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Inheritance of Antioxidant Activity of Bread Wheat under Terminal Drought Stress

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Abstract: To evaluate the inheritance of antioxidant activity of bread wheat, the F1 seeds of a 7×7 half diallel along with their parents were grown in greenhouse in well watered and terminal drought conditions at Islamic Azad University, Ardebil, Iran in 2008. After exposure of plants to drought stress, the activity of Ascorbate peroxidase (APX), Catalase (CAT) and Superoxide dismutase (SOD) enzymes were measured. In general all of traits had very high broad sense heritability. Among the traits APX had high narrow sense heritability (Hn), fallowed by moderate Hn for CAT and low Hn for SOD. Presence of over dominance and greater importance of dominance effects in control of traits was also observed. Regarding to the significant correlations between antioxidant activity and drought tolerance in the literature and considering the highest heritability of antioxidant enzymes in this study, it can be concluded that they can be considered as good criteria for selecting drought tolerance in wheat.

Key words: Heritability · Diallel · Antioxidant · Drought

INTRODUCTION

Drought is a major abiotic stress, limiting crop production in arid and semi-arid climates. Stress resistance in plants is a complex character that depends on many genes and thus is determined by the interactions of many morphological, physiological and biochemical processes. Photosynthesis is particularly sensitive to water deficit because the stomatal closure to conserve water, depletes intercellular CO_2. This process reduces the availability of CO_2 for photosynthesis, which can lead to the formation of reactive oxygen species (ROS) from the misdirecting of electrons in the photosystem [1,2]. These cytotoxic oxygen species are highly reactive and in the absence of any protective mechanism they can seriously disrupt normal metabolism through oxidative damage resulting in lipid peroxidation and consequently membrane injury, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands [3,4]. Higher plants have active oxygen scavenging systems consisting of several antioxidant enzymes, such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR) and some low molecules of non-enzyme antioxidants [5,6]. The activities of the antioxidant enzymes in plants under stress usually regarded as indicators of the tolerance of genotypes against stress conditions [7,8]. They also can be measured easily and are nondestructive to whole plant. So they potentially can be considered as selection criteria if they also have high heritability. The objective of this study was to evaluate the inheritance of antioxidant activity of bread wheat under terminal drought stress.

MATERIALS AND METHODS

Seven bread wheat varieties (Table1) were crossed in half diallel fashion at the experimental farm of Islamic Azad University, Ardebil, Iran in 2007. The F1 seeds, along with their parents were grown in greenhouse in well watered and terminal drought conditions using randomized complete block design with two replications in 2008. Grains of the genotypes were sown in plastic pots filled with 10 kg of soil composed of a mixture of garden soil, compost and sand (1:1:1, v/v). Well-watered plants were irrigated to water-holding capacity in 3 day intervals. The drought stress was started at the mid-flowering stage. Drought was imposed by withholding water until 80% soil moisture depletion and then adding only 25% of the water given to the control pots. Two weeks after onset of drought stress, flag leaves were collected from both control and stress treatments for enzyme assay.

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Table 1: List of cultivars used in the study

Cultivar	Pedigree/Origin	G.H.	P.H.	D.R.
Sabalan	908/Fn A12//1-32-4382	W	T	R
Azar2	Kvz/Tr71/3/Maya"s"//Bb/Inia/4/Sefid	W	T	R
Pishtaz	Alvand//Aldan"S"/Ias58 40072-48	S	M	MR
Alvand	1-2-7 6275/CF 1770	F	M	MR
Mv17	Introduction Cultivar-Hungary	W	S	S
Gaspard	Introduction Cultivar-France	W	S	S
Siosson	Introduction Cultivar-France	W	S	S

G.H. =growth habit, W=winter, F=facultative, S=spring, P.H. =plant height, S=short (<70cm), M=medium (70to85cm), T=tall (>85cm),

D.R.=drought Resisitance, R=resistant, M.R.=moderately resistant, S=susceptible

Enzyme Extraction and Determinations of Enzymes Activity: Leaf tissues were ground to fine powder in liquid nitrogen, then enzyme extraction was done according to Sairam *et al*., [7]. The activity of SOD was measured according to the method of Giannopolities and Ries [9]. Catalase was assayed by the method of Chance and Maehly [10]. The assay of APX activity was performed as described by Nakano and Asada [11]. The enzyme activities were expressed in terms of specific activity (Unit/mg Fresh Weight).

Statistical Analysis: The diallel analysis was done according to the theoretical basis developed by Hayman [12], adapted for the half diallel by Walters and Morton [13]. The goodness of fit of the additive-dominant model was performed b ased on the analysis of variance of Wr-Vr and linear regression of Wr on Vr [12]. The genetic components: D, H1, H2, F and h^2 were estimated according to Singh and Singh, [14]. Standard errors of these components were calculated from expected and observed values of *Wr*, *Vr*, $\overline{Vr}$, *Vp* and $(mL1 - mL0)^2$ over replications [12]. From the estimates of the genetic components, the genetic parameters presented in Table 4 were estimated. Average degree of dominance, broad sense heritability and narrow sense heritability were calculated according to Mather and Jinks [15]. Analysis of variance of diallel was performed using the DIAL98 software [16] and genetic components were estimated by electronic spreadsheets in the Excel program (Microsoft® Excel 2003).

RESULTS

The results of the goodness of fit of the additive-dominant model are shown in Tables 2 and 4. Non significant Wr-Vr mean squares for treatment (crosses) indicate the adequacy of additive dominant model for all of traits. However for CAT and APX activities in normal condition, the slop of linear regression was significantly lower than unit and additive-dominant model was not satisfied (Table 4). The Analysis of variance of the diallel is shown in Table 3. For APX and CAT activity, Additive variance ("a" component) was highly significant in stress conditions indicating the presence of additive effects in the control of these traits. However this component was not significant in activity of SOD. The significance of "a" in table 2 was in accordance with the significance of additive effects ("D" component) in Table 4. The "b" source of variation (dominant genetic effects) also showed highly significant effects for all of traits under stress conditions. This indicates the importance of dominant genetic effects as well as additive effects in the control of all of traits. The "b1" component which measures the mean deviations of the F1s from the mid-parental values was highly significant for APX and SOD activity (Table 3). The significance of the "b1" component indicates that the dominance was predominantly in one direction and measures average heterosis [14]. Significance of b1 component was generally in accordance with higher magnitude of dominance ($\overline{F}_1 - \overline{P}$) where F1s had higher enzymatic activity than their parents under stress. The "b2" component was significant only for APX activity under stress. The significance of the b2 item indicated that the mean dominance deviations of the F_is from their mid parental values differed significantly over the F_1 arrays; this implies the presence of asymmetry in the distribution of alleles among the parents [17]. This means that there was evidence that some parents had a significantly better performance than others [18]. The proportion of positive and negative genes was estimated by calculating (H2/4H1) in Table 4. This ratio was lower than 0.25 in APX (under stress) and CAT activity, indicating the presence of asymmetry in the distribution of the positive and negative alleles in the parents. This is also substantiated by H1 being greater than H2 in these cases. The "b3"

Table 2: Goodness of fit of additive-dominant model based on ANOVA of Wr-Vr

		APX		CAT		SOD	
S.V.	d.f	Normal	Stress	Normal	Stress	Normal	Stress
Replication	1	0.074**	1.69*	0.00858*	0.00021 ns	0.067 ns	1.486**
Treatment	6	0.005 ns	0.775 ns	0.000926 ns	0.00424 ns	0.110 ns	0.088 ns
Error	6	0.002	0.225	0.000818	0.00325	0.032	0.043

Table 3: Analysis of variance of the diallel tables for the evaluated traits

		APX		CAT		SOD	
S.V.	d.f	Normal	Stress	Normal	Stress	Normal	Stress
REP	1	0.41 ns	5.02**	0.00022 ns	0.0924**	0.05 ns	1.41 ns
a	6	0.20 ns	3.56**	0.01190*	0.044**	0.42 ns	0.50 ns
b	21	0.17 ns	4.95**	0.0094*	0.0201**	0.37 ns	1.63**
b1	1	0.55 ns	20.59**	0.0103 ns	0.007 ns	0.42 ns	13.05**
b2	6	0.10ns	1.67**	0.0084 ns	0.0118 ns	0.31 ns	0.20 ns
b3	14	0.18 ns	5.03**	0.0097*	0.0247**	0.39 ns	1.43**
Error	27	0.14	0.37	0.0044	0.00629	0.41	0.37

Table 4: Estimates of genetic components and related statistics in half- diallel design

	APX		CAT		SOD	
	Normal	Stress	Normal	Stress	Normal	Stress
D=	0.084^{ns}±0.06	1.67**±0.31	0.007**±0.0022	0.021**±0.0028	0.117^{ns}±0.12	0.122^{ns}±0.19
H1=	0.306^{ns}±0.15	3.76**±0.77	0.012*±0.0054	0.027*±0.0068	0.24^{ns}±0.29	2.44**±0.45
H2=	0.317*±0.13	3.21**±0.69	0.007^{ns}±0.0048	0.021*±0.0060	0.247^{ns}±0.26	2.50**±0.40
F=	0.033^{ns}±0.14	-1.0 ^{ns} ±0.75	0.0048^{ns} ±0.005	0.012^{ns} ±0.007	-0.116^{ns}±0.29	-0.16^{ns}±0.45
h^2=	0.170^{ns}±0.09	6.83**±0.47	0.004^{ns}±0.0032	0.0042^{ns} ±0.004	1.316**±0.17	3.67**±0.27
E	0.07**±0.002	0.185^{ns}±0.12	0.0022*±0.0008	0.0031**±0.001	0.205**±0.043	0.186*±0.067
Averag d	1.91	1.50	1.31	1.12	1.43	4.46
H2/4H1	0.259	0.214	0.144	0.199	0.258	0.256
KD/KR	1.228	0.664	1.69	1.71	0.486	0.747
h2/H2	0.535	2.126	0.634	0.199	5.326	1.47
Hn	0.119	0.620	0.483	0.460	0.297	0.119
Hb	0.587	0.929	0.711	0.805	0.460	0.798
F1-P%	-1.18	6.47	-1.27	1.03	-16.7	118
rYr(Wr+Vr)	-0.626 ^{ns}	-0.327 ^{ns}	-0.139 ^{ns}	-0.489 ^{ns}	0.369 ^{ns}	-0.0825 ^{ns}
b(Wr/Vr)	0.109**±0.107	0.598^{ns}±0.19	0.40**±0.21	0.937^{ns}±0.695	0.386 ^{ns}±0.244	0.635^{ns}±0.154
A(intercept)	0.028^{ns}±0.023	6.7×10^{-5ns} ±10^{-4}	0.00032^{ns}±0.001	-0.003 ^{ns} ±0.008	-0.008 ^{ns} ±0.08	-0.05 *±0.019

ns,* and ** non-significant, significant at p<0.05 and 0.01 respectively, D= Additive genetic variance, H_1=Uncorrected dominance genetic variance, H_2=corrected dominance variance, F=average covariation of additive and dominance effects, h^2 = dominance effects (as algebraic sum over all loci in heterozygous phase in all crosses), Average d = average degree of dominance, $H_2/4H_1$=relative distribution of positive and negative genes among parents, KD/KR=relative distribution of dominant and recessive genes among parents, h^2/H_2 = The number of effective factors that show dominance Hn= narrow sense heritability, Hb= broad sense heritability, E= environmental variance (error mean square of simple ANOVA divided by number of replications), rYr(Wr+Vr)= relation between the favorable alleles and dominance, F_1-P= magnitude of dominance, b(Wr/Vr)= slope of regression line of Wr on Vr.

component which is equivalent to specific combining ability variance was significant for all of traits under stress. The estimate of the genetic component F was non significant in all cases which is an indication of symmetry in the distribution of dominant and recessive alleles in the parents. However the ratio of the total number of dominant and recessive alleles in the parents (KD/KR) was higher than 1 for CAT activity, demonstrating a higher frequency of dominant alleles in the parents, this ratio was lower than 1 in the case of SOD and APX (under stress) activity. Positive values for F substantiated by (KD/KR) being greater than 1 and vice versa. For most of traits positively significant h^2 values were recorded except for CAT activity (Table 4). The degree of average

dominance was higher than 1, indicating the presence of over dominance in control of these traits. The degree of average dominance also was shown by the intercept point between the regression line and origin. As shown in Table 4, only the intercept of SOD activity (under drought stress) was significantly lower than zero confirming its over dominance. Contribution of over dominance also confirmed by higher heterosis(118%) in SOD activity (Table 4). The number of groups of genes that control the character and exhibit dominance (h2/H2) ranged from 0.199 to 5.32. In general SOD had the highest number of dominant genes followed by APX and CAT activity. Narrow sense heritability (Hn) of traits ranged from 0.119 to 0.620. Among the traits APX activity had the highest Hn, followed by CAT with moderate Hn and SOD with low Hn. The values of the broad sense heritability (Hb) ranged from 0.460-0.929. The differences observed between the Hn and Hb reflected the presence of the dominant effects. Non-significant correlation coefficients between the parental means and order of dominance rYr(Wr+Vr) were observed for all traits indicating that there is not a strong relation between dominance and favorability of traits.

DISCUSSION

Based on the results of this experiment it was concluded that the activity of antioxidant enzymes under drought stress adequately can be described by additive-dominance model. For APX and CAT activity under stress, additive effects as well as dominant effects were significant. However in SOD activity, additive effects were not significant. Since the degree of average dominance was higher than 1, the presence of over dominance and greater importance of dominance effects in control of traits was suggested. Due to the importance of dominance in the control of characters under study, especially APX and CAT activity, it was suggested that the evaluations of genotypes must be done at advanced generations of inbreeding. The results also showed that, dominance was predominantly in one direction except for CAT activity, indicating the presence of heterosis in the control of these 2 traits. Among the traits APX had the highest narrow sense heritability, fallowed by moderate heritability for CAT and low heritability for SOD. In general all of traits had high broad sense heritability and its magnitude was higher under stress than normal conditions, but such trend did not observed in narrow sense heritability. Regarding to the highest heritability of antioxidant enzymes under study, it can be concluded that they can be considered as good candidates for selecting drought tolerance in wheat. Since CAT and APX were controlled by relatively fewer numbers of dominant genes than SOD, it seems that they can be more easily manipulated in plant breeding programs.

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