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Response of Durum Wheat (Triticum durum L.) Genotypes to Drought stress at Early Growth Stage

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Abstract. Early drought is a great threat limiting wheat production in semi-arid region resulted in poor emergence and weak seedlings. This research experiment attempts to evaluate the drought-tolerance potential of four local Triticum durum L. genotypes at germination stage. Hawler-1, Akassad, Semito and Barcelona subjected to drought stress of about 25 % of the soil water holding capacity (SWHC) and compared to their control 100 % SWHC in a factorial experiment designed in a complete randomization. Under stressed condition Semito genotype showed the significantly highest final germination percentage 54.66 %. Lowest MGT was 12 recorded by Semito and Aksaad. The heaviest roots, shoot dry weight and root: shoot ratio were observed in Akassad; 4.09 and 3.95 g and 1.28 respectively. The longest shoot averaged over all treatments was observed in Semito (23.05 cm). The highest leaves relative water content (LRWC) and membrane stability index (MSI) were 56.39 and 62.05 recorded by Semito. Akassad had the highest content of chlorophyll a, b and total; 2.40, 1.51 and 3.91 mg/ g fresh weight. Highest significant proline content was 0.548 mg/ g fresh and sugar content was 2.94 mg g dry weight accumulated in Semito leaves. Genotypes with a fast germination, more vigorous root system and highest root: shoot ratio are very desirable for the rapid establishment of seedlings and could have best physiological response as well as accumulate more osmoticum in their cytosol. Thus, Akassd and Semito had best potential adaptability to drought stress at early growth stage.

Keywords. Durum wheat, Drought, proline, Chlorophyll, Germination.

1. Introduction

Wheat (*Triticum* spp.) is one of the most produced cereal crops in the world, is a widely cultivated crop, grown in wide range of environmental conditions with a short growing season and plays an essential role in world trade (Mohammed and Kadhem, 2017). The most important wheat species are *Triticum aestivum* L. for making bread, followed by *Triticum durum* L. for pasta and lastly *Triticum* comactum L.; a soft type of wheat that is used for making cakes, crackers, cookies and flours (Marti and Slafer, 2014). Environment inadequate conditions such as salinity, drought and heat, progressive global climatic changes and increasing shortage of water resources can cause reductions in morphological and agronomical parameters, as well as disorders at physiological, biochemical and molecular levels (Zhu, 2002). In semi-arid regions, water is also one of the most important limiting

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factors for seed germination and early growth stage of wheat (Qadir, 2018). Water performs a vital role in activating variety of enzymes in addition to its role in solubilize and transport of metabolites. also act in hydrolytic breakdown of stored proteins, lipids, and carbohydrates in germinated seeds (Biaecka & Kepczynski, 2010). In semi-arid regions, the seedlings that in exposure to drought at early stage might result in poor emergence. In addition, severe drought at early growth stages may cause a complete failure of seedlings and therefore a total loss of the crop (Sahnoune et al., 2004).

Vital drought stress responses consist of root elongation maintain and complete inhibition of shoot growth in few plant species. Root: hoot dry weight ratio will increase as water availability decreases because of relative reductions in shoot dry weight (Qadir et al., 2019). Cells shrinks due to low water content can bring about decrease in relative water content in leaves (LRWC). It regarded as important indicator to examine the sensitivity of genotypes to water deficit (Sánchez-Rodríguez et al., 2010). Under water stress reactive oxygen species liberated lead to lipid peroxidation and gradual decreases membrane stability (MS) which award the degree of drought resistance of the plant species (Tripathy et al., 2000). Chlorophyll content is an indicator of photosynthetic capability of plant tissues. The chlorophyll content of several plant species didn't affect by decreasing water content in the soil, but it depends on shortage intensity and exposure period (Gholamin et al., 2010). Osmotic adjustment consisting of proline and sugar accumulation can partially protect the plant against slight drought stress (Alexieva et al., 2001). The evolvement of resistance to drought even at early growth stage is a complex phenomenon from the combination of several genetic, physiological, biochemical, and morphological adaptation mechanisms (Mattana and Biazzi et al., 2005).

Research experiments should focus on identifying and evaluating new genotypes with short-term development, water-conserving, and drought-resistant properties to make wheat production economical. This experiment is an attempt to evaluate the growth of different durum wheat genotypes under drought-induced stress at early germination stage to promise its render to the stress at other advanced growth stages.

2. Materials AND Methods

2.1. Treatment and Experimental Design

The experiment was conducted to study the tolerance ability of four durum wheat genotypes; Hawler-1, Akassad, Semito and Barcelona to examine their ability to water shortage at early growth life in a glass house experiment at the biology department of the College of Education/ University of Salahaddin- Erbil. The seeds were surface sterilized using 1% Sodium hypochlorite solution for 3 mints, later rinsed with tap water. Ten seeds were sowed per plastic pot contains previously sieved soil about 3 kg. Seeds were considered germinated with the emergence of plumule up to 2 mm and the number of germinated seeds was monitored daily up to 10 days continuously. Thinning was performed to only two seedlings per pot after the 10th day of seedling emergence. The first dose of recommended fertilizer 30 Kg/dounum DAP (N= 18% and P=46%) was added to 10 days old seedlings. Respectively the second dose of Urea (N= 46%) added after 30 days old. The water holding capacity of the soil (SWHC %) of the soil was determined by saturating the soil pot, covering the tops with aluminum foil and weighing daily until there was no weight loss within 24- and 48-hours' period. And on this base 400ml and 120ml water added respectively as; 100 % and 25 % SWHC. Factorial experiment laid out in a completely randomized design (CRD). Include the interaction of two factors; four durum wheat genotypes and two SWHC % each replicated three instances. Duncan's multiple range test (DMRT) applied to compare among means.

2.2. Studied Parameters

2.2.1. Germination Indices and Seedling Growth Characters

Daily monitored during the experiment period was done every 24 hours. Two germination indices were determined; Final Germination Percentage (FGP = Ng / Nt x 100, Ng=Total number of germinated seeds, Nt=Total number of seeds evaluated. Mean Germination Time (MGT) = $\Sigma D n / \Sigma n$; where n is the number of germinated seeds on day D, and n; the number of days counted from the beginning of germination, calculated according to Qadir (2018). After one month the final germination growth characters were measured; shoot length (SL) (cm), root length (RL) (cm), shoot dry weight (SDW) (g), root dry weight (RDW) (g) and root: shoot (R: S) ratio. The dry weight (DW) was obtained after drying the seedlings for 48 h at 72ºC (BAĞCI and Yilmaz, 2003).

2.2.2. Physiological Indices

Leaf relative water content (RWC): fresh leaves were floated on water for 24 hours to saturate and weight, dried at 60 ℃ until constant weight was reached (Qadir et al., 2019).

$$\text{RWC (\%)} = (\text{Fresh weight} - \text{dry weight}) / \text{Turgid weight} - \text{dry weight}) * 100$$

Membrane stability index (MSI): Leaf discs (100 mg) were thoroughly washed in running tap water followed by washing with double distilled water and there after the discs was heated in 10ml of double distilled water at 40 for 30 min. Then electrical conductivity (C1) was recorded by EC mater, subsequently the same were placed in a boiling water bath (100℃) for 10 min and their electrical conductivity was also recorded (C2) (Hofmann et al., 2003 and Qadir et al., 2019). The MSI was calculated: Membrane stability index (MSI) = {1 - (C1/C2) *100.

Photosynthetic pigments: 3ml of 99.5% methanol was add to 50mg of fresh leaf and incubated for 2hours in dark. Samples were homogenized and centrifuged at 10000 rpm for 10 min. Absorbance of the supernatant was read at 650 nm and 665 nm by the UV spectrophotometer (Genesys 10 SUV - Vis spectrophotometer) (Horii et al., 2007). Chl. a, Chl. b and total Chl. content were counted using below equations:

$$\text{Chlorophyll a } (\mu g / ml) = 16.5 * A665 - 8.3 * A650$$

$$\text{Chlorophyll b } (\mu g / ml) = 33.8 * A650 - 12.5 * A665$$

$$\text{Total Chlorophyll } (\mu g / ml) = \text{chlorophyll a} + \text{chlorophyll a}$$

2.2.3. Osmotic Adjustment Response

Proline content (mg/g fresh weight): was determined following the method of Bates et al. (1973). 0.1 g of fresh sample of leaves was added in 5 ml of 3% sulfosalicylic for extraction. Then 2 ml from supernatant was mixed with 2 ml of glacial acetic acid and 2 ml ninhydrin reagent and was boiled for 1 h in water bath at 100°C. After 1 h, the reaction was stopped in ice and finally 4 ml of toluene was added, vortexed and the absorbance of the supernatant was read at 520 nm on the UV Spectrophotometer (Biochem, 2100).

Total soluble sugars (mg g^{-1} dry weight): 0.1g of leaf dried powdered used to exract suger with ethonal (80%) total sugar sugars estimated using anthrone reagent. The absorbance was measured at 630 nm in spectrophotometer. Total sugar content was calculated by using the glucose standard curve and expressed in mg g^{-1} dry weight of leaf sample (Dubois et al., 1956).

3. Results and Discussion

3.1. Germination Indices and Seedling Growth Response

Germination and seedling growth are important and sensitive stage for wheat crop growth and might be delayed due to water shortage, however, this depends on the shortage severity. The survival ability of the four durum genotypes determined during germination stage as a drought tolerance screening tool. Data pertain the effect of drought stress (25 % SWHC) on final germination percentage (FGP) and mean of daily germination (MDG) are given in table (1). Under stressed condition Semito genotype showed the significantly highest final germination percentage 54.66 % over other genotypes, while Barcelona genotype had the lowest FGP; 30.00 %. Differences in seed germination among the genotypes under drought stress condition due to genetic variation could be used a screen tool of genotypes adapted to unfavorable conditions in arid and semi-arid regions (Chachar et al., 2014). The finding in parallel with those reported by Liu et al. (2015), Qadir (2018) and Abdel-Ghani et al. (2020). which showed significant reduction in germination because of decreasing potential gradient between seeds and their environment. That directly affect water imbibition and later decrease in germination rate and viability of the seedlings. There was a significant increase in mean daily of germination period MGT. The highest period of daily germination under drought condition was recorded by Hawler-1 was 16.33 as compared to control condition (12.33). As well as Barcelona responded a same increase from 10 to 15.33 days, but the lowest MGT was 12 recorded by Semito and Aksaad. Which is in parallel to the findings of Abdoli & Saeidi (2012) and Dodig et al., (2014) found that stress caused by water deficiency augmented MGT of wheat cultivars. This response is due increasing in adequate time required for germination under water scarcity condition (Ahmad et al., 2017). Greater biomass under water-limited conditions tended to be the indicator of tolerance of genotypes grown under drought stress condition.There was a high variation in seedling growth traits under drought condition as compared to control condition (Table 1). The variation in root length ranged from 24.62 cm in Akassad to 14.72 cm in Hawler-1. Regarding the root dry weight, the heaviest roots were observed in Akassad; 4.09 g followed by Semito (3.32 g), Barcelona (3.85 g) and Hawler-1 (3.12 g) respectively. Rana *et al*., (2017) reported that early and rapid elongation of roots is an important indicator of drought resistance. And the decrease in root and shoot length, which were an obstacle to cell division in shoot and root elongation, and to seed reserve utilization. The longest shoot averaged over all treatments was observed in Semito (23.05 cm), while the shortest one recorded by Barcelona (17.70 cm). Water deficit significantly affected root-related traits. Faisal *et al*., (2017) and Othmani et al., (2021) also showed a significant effect of water stress on durum and bread wheat root characters. As well as the highest mean of shoot dry weight recorded by Semito (3.95 g) and the lowest mean observed in Barcelona (3.01 g). The size of a plant's root system is a key trait to acquit soil resources but only in relation to the size of shoot system, relative to either leaf area, shoot, or whole plant size (Figueroa-Bustos et al., 2018). The highest root: shoot ratio was which indicates its tolerance adaptability observed in Akassad (1.28). While minimum ratio was 0.84 recorded by Hawler-1. Many plants respond to drought by increasing the proportion of assimilate diverted to root growth to form deeper root and thus, increase the root: shoot ratio to tap extra water from the soil profile as previously found by; Ahmad *et al*., 2017, Qadir, 2018 and Othmani et al. (2021). Due to the induction of root-to-shoot hormonal signaling and higher dry matter and soluble sugar content in roots, due to an increase in enzyme activity while the root system is subjected to drought stress (Xu *et al.*, 2015).

Table 1. Germination indices and seedling growth response of different *Triticum durum* L. genotypes under drought condition.

Irrigation type	**Genotypes**	**Germination indices**		**Seedling growth characters**				
		FGP %	**MGT**	**RL (cm)**	**SL (cm)**	**RDW (g)**	**SDW (g)**	**R:S**
100 % SWHC	Hawler-1	56.66 ± 3.22 a	12.33 ± 1.01 b	19.50 ± 1.87 b	25.82 ± 2.13 a	3.63 ± 0.04 b	4.54 ± 0.05 a	0.79 ± 0.001 b
	Akassad	53.33 ± 2.87 a	10.00 ± 1.12 b	26.04 ± 1.98 b	20.92 ± 1.09 b	4.31 ± 0.06 a	4.02 ± 0.06 b	1.07 ± 0.08 a
	Semito	60.66 ± 4.33 b	11.00 ± 1.06 b	20.50 ± 0.87 b	26.14 ± 1.21 a	3.50 ± 0.04 b	4.22 ± 0.08 a	0.83 ± 0.03 b
	Barcelona	53.33 ± 3.78 a	10.00 ± 1.03 b	30.21 ± 2.01 a	23.52 ± 1.12 a	4.24 ± 0.05 a	3.83 ± 0.05 c	1.11 ± 0.05 a
25 % SWHC	Hawler-1	35.00 ± 2.33 c	16.33 ± 1.67 a	14.72 ± 1.02 c	19.76 ± 0.98 b	3.12 ± 0.04 bc	3.61 ± 0.04 c	0.84 ± 0.03 b
	Akassad	36.33 ± 2.67 c	12.00 ± 1.65 b	24.62 ± 1.21 b	18.30 ± 0.78 b	4.09 ± 0.08 a	3.30 ± 0.02 d	1.28 ± 0.07 a
	Semito	54.66 ± 4.23 a	12.00 ± 1.45 b	18.32 ± 1.09 c	23.05 ± 1.03 a	3.32 ± 0.05 b	3.95 ± 0.07 c	0.86 ± 0.09 b
	Barcelona	30.00 ± 2.11 c	15.33 ± 1.22 a	23.42 ± 2.01 b	17.70 ± 0.98 c	3.85 ± 0.06 b	3.01 ± 0.05 d	1.24 ± 0.10 a

3.2. Physiological Response

Relative water content of leaves (LRWC) has been reported as important indicators of water stress in crop plants (Arjenaki *et al*., 2012). LRWC decreased significantly under drought condition, the highest mean 56.39 was recorded by Semito, it was followed in descending order by Akassad (45.26,) Hawler-1 (40.01) and Barcelona which possessed the lowest mean; 33.54. (Table 2). Under drought conditions relative water content of leaves decreased due to decrease in osmotic potential to maintain pressure potential. The decline in solute potential (Ψs) could be a result of either simple and passive diffusion of solutes due to dehydration or net accumulation of osmolytes as suggested by Qadir, 2018 and Karimpour (2019) noted the same decline in LRWC% under water deficit conditions.

membrane structure and function impaired due to water content loss of the plant tissues under limited water condition. that lead to decrease the stability of membrane systems in plant cells (MSI). Because of their effect on lipid peroxidation by the reactive oxygen species (ROS) (Zada et al., 2020). Membrane stability decreased significantly of all durum genotypes under 25 % SWHC (table 2). The highest mean 62.05 was recorded by Semito followed by Akassad (56.46), Barcelona (51.66) and the lowest RWC was 46.99 found in leaves of Hawler-1 respectively. Qadir et al. (2019) determined that tolerant genotypes considerably had higher MSI % as compared to susceptible genotypes. The results are much like those found by Razzaq et al. (2013); Qadir et al. (2019) and Zada et al. (2020).

Chlorophyll retention or ‘stay green’ is regarded as a key indicator of stress adaptation (Ananthiet al., 2013). Drought stress significantly decreased chlorophyll a, b and total content of all genotypes under drought stress condition (Table 2). Akassad had the highest content of chlorophyll a, b and total; 2.40, 1.51 and 3.91 mg/ g fresh weight. High chlorophyll content is a desirable characteristic under drought stress because it indicates a low degree of photo inhibition of photosynthetic apparatus (Ananthi, et al., 2013). While Hawler^{-1} had lowest chlorophyll amount content; 1.36, 0.69 and 2.08 mg/ g fresh weight. Drought stress reduces leaf chlorophyll mainly due to chloroplast damage caused by the genesis of reactive oxygen species (ROS) (Ommen et al., 1999). These results are in line with those reported by Hussein and Khursheed (2014); Farshadfar et al. (2015); Chachar et al. (2016) and Karimpour (2019).

Table 2. Physiological response of different *Triticum durum* L. genotypes under drought condition.

Irrigation type	**Genotypes**	**LRWC %**	**MSI %**	**Physiological indices**		
				Chlorophyll a (mg/g Fwt.)	**Chlorophyll b (mg/g Fwt.)**	**Total chlorophyll (mg/g Fwt.)**
100 % SWHC	Hawler-1	79.93 ± 0.33 b	60.32 ± 0.33 c	2.63 ± 0.048 a	1.25 ± 0.42 b	3.36 ± 0.03 bc
	Akassad	74.03 ± 0.67 c	73.22 ± 0.66 ab	2.52 ± 0.045 ab	1.67 ± 0.56 a	4.19 ± 0.01 a
	Semito	84.21 ± 0.67 a	73.84 ± 1.00 a	1.87 ± 0.067 d	0.88 ± 0.29 c	2.75 ± 0.01 d
	Barcelona	82.86 ± 0.66 ab	70.17 ± 0.66 b	2.63 ± 0.033 a	0.91 ± 0.30 c	3.51 ±0.06 b
25 % SWHC	Hawler-1	40.01 ± 0.68 f	46.99 ± 0.89 f	1.36 ± 0.036 e	0.69 ± 0.23 c	2.08 ± 0.01 e
	Akassad	45.26 ± 0.87 e	56.46 ± 1.54 d	2. 09 ± 0.01 c	0.74 ± 0.25 c	2.83 ± 0.01 c
	Semito	56.39 ± 0.56 d	62.05 ± 1.23 c	1.75 ± 0.01 d	0.83 ± 0.28 c	2.71 ± 0.08 d
	Barcelona	33.54 ± 0.61 g	51.66 ± 3.22 e	2.40 ± 0.01 b	1.51 ± 0.50 a	3.91 ± 0.07 b

Values sharing the same letter in a column do not differ significantly at P = 0.01.

3.3. Osmotic Adjustment Response

Proline is the most widely studied osmolyte, because of its considerable importance in the stress tolerance (Ashraf and Foolad, 2007). A significant accumulation of proline observed in all the genotypes under the drought condition (Figure 1). Highest significant proline content was 0.548 mg/ g fresh weight accumulated in Semito leaves. While Aksaad, Hawler-1 and Barcelona accumulated the lowest amount of priline in their cytosol. Increased proline in the stressed plants may be an adaptation mechanism to overcome the stress conditions. As a result of solute accumulation, the osmotic potential of the cell is lowered, which attracts water into the cell and helps with the maintenance of turgor. By means of osmotic adjustment cell water balance and a sink for excess resistance and a store of carbon and nitrogen for use after relief of water deficit (Zhu, 2002). Proline not only acts as an osmolyte, but it supplies energy for growth and survival, might have the function of scavenger of reactive oxygen species or act as an osmolyte and thereby helps the plant to tolerate stress (Mafakheri *et al*., 2010). Similar results of proline accumulation in plants under water deficit were found by Anjum *et al.*, (2011); Hussein and khursheed (2014) and Qadir, (2018).

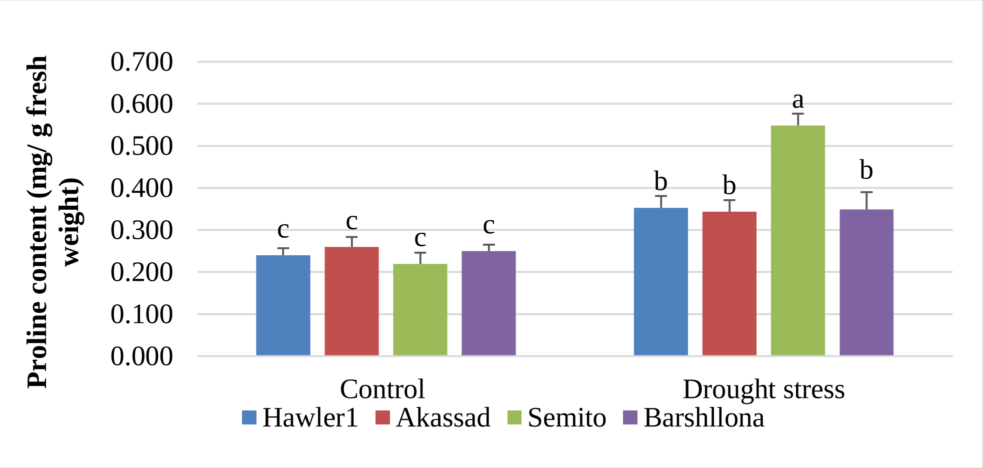

Figure 1. Proline accumulation in different *Triticum durum* L. genotypes in response to drought condition.

Soluble sugars have been shown to increase under water stress. They are considered to play an important role in osmotic adjustment which is widely regarded as adaptive response to water stress conditions (Kameli and Lösel, 1995). A significant increase in the total sugar content observed in durum genotypes under drought stress condition (Figure 2). The highest sugar content was 2.94 accumulated in Semito leaves. Followed by Barcelona which had 2.31, then Aksaad had 2.31 and finally Hawler-1 had 1.58 mg soluble sugar/ g. Sugar is being whose concentration increased under water stress condition due to stimulation of α- amylase and other hydrolytic enzymes to promote the hydrolysis of reserves. Or may be due to low concentration of K^+ under water stress condition because K^+ act as an activators starch synthesis. Meanwhile the starch is degraded, and this degradation is promoted by the amylase enzyme action due to decrease of photosynthetic product (Assmann and Haubrick, 1996). These results are partially agreed with those obtained by Anjum *et al.,* (2011), Chorfi and Taïbi, (2011); Hussein and khursheed (2014) and Qadir (2018).

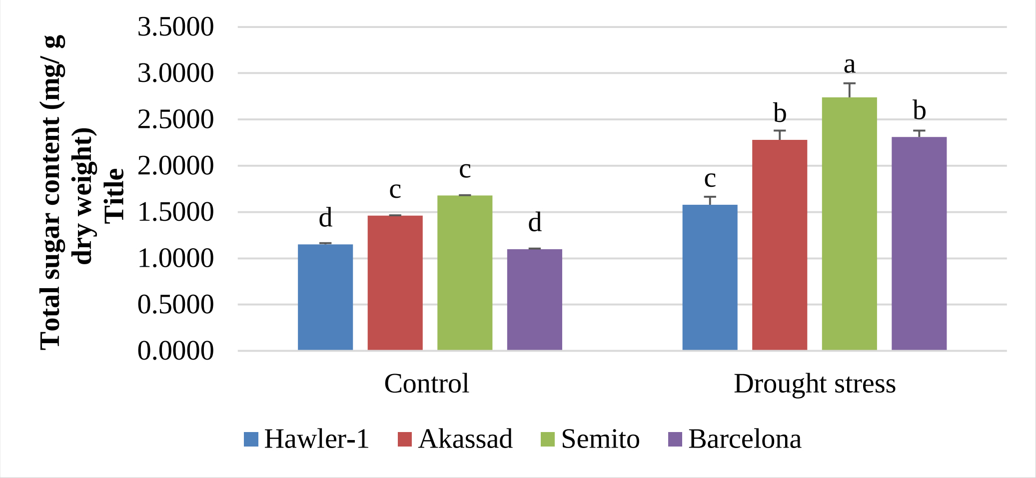

Figure 2. Sugar accumulation in different *Triticum durum* L. genotypes in response to drought condition

Conclusion

Germination and seedling growth are the first are most susceptible stages susceptible to drought Genotypes as Akassd and Semito had best potential adaptability to drought stress at early growth stage to be culture in semi-arid regions. Because they had fast germination, more vigorous root system and highest root: shoot ratio are very desirable for the rapid establishment of seedlings and could have best physiological response as well as accumulate more osmoticum in their cytosol. While Hawler-1 and Barcelona be the most susceptible genotypes to drought stress at early growth stage.

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