

Assessment of genetic variability, heritability and genetic advance for agro-morphological and some *in-vitro* related traits in durum wheat

Shadi Heidari^{1*}, Peivand Heidari¹, Reza Azizinezhad¹, Alireza Etminan² and Mahmood Khosroshahli¹

¹Islamic Azad University, Department of Plant Breeding, Science and Research Branch, P. O. Box 14515/775 Tehran, Iran

²Islamic Azad University, Department of Plant Breeding, Kermanshah Branch, Kermanshah, Iran

*Corresponding author: shadiheidari47@gmail.com

Abstract

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Sixteen advanced durum wheat breeding lines were evaluated under rain-fed and supplementary irrigation conditions in a randomized complete block design (RCBD) with three replications on seventeen agro-morphological characters to examine genetic value of yield and yield-related traits. Most traits revealed the highest coefficients of variation (CV). Results showed that the maximum phenotypic variance (PCV) of traits were generally higher than genotypic coefficients of variance (GCV) under both conditions. Obtained heritability accompanied with high genetic advance for several traits indicates that most likely the heritability is due to additive gene effects and selection may be effective in early generations for these traits. These parameters were estimated for a number of traits *in vitro* cultures as well. Mean comparisons demonstrated that the lines G6 and G9 achieved higher levels of callus size and relative water content under the both optimal and stress conditions, while maintaining this ability in stress condition compared to other lines. Moreover, the lines G4 and G16 had the minimum difference between fresh and dry weights in optimal and stress conditions. So, these lines had the higher genetic potential for response to drought stress *in vitro* environments.

Keywords: durum wheat; agro-morphological and *in-vitro*; traits; genetic variability; genetic advance; heritability

Introduction

Durum wheat is one of the most consequential cereal crops that cultivated in diversified environmental conditions such as the arid and semi-arid regions. Normal rainfall in these area in Iran is about < 300 mm which this is one third of average rainfall in the world. A total of 0.6 million tons durum wheat was produced during the crop season 2014-15 in Iran (Heidari et al., 2017). Drought stress is the most prevalent adverse constraint environmental condition that can tremendously decrease crop productivity. One of the main purposes of breeding program is

to produce high-yielding and better-quality genotypes for release as cultivars in any conditions. To acquisition these intentions, existence adequate amount of variability in the material for yield and its components, in which favorable genotypes have to be designated for more crop improvement is, therefore, a prerequisite. Despite workload of improvement research in durum wheat the grain yield in the dry land and rain fed conditions is lower than that realizable in other conditions. To survive against to drought stress, plants have adopted considerable genetic variation for morphological, physiological, biochemical, and metabolic traits which contribute to grain yield and greatly affected

by many environmental conditions. Breeding programs depend on the understanding genetic systems controlling inheritance of these traits that influence their performance, so it is important to separate the total variation into heritable and non-heritable components. However, exploitation of genetic resources variability and enhancement of high-yielding cultivars requires commensurate systematic evaluation (Belay et al., 1993). Genetic parameters have been used to assess the nature and magnitude of variation in durum wheat breeding material.

The magnitude of the components of variances has been obtained from analysis of variance to appraise the different genetic parameters as described by Singh and Chaudhary (1985) and Falconer (1989). Genetic variability, which is due to the genetic differences among individuals within a population, is the main aim of plant breeding programs, because proper management of diversity can produce permanent gain in the performance of plant and can buffer against seasonal fluctuations (Sharma, 1998). Genetic variability among traits is important for breeding and in selecting desirable types. As the breeders are interested in selection of superior lines based on phenotypic performance, the foremost function of heritability is its predictive role to represent the reliability of phenotypic performance as an indicator to breeding value and to provide information on transmission of character from the parent to progeny. Heritability studies provide opportunities for breeders to predict about the interaction of genes in successive generations and are essential for effective breeding programs.

For effective genetic improvement of grain yield, it is important to understand how the proportion of genetic component and genetic advance are affected by environments. It has been noted that, heritability alone is not enough to make sufficient improvement through selection generally in advance generations unless accompanied by substantial amount of genetic advance. The utility of heritability therefore increases when it is used to calculate genetic advance, which indicates the degree of gain in a trait obtained under a particular selection pressure. Thus, genetic advance is yet another important selection parameter that aids breeder in a selection program. Estimates of heritability and genetic advance will help in knowing the nature of gene action affecting the concerned traits (Shukla et al., 2004). Previous studies have revealed considerable variation in wheat (Tewodros et al., 2014). Khan et al. (2015) reported that the high phenotypic and genotypic coefficients for the yield and yield components traits in wheat genotypes. Tambe et al. (2013) reported the high values of GCV and PCV for grain yield, number of effective tillers, spike length and 1000 grain weight. Lone et al. (2017) reported the expected

genetic advance in wheat were high for number of grains per ear head, ear head length, peduncle length, number of effective tiller and plant height. The high heritability combined with high genetic advance was observed for grain yield indicated that selection may be effective for this character. In the study conducted by Wolde et al. (2016), the high values of PCV and GCV were recorded for fertile tillers, the number of grains per spike, thousand grains weight, grain yield and harvest index. The current study aimed to estimate the genetic variability, heritability and genetic advance for several agro-morphological and *in-vitro* traits for selection of high yielding durum wheat lines under diverse conditions.

Materials and Methods

This study was carried out under rain-fed and supplementary Irrigation (two irrigations at flowering stage until each stage reach to 25 mm) conditions in a randomized complete block design (RCBD) with three replications across two years (2011-2013). Sixteen advanced breeding lines of wheat including three control lines Saji, Zardak and Sardari along with 13 advanced lines were evaluated. The unit plot size was 1.2 m x 6 m, consisting of 6 rows with 20 cm space between rows. The plants in full maturity stage were used for analysis. The origin and pedigree of these materials are presented in Table 1. Data were measured from each plot on seventeen characters (Table 1).

In callus culture, the sample seeds were disinfected with sodium hypochlorite 1.5% for 10 minutes. Then, they were rinsed with distilled water. The MS medium (Murashige & Skoog, 1962) was prepared with 15 g sucrose, 7 g agar pH = 5.8. The medium was then autoclaved. The sub-samples were maintained in a MS medium containing 2,4-D hormone concentrations and a medium under osmotic stress with mannitol 2% until callus induction. Measured traits in callus culture are presented in Table 2 and were evaluated in (RCBD) with three replications. Both morpho-physiological and *In vitro* traits under normal and stress conditions were subjected to analysis of variances according to the formula suggested by Steel and Torrie (1960). The genotypic and phenotypic variances were estimated according to the formula suggested by Johnson et al. (2003). GCV and PCV were computed as percent the method suggested by Burton & Devane (1998). Heritability in broad sense ($h^2_{b.s}$) was computed using the formula adopted by Allard (1960). The expected genetic advance (GA) for different characters under selection was estimated using the formula suggested by Johnson et al. (1955). Genetic advance over mean was estimated by using the following formula proposed by Comstock & Robinson (1952).

Table 1. The names of evaluated wheat lines along with their origins; studied traits and measuring units with the abbreviation of names

Lines	Name/Pedigree	Trait	Measuring units	Abbreviation
G1(C = Control)	Saji*	1 Plant height	cm	PH
G2 (C)	Zardak	2 Spike length	cm	SL
G3 (C)	Sardari	3 Peduncle length	cm	PL
G4	19E-TOPDY	4 Peduncle extrusion	cm	PE
G5	19E-RASCON	5 Flag length	mm	FL
G6	19E-M84859	6 Thousand kernels weight	g	TKW
G7	19E-M141979	7 Grain yield	g/m ²	GY
G8	19E-M141982	8 Biological yield	g/m ²	BY
G9	19E-M141994	9 Harvest index	%	HI
G10	19E-M141995	10 Number of grain/spike	m ²	NSPS
G11	18E-M142005	11 total number of stem	m ²	NTS
G12	19E-M142017	12 total number of fertile stem	m ²	NFS
G13	19E-M142025	13 Straw yield	g/m ²	SY
G14	19E-M142038	14 Days to physiological maturity	day	DM
G15	19E-M142045	15 Days to heading	day	DH
G16	19E-M142069	16 Canopy temp. centigrade	(°C)	CT
		17 Stomatal conductance	m ⁻² s ⁻¹ mmol	SC

*The origin of all lines was dry land agricultural research institute of Sararood (DARI)

The Duncan's method was employed to compare the mean values of durum wheat lines in relation to the tissue culture traits. The physiological traits were measured at the seedling stage of durum wheat lines. The abbreviated names along with measurement methods and units of measurement for traits and enzymes are given in Table 2.

To study of relationships among tested durum lines and measured traits factor analysis and hierarchical cluster analysis (HCA) were performed by NTYSYSsps 2.02 software (Rohlf, 2000). The mean values of durum wheat lines were

compared through the Duncan's method for measured physiological traits under optimal and stress conditions.

Results and Discussion

Evaluation of genetic variability for agro-morphological traits

Analysis of variance showed high significant differences ($P < 0.05$) for 17 quantitative characters studied (data not shown), indicating that there is variability among the studied

Table 2. The abbreviated names along with measurement methods and units of measurement for callus culture traits and physiological traits

Trait	Abbreviation names	Measurement method	Unit of measurement
Callus culture traits			
Callus size	CS	–	millimeter
Fresh weight	FW	–	gram
Dry weight	DW	–	gram
Relative water content	RWC	–	gram
Physiological traits			
Malondialdehyde	MDA	Stewart & Bewley (1980)	uM/g FW
Superoxide dismutase	SOD	Moon & Terao (1998)	unit /mg protein
Catalase	CAT	Chaoui(1987)	mmol / g FW min
Ascorbate peroxidase	APX	Nakano & Asada (1987)	mmol / g FW min
Peroxidase	PRX	Abeles & Biles (1991)	OD470 /gFW min
Relative water content	RWC	Dispersed	mg
Chlorophyll	CHA, CHB, CHT	Lichtenthaler & Wellurn (1983)	mg/g ⁻¹ FW
Fresh & Dry biomass	SFW-SDW		mg

lines and would respond positively to selection. The coefficients of variation and standard error (SE) were high except for CT, DH, DM and SL in all conditions. The best mean performance of lines was in irrigation condition 2012-13 for most of characters. The result of mean comparison of grain yield and its related traits showed the highest values for all characters in the optimal condition than rainfed environment. Grain yield ranged from 84.6 and 2010 gm². ADWL-12, ADWL-10, and (ADWL-1 as control) were identified as the high-yielding lines (result not shown). To examine genetic value of yield and its components, several genetic parameters like phenotypic variance (σ^2_p), genotypic variance (σ^2_g), phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV), heritability (h^2) and genetic advance (GAM) were estimated. A wide range of genotypic and phenotypic variances was observed for these traits in the both rainfed and irrigated conditions. Maximum genotypic and phenotypic variances in both conditions 2011-12 was exhibited for GY, NTS, NFS, BY, SC and SY. Maximum of these amounts in rainfed condition 2012-13 was exhibited for GY, NTS, NFS and maximum of these amounts in irrigated condition 2012-13 was exhibited by GY, NTS, NFS, BY and SY. Rohani and Marker (2016) reported maximum genotypic and phenotypic variances for plant height followed by harvest index (186.54 and 145.25, respectively).

In the present study, at the first year, the contribution of genotypic variance was larger than the total contribution of non-heritable components for PL, PE, DH, FL, DM, PH and NSPS in the rainfed condition and also for CT, DH, FL, DM, NTS, NSPS and HI in the optimal condition. Similarly, at the second year, CT and DH in the rainfed condition and CT, DH, NFS and SL in the optimal condition showed the highest GCV. These results indicated that phenotypical variation was not influenced by the environmental component, indicated a few effects of environment on the genotypical effect. Selection in the breeding programs is based on measurements of phenotypic character and genotypic variability is measured through analysis of variance (Singh, 1990). In the present study, assessing magnitude of these traits variation is important to success in crop improvement program. It is very important to select the desirable line which could transmit high yield and other desirable traits. In the first year, the maximum GCV and PCV in the rainfed condition 2011-12 were observed for GY (25.87 and 36.88), NFS(16.12,29.88), PL(34.85,43.87), SC(21.54,30.52) and NSPS(18.29,22.71). Maximum GCV and PCV in irrigated condition 2011-12 were observed for GY(22.30,34.01), NTS(17.66,26.63), NFS(17.97,26.96), BY(17.46,30.53), SC(20.91,35.17) and NSPS(17.45,24.52). Maximum of these amounts for rain-

fed condition 2012-13 were obtained for GY(21.64,40.85), NTS(21.10,34.03), NFS(21.65,34.98), PE(18.54,42.88) and HI(16.08,35.87) and maximum of these amounts for irrigated condition 2012-13 were obtained for NTS(19.88,24.66), NFS(20.04,24.94), PE(17.39,32.28) and SC(27.03,60.50).

In these traits, estimates of PCV were generally higher than GCV. Singh et al.(2013) reported high magnitude of PCV and GCV for grain yield, number of effective tillers per plant and number of grain per spike. In the present study, maximum heritability estimates were also shown for GY, NFS, NTS, NSPS, PE, SC, DH, HI, DM, PH and SL in both irrigated and rainfed conditions. Similar to the result of this study, many researchers reported maximum heritability for numbers of tillers per plant, number of grain per spike and harvest index in wheat (Rohani & Marker, 2016; Rahman et al., 2016; Jan & Kashyap, 2016), while grain yields had lowest heritability estimate which their findings were not in accordance with present study. Sharma et al.(1995) obtained high heritability for thousand grain weight, biological yield/plant, harvest index, plant height and number of grains/spike. The heritability estimates help the breeders in selection based on the phenotypic performance and shift in gene frequency towards superior side under selection pressure is termed as genetic advance. Johnson et al. (1955) suggested that estimates of heritability and genetic advance should be considered together for more reliable conclusions. Estimates revealed that characters having maximum PCV and GCV, exhibited approximately moderate to high heritability and GAM viz., GY(49.23,37.43 heritability and GAM respectively), NFS(29.10,17.93), PL(63.10, 57.09), SC(49.80,31.36) and NSPS(64.89,30.36) for rainfed condition 2011-12. GY(43.01, 30.17), NTS(43.98,24.12), NFS(44.46,24.69), BY(32.71,20.60), SC(35.36,25.61) and NSPS(50.66,25.59) for irrigated condition 2011-12. GY(28.07,23.59), NTS(38.47,26.95), NFS(38.30,27.64) for rainfed condition 2012-13 and NTS(65.01,33.07), NFS(64.53,33.19) and PE(29.03,19.31) for irrigated condition 2012-13. The high heritability accompanied with high genetic advance in case of these traits indicates that most likely the heritability is due to additive gene effects and selection may be effective in early generations for these traits. Similar findings have been reported by Lone et al. (2017) that the high PCV and GCV values with a high heritability were recorded for the total phenols trait. Genetic advance as was also high for this trait suggesting further improvement of lines for the character for further selection and subsequent use in breeding program. Rohani & Marker (2016) recorded high heritability combined with high genetic advance for grain weight per spike followed by harvest index.

Table 3. Analysis of variance for several measured traits of durum wheat lines under *in-vitro* condition

Characters	Mean squares			
	stress	line	line× stress	Error
Df	1	16	16	68
Callus size	217.482**	5.022**	0.573*	0.312
Fresh weight	0.042**	0.004**	0.001*	0.001
Dry weight	0.001**	0.0001**	1.613E ⁻⁵ ns	1.721E ⁻⁵
RWC	0.367 ns	202.325**	31.753 ns	26.010

**Significant at the 1% probability level

Evaluation of genetic variability in some of in-vitro traits

Analysis of variance revealed significant differences among lines for callus size, fresh and dry weight and RWC traits in callus culture (Table 3) indicating the presence of genetic variability for these traits and it shows the line and stress significantly affected callus induction and characteristics. Ozgen et al. (1996) in a study on embryonic callus induction of wheat varieties, found that callus water content, callus induction percentage and callus dry and fresh weights were influenced by line.

The stress effect was significant for all traits except the callus relative water content. This indicates that applying the stress could change properly the environmental conditions. The line×stress interaction was significant for callus size and callus fresh weight. This indicated that both environmental and genotypic factors do not function independently for these two traits and their effects pertain together. In fact, some lines indicated better situation in one environment than the others. Similar results have been reported on drought tolerance of sugar beet monogerm hybrids *in vitro* (Ghafari et al., 2014). Cheghakabodi et al. (2012) showed the analysis of variance for the studied traits in callus culture of canola lines revealed that the lines were significantly different for all traits and there was a significant difference between stress levels in all traits. Furthermore, they showed the line×stress interaction was significant for these traits. It reflected the dependence of the two factors on the measured traits *in-vitro*

culture (Cheghakabodi et al., 2012). The results showed a high level of variation among lines. The highest and lowest values as well as average are given in Table 4.

Genetic diversity helps in the selection of lines with higher performance. Values of genotypic (s^2_g) and phenotypic variance (s^2_p) for all traits in normal and stress conditions are given in Table 4. High levels of these parameters indicated that sufficient diversity can be useful in the selection process. Mahto et al. (2002) and Vashistha et al. (2013) reported similar results on corn for agro-morphological traits. The evaluation of GCV indicated the total value of genotypic variance which transmitted from parents, to offspring and reflected by heritability. According to Deshmukh et al. (1986), amount of PCV and GCV nearly over 20% were considered as high amount. Whereas, amount of less than 10% were considered as a low amount, and amount of between 10% and 20% counted as a moderate. Therefore, the GCV was high for callus size under stress conditions (22.62), (40.54 and 47.58) for fresh weight in both normal and stress conditions, (45.30 and 56.77) for dry weight in both conditions, and was moderate for callus size in normal conditions (18.26). The values were low (2.94 and 8.56) for relative water content in both normal and stress conditions (Table 4). Yuce et al. (2008) and Wolie et al. (2013) reported similar results on millet. In the present study, general heritability ranged from 11.81% for relative water content in normal conditions to 66.61% for callus size, and from

Table 4. Mean, ranges, genotypic and phenotypic of variances, genotypic (GCV) and phenotypic (PCV) coefficient of variations, heritability and genetic advance as percent of mean (GAM) of callus measured traits in durum wheat lines

Range		Mean	Min	Max	s^2_g	s^2_p	GCV	PCV	h ² (%)	GAM
Characters										
Callus size	N	5.95	3.62	10.5	1.18	1.77	18.26	22.37	66.61	30.67
	S	3.03	2.10	5.10	0.47	0.50	22.62	23.33	94	44.73
Fresh weight	N	0.078	0.02	0.29	0.001	2E ⁻³	40.54	57.33	50	52.82
	S	0.038	0.011	0.070	3.27E ⁻⁴	3.43E ⁻⁴	47.58	48.73	95.33	92.89
Dry weight	N	0.010	0.0018	0.0353	2.22E ⁻⁵	5.53E ⁻⁵	45.30	71.50	40.14	58.65
	S	0.004	0.0020	0.019	7.74E ⁻⁶	9.02E ⁻⁶	56.77	61.29	85.80	108.16
RWC	N	85.92	56.75	98.5	6.41	54.25	2.94	8.57	11.81	2.08
	S	86.04	60.9	92.85	54.27	58.45	8.56	8.88	92.84	16.98

85.80% for dry weight to 95.33% for fresh weight under stress conditions (Table 4). High general heritability indicated the low effect of environment on the studied traits.

According to Singh (2001), if heritability of one trait be high, it will be selected easily and achieve good efficiency. The heritability of callus size (94), fresh weight (95.33) and dry weight (85.80) were high under stress conditions, whereas heritability of callus size (66.61) and fresh weight (50) were moderate under normal conditions. In low-heritability traits, the selection may have considerably poor efficiency. General heritability indicated both additive and non-additive genetic effects. Genetic advance (GA) under selection refers to improvement of traits in genotypic values for new population compared to the base population under a selection cycle in certain selection intensity (Singh, 2001). The maximum amount of genetic advance as percent of the mean (GAM) at selection intensity of 5% was estimated to be (108.16%) for callus dry weight and (92.89%) for callus fresh weight in stress condition.

Heritability evaluated along with genetic advance will be more effective in prediction of efficiency under selection. Therefore, genetic advance is another important parameter, assisting the plant breeders in the selection program (Johnson et al., 1955). Similarly, this study demonstrated that accurate selection can be effective for callus fresh and dry weights, and relatively for callus size (Table 4). Dyulgerova & Valcheva (2014) examined 23 barley doubled haploid lines for diversity, heritability and genetic advance for 6 traits. The PCV and GCV values were high for a number of traits. High heritability with high genetic advance in these traits reflected the importance of traits in improvement of barley lines. In an experiment conducted by Sabiel et al. (2014), 12 millet genotypes were evaluated for genotypic and phenotypic variance, heritability and genetic advance. These parameters were estimated for a number of traits. The results of comparing mean values for durum wheat lines demonstrated that G6 and G9 achieved higher levels of callus size and RWC under both normal and stress conditions while maintaining this ability in stress conditions compared to other lines. Moreover, G4 and G16 had minimum difference between fresh and dry weights in normal and stress conditions so, these lines had higher genetic potential for response to drought stress *in vitro*.

Estimation of seedling physiological traits under drought and normal conditions

The principal coordinate analysis was conducted on genetic diversity of durum wheat lines for eleven physiological traits measured in seedling stage under normal and stress conditions. The first four components explained 68.61% and 73.87% of total data variance in normal and stress condition

respectively, based on which cluster analysis was conducted to categorize the lines. The dendrogram obtained from cluster analysis for durum wheat lines has not been illustrated. Cut of dendrogram in 2.74, clustered lines into 4 groups in normal condition and cut of dendrogram in 2.70, clustered lines into 3 groups in stress condition. The mean comparison values for durum wheat lines for eleven physiological measured traits were analyzed (results not shown). The physiological traits of the plant, including the increase in activity of antioxidant enzymes, stomata closure, variation in the pattern of growth regulators and accumulation of metabolites indicate the adaptation to stress conditions. Hence, the effects of drought stress can be examined based on amount of enzymes to more quickly identify the resistant rootstocks of a plant. That is because there is a strong correlation between tolerance to environmental stresses and variations in the concentration of antioxidant enzymes in photosynthetic plants. Since the synthesis of every substance in cells are controlled by genes, the drought resistant rootstocks can be more quickly produced by identifying the genes responsible for synthesis of substances and their transfer to other plants (Khavari et al., 2017).

In this study higher levels of ascorbate peroxidase (APX), catalase, peroxidase, malondialdehyde enzymes were produced in lines under stress conditions. Intensified drought can increase the antioxidant enzymes. There are numerous reports in this regard. For instance, high temperatures during drought can escalate the activity of SOD, APX, and CAT in compatible wheat genotypes (Sairam et al., 2001). Previous studies showed that higher antioxidant enzymes activity substantially enhanced the tolerance for stress through dampening the effects of peroxide under various stresses in wheat, oats, soybeans and peas (Kafi et al., 2000; Ghorbanli et al., 2004). With respect to superoxide dismutase, the production of this enzyme was reduced under stress conditions. In present study, under stress, values for all chlorophylls (a, b and T) decreased. Chlorophyll stability recognized as an indicator of drought stress, high chlorophyll stability implies that the effect of stress on the plant is low, providing the plant with better access to light. The total amount of chlorophyll varies due to drought.

Ahmadi et al. (2013), showed the chlorophyll content of bean leaf decreased under drought stress, which in turn was linked directly to biomass production. The variations in the concentration of chlorophylls a and b were adopted as a short-term response to stress and a measure of ability to maintain the source power in drought stress conditions. In present study, lines that produced greater amounts of chlorophylls in comparison to other lines under stress condition were identified. Relative water content was reduced under

stress condition and line that achieved to maximum relative water content was identified. Line G8(19E-M141982), maintained RWC capacity under stress. Capable of retaining water in their tissues (i.e. higher relative water content), lines tended to be more drought resistant and perform more efficiently.

For fresh and dry biomass in both normal and stress conditions, the lowest difference between fresh biomass under normal and stress conditions were observed in several lines. In terms of this trait, the lines responded more desirably to stress by losing less water. The germplasm evaluation based on physiological traits showed different levels of variation among lines for some antioxidant enzyme activities and biomass of wheat lines under both normal and stress conditions. The results revealed high genetic diversity among the lines under experiment, which can be applied in dry durum wheat breeding programs.

Conclusions

In the present investigation, most of measured traits revealed the highest coefficients of variation and PCV values of traits were generally higher than GCV. Obtained heritability accompanied with high genetic advance for several traits indicates that most likely the heritability is due to additive gene effects and selection may be effective in early generations for these traits. According to mean comparison results for some traits *In vitro* culture, the lines G6 and G9 achieved higher levels of callus size and relative water content under the both optimal and stress conditions. The lines G4 and G16 had the higher genetic potential for response to drought stress *In vitro* environments.

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