was crushed using mortar and pestle and was homogenized with 5.0 ml of 3% sulfosalicylic acid. The homogenate was centrifuged at 6,000 rpm for 15 min. One ml of the supernatant was taken and 1.0 ml each of nynhidrin and acetic acid were added to it. This was heated in water bath for 1 h and was incubated in ice for 5 min. Two ml of the solution was taken and extracted with 2.0 ml of toluene and quickly shaken with a vortex until chromoporm was formed. The upper phase of the chromoporm was taken and the absorbance was measured with a spectrophotometer at 520 nm wavelength. To determine the proline content of corn cultivars, a standard curve was made using pure proline. The content of proline was expressed in units of $\mu molg^{-1}$ fresh weight ($\mu molg^{-1}$ FW).

Data analysis

The data obtained were analyzed using ANOVA to determine the effect of drought stress on the corn responses. Means comparison was conducted using the Duncan's multiple range test (DMRT) at 5% probability level. ANOVA and DMRT were performed by R software (R Core Team, 2020; De Mendiburu, 2009) [37, 12].

Results and Discussion

The analysis of variance presented in Table 3 clearly indicated existence of substantial magnitude of variations among 80 maize genotypes included in the present investigation for both of the biochemical parameters investigated.

In general, the increased magnitudes of proline and total soluble sugar content was investigated under the partial irrigated condition and the lowest under the irrigated condition (Table 2; Fig.1; Fig.2). Soluble sugars are the key osmotic modification constituents and therefore are significant pointers of tolerance/resistance in genotypes. Under irrigated condition, among 80 maize genotypes sugar content ranged between 0.82$\mu molg^{-1}$ FW to 1.41 $\mu molg^{-1}$ FW with a mean value of 1.06 $\mu molg^{-1}$FW (Table 2; Fig.1). Under irrigated condition the highest sugar content was recorded in genotype IL1XIL4 (1.41) followed by IL3XIL5 (1.41), IL6XIL9 (1.41), HKI1128 (1.41) and IL1XIL3 (1.35) while the lowest in genotype IL2XIL9 (0.82). However, under partial irrigated condition it ranged between 1.48$\mu molg^{-1}$ FW to 0.91$\mu molg^{-1}$ FW with a mean worth of 2.49 $\mu molg^{-1}$ FW. Under partial irrigated condition the highest value was obtained in genotype IL8 (2.49) tracked by IL2XIL5 (2.4), IL2XIL11 (2.37), IL7XIL9 (1.93) and IL6XIL10 (1.93), whereas the lowest value was documented in genotype IL2XIL9 (0.91). Genotypes pursuing higher sugar content might be drought tolerant as reported earlier by Irigoyen *et al*. (1992) [15], Kachare *et al*. (2019) [17], Choudhary *et al*. (2021c) [11], Mishra *et al*. (2021e) [28] and Sharma *et al*. (2021) [39] as they stated that the sugar content in leaves of the plant increased under drought conditions. Arabzadeh (2012) [1] itemized that the sugar dissolves compatible metabolites and

absorption increases with increased drought stress and reduced soil water content. Moreover, one of the mechanisms plants use to withstand drought stress is by regulating osmotic potential of the cell, especially if drought stress increases gradually from mild stress to severe one (Lisar *et al*., 2012, Lipiec *et al*., 2013; Naghavi *et al*., 2013) [22, 21, 33].

When plants are faced by drought stress, the osmotic pressure of the plant cell regulates many processes through the accumulation of non-toxic solutes inside the cell (Lisar *et al*., 2012; and Lipiec *et al*., 2013) [22, 21]. This osmotic accumulation occurs because the cell water potential decreases thereby increasing the concentration of dissolved material to maintain turgidity of the cell. Moreover, compatible solutes prevent interaction between the ions and cellular components by replacing the water molecules around the component, thus preventing destabilization during drought. Osmolyte accumulation is also owing to increased biosynthesis without degradation (Lisar *et al*., 2012; Sinay *et al.*, 2014) [22, 44]. Osmotic cell potential can be adjusted by increasing the concentration of sugar which can decrease water potential of cells without inhibiting the function of the enzyme and does not reduce turgidity of the cell. Sugar accumulation in drought stress conditions helps to maintain the stability of the membrane, prevent and protect membrane fusion and; keep protein so as to remain functional (Xonostle-Cazares *et al*., 2011; Arabzadeh, 2012; Lisar *et al*., 2012; Lipiec *et al*., 2013; Sinay *et al.* 2014) [48, 1, 22, 21, 44].

Proline is also trusted as an authoritative drought tolerance pointer. As similar under irrigated condition proline content ranged between 16.4 µmolg^{-1} FW to 53.8µmolg^{-1} FW with an average value of 30.92 µmolg^{-1} FW (Table 2; Fig.2). The highest proline content attained in genotype IL7XIL11 (53.8) persuaded by genotypes IL5XIL11 (46.46), IL8 (45.4), IL1XIL8 (45.1) and IL1XIL7 (41.9) whilst the lowest value was evidenced in genotype IL4XIL8 (16.4). Nevertheless, under partial irrigated condition, proline content was arrayed between 21.01 µmolg^{-1} FW to 63.41 µmolg^{-1} FW with a mean worth of 35.99 µmolg^{-1} FW. Under partial irrigated condition the highest proline content value was recorded in genotype IL7XIL11 (63.41) intimately tracked by IL9XIL10 (56.16), IL8 (53.71), IL5XIL11 (50.41) and IL1XIL7 (45.11), whilst the lowest value was evidenced in genotype IL9 (21.01). Genotypes exhibiting higher proline content may have drought tolerance as advised by Kachare *et al.* (2019) [17], Mishra *et al.* (2021f) [29] and Sharma *et al.* (2021) [39] as they also concluded during their study on screening of soybean genotypes. It may be perhaps owing increased proline content maintains cell water level under drought (Choudhary *et al*., 2021c) [11]. According to Umezawa *et al.* (2006) [46] and Krasensky and Jonak (2012) [20], plants have the ability to accumulate non-toxic compounds such as proline which protects cells damage due to low water potential of cells, which is a way of adaptation of plants to drought stress tolerance.

Corn is very sensitive to water stress (Shaddad *et al*., 2011) [38]. The differences in the responses to drought stress among 80 maize genotypes show that each cultivar has different ability to synthesis proline and total soluble sugar with an increase in drought stress treatment. Based on these findings, it is clear that the sugar content and proline content in the leaves was significantly increased due to the increase in the level of drought stress. Similar results were also addressed by Mohammadkhani *al.* (2008) and Sinay *et al.* (2014) [32, 44].

Table 1: List of inbred lines with their parentage used in study

S. No.	Lines/genotypes	Parentage	Source
1	IL-1	CM-13	SHUATS, Allahabad
2	IL-2	CML-193	SHUATS, Allahabad
3	IL-3	CML-439	SHUATS, Allahabad
4	IL-4	NBPGR-36417	SHUATS, Allahabad
5	IL-5	NBPGR-36417 X NBPGR-33000	SHUATS, Allahabad
6	IL-6	(103) NBPGR-36548 X (97) NBPGR-36407	SHUATS, Allahabad
7	IL-7	DMR-N 21 X NBPGR-32809	SHUATS, Allahabad
8	IL-8	LM- 13 X NBPGR-31899	SHUATS, Allahabad
9	IL-9	CML-224-1 X NBPGR-32809	SHUATS, Allahabad
10	IL-10	NBPGR-36550 X NBPGR-36407	SHUATS, Allahabad
11	IL-11	KL- 153237 X VL- 1016536	SHUATS, Allahabad
12	IL-12	CML- 161 X VL- 1056	SHUATS, Allahabad
13	DS	HKI 1128	
14	DT	HKI 1105	

DS- drought stress, DT- drought tolerance

Table 2: Level of sugar content and proline content of the maize leaf under Irrigated (I) and Partial irrigated (PI) condition

Sr. No.	Genotype	Sugar content (µmol/g FW)		Proline content (µmol/g FW)	
		I	PI	I	PI
1	IL1 X IL2	1.14	1.3	32.33	42.91
2	IL1 X IL3	1.35	1.6	35.1	38.11
3	IL1 X IL4	1.41	1.3	39.45	42.76
4	IL1 X IL5	1.3	1.41	34.9	42.45
5	IL1 X IL6	0.85	1.02	32.86	33.76
6	IL1 X IL7	0.87	1.17	41.9	45.11
7	IL1 X IL8	0.94	1.24	45.1	38.86
8	IL1 X IL9	1.3	1.32	38.35	42.71
9	IL1 X IL10	1.15	1.48	38.7	41.08
10	IL1 X IL11	1.35	1.56	32.66	34.36

11	IL1 X IL12	1.33	1.57	36.46	39.01
12	IL2 X IL3	0.96	1.26	37.61	40.21
13	IL2 X IL4	0.96	1.39	37.1	40.06
14	IL2 X IL5	0.94	2.4	36.25	42.81
15	IL2 X IL6	0.99	1.11	37.41	39.01
16	IL2 X IL7	0.93	1.18	35.01	36.76
17	IL2 X IL8	0.9	0.99	35.36	37.51
18	IL2 X IL9	0.82	0.91	34.3	38.26
19	IL2 X IL10	0.87	1.02	37.95	40.27
20	IL2 X IL11	0.94	2.37	37.81	40.96
21	IL2 X IL12	1.14	1.3	31.46	33.46
22	IL3 X IL4	1.35	1.59	20.05	29.62
23	IL3 X IL5	1.41	1.6	39.4	40.51
24	IL3 X IL6	1.3	1.62	36.95	40.81
25	IL3 X IL7	0.85	1.3	33.86	36.01
26	IL3 X IL8	0.87	1.05	34.61	36.31
27	IL3 X IL9	0.94	1.18	35.36	36.61
28	IL3 X IL10	1.3	1.48	20.9	23.61
29	IL3 X IL11	1.15	1.47	27.36	30.76
30	IL3 X IL12	1.35	1.54	35.26	39.76
31	IL4 X IL5	1.33	1.6	38.46	42.21
32	IL4 X IL6	0.96	1.15	29.26	30.46
33	IL4 X IL7	0.96	1.14	33.01	34.36
34	IL4 X IL8	0.94	1.44	16.4	23.71
35	IL4 X IL9	0.99	1.41	21.8	28.56
36	IL4 X IL10	0.93	1.14	31.56	35.86
37	IL4 X IL11	0.9	1.44	23.6	29.56
38	IL4 X IL12	0.82	1.41	22.9	28.96
39	IL5 X IL6	0.87	1.17	24.45	27.91
40	IL5 X IL7	0.94	1.33	16.4	22.65
41	IL5 X IL8	0.94	1.45	21.3	25.94
42	IL5 X IL9	0.87	1.84	33.81	38.41
43	IL5 X IL10	0.88	1.81	34.06	38.56
44	IL5 X IL11	0.9	1.17	46.46	50.41
45	IL5 X IL12	0.99	1.33	17.1	31.81
46	IL6 X IL7	1.14	1.38	25.86	30.82
47	IL6 X IL8	1.35	1.51	18.05	32.11
48	IL6 X IL9	1.41	1.8	17.6	37.36
49	IL6 X IL10	1.3	1.93	21.45	22.72
50	IL6 X IL11	0.85	1.81	22.25	40.36
51	IL6 X IL12	0.87	1.51	20.9	34.21
52	IL7 X IL8	0.94	1.8	21.85	30.76
53	IL7 X IL9	1.3	1.93	25.7	34.81
54	IL7 X IL10	1.15	1.81	24.95	42.96
55	IL7 X IL11	1.35	1.51	53.8	63.41
56	IL7 X IL12	1.33	1.8	21.65	31.21
57	IL8 X IL9	0.96	1.93	36.85	39.01
58	IL8 X IL10	0.96	1.81	28.06	31.06
59	IL8 X IL11	0.94	1.51	29.61	33.31
60	IL8 X IL12	0.99	1.8	19.7	22.41
61	IL9 X IL10	0.93	1.93	25.55	56.16
62	IL9 X IL11	0.9	1.81	23.3	31.61
63	IL9 X IL12	0.82	1.14	26.41	31.38
64	IL10 X IL11	0.87	1.35	33.16	36.31
65	IL10 X IL12	0.94	1.41	29.21	31.21
66	IL11 X IL12	1.14	1.3	26.11	28.41
67	HKI 1105	1.35	1.14	37.61	39.91
68	HKI 1128	1.41	1.35	29.26	31.51
69	IL1	1.3	1.41	29.96	31.51
70	IL2	0.85	1.3	35.6	41.71
71	IL3	0.87	1.35	27.56	29.56
72	IL4	0.94	1.54	29.71	31.36
73	IL5	1.3	1.71	38.95	42.61
74	IL6	1.15	1.45	37.21	39.61
75	IL7	1.35	1.3	28.06	28.96
76	IL8	1.33	2.49	45.4	53.71
77	IL9	0.96	1.12	24.7	21.01

78	IL10	0.96	1.72	37	41.26
79	IL11	0.94	1.78	25.35	37.21
80	IL12	0.99	1.84	32.56	33.91
Mean		1.06	1.48	30.92	35.99
CV		8.74	10.10	11.85	5.70
$CD_{0.05}$		0.185	0.298	7.290	4.080

Table 3: Analysis of variance for proline and sugar contents

Level of variance	Df		SS		MSS		F value		Probability (>F)	
	PC	SC	PC	SC	PC	SC	PC	SC	PC	SC
Replication	1	1	184.3	2.659	184.35	2.659	20.13	105.22	0.00 ***	0.00 ***
Treatment (A)	79	79	16057.9	13.300	203.26	0.168	22.20	6.66	0.00 ***	0.00 ***
Drought stress(B)	1	1	2059.1	14.074	2059.13	14.074	224.86	556.89	0.00 ***	0.00 ***
Interaction (AxB)	79	79	2221.3	8.792	28.12	0.111	3.07	4.40	0.00 ***	0.00 ***
Errors	159	159	1456.1	4.018	9.16	0.025				

***Significantly different at α<0.0001
PC=Proline content
SC=Sugar content

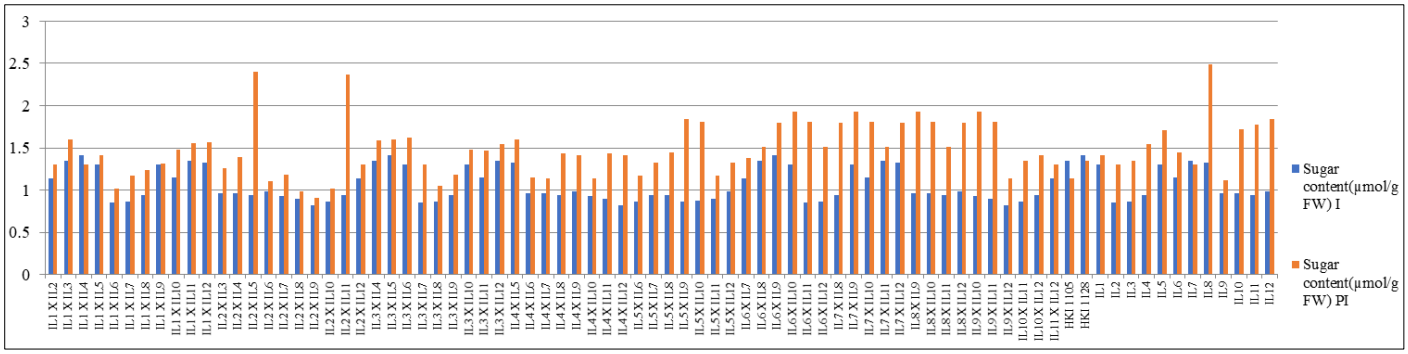

Fig 1: Comparative mean performance of sugar content (µmolg^{-1} FW) of maize genotypes under irrigated (I) and partial irrigated (PI) condition

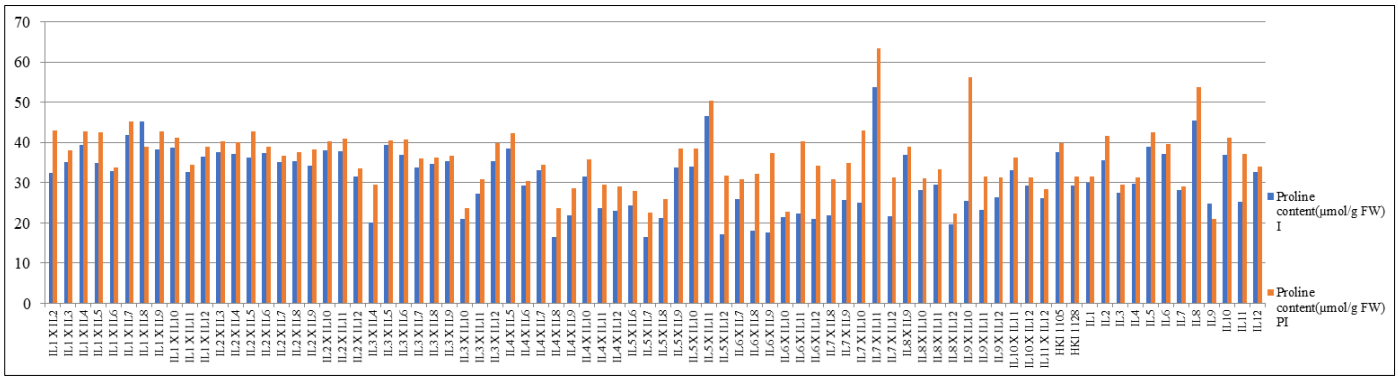

Fig 2: Comparative mean performance of proline content (µmolg^{-1} FW) of maize genotypes under irrigated (I) and partial irrigated (PI) condition

Conclusion

In this investigation, eighty maize genotypes were evaluated on the basis of sugar and proline content. Increased level of sugar and proline content was evidenced with increase in drought stress. The findings suggest that, genotypes IL1XIL4, IL3XIL5, IL2XIL5, IL2XIL11, IL7XIL11, IL9XIL10, IL8, IL5XIL11, IL1XIL7 and IL1XIL8 may be used for further crop improvement programme to breed drought tolerant cultivar(s) as increased level of sugar and proline was evidenced in these genotypes.

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Conflict of Interest: None

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