Biological Forum – An International Journal **7**(2): 43-47(2015)

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Genotypic Variation and Heritability of Antioxidant related Traits in Wheat Landraces of Iran

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(Received 12 May, 2015, Accepted 15 June, 2015)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: In respect to the importance of genetic variation and resolution of population structure for crop improvement, this study were carried out to investigate the interrelationships of biochemical traits and the extent of genetic variation in wheat landrace varieties. The research work was conducted at the agricultural research station of Razi University, Kermanshah, Iran in 2013-2014 growing season. Twenty Iranian landraces of bread wheat (*Triticum aestivum* L.) were cultivated in a randomized complete block design with three replications. Biochemical and antioxidant related traits such as superoxide dismutase activity, peroxide activity, catalase activity, ascorbic peroxide activity, proline content, carotenoid content, hydrogen peroxide, and malondialdehyde content were measured in landraces. The overall results showed a high genotypic variation for enzymatic antioxidant activity, and also high heritability for all measured parameters. These results showed that screening program for obtaining high antioxidant activity and lower oxidant content could be performed in this population. Phenotypic correlation between hydrogen peroxide content with proline and with carotenoid content were positive but these correlation were genotypicaly negative indicating that genetically and naturally, proline and carotenoid content are working for detoxification of hydrogen peroxide but environment affects this relationship. So that, screening genotypes for higher content of proline and carotenoid content must performed precisely to gain higher detoxification of ROSs.

INTRODUCTION

Growing population in developing countries accompanied with high food consumption lead to high global demand on food. On the other hand, massive using of bred lines or synthetic cultivars results in germplasm uniformity and consequently crop vulnerability against biotic and abiotic stresses(STAT 2010). Expanding genetic diversity guarantees feeding world population and crop germplasm against adverse effects of environmental stresses. Variations in crop germplasms also increase the chance of finding high yielding plants for cultivation. Screening various germplasms is the first step of a basic program for selection of candidate genotypes for cultivation under targeted regions. One of the rich genetic resources are landrace varieties harboring valuable genes against adverse effects of biotic and abiotic stresses (Zeven 1998). A landrace is a local variety of a domesticated plant species which has developed largely by natural processes, by adaptation to the natural and cultural environment. Landrace differs from a formal breed which has been selectively bred deliberately to conform to a particular trait.

Landraces are usually more genetically and physically diverse than formal breeds. The features of landrace varieties could be incorporated into commercial cultivars and inbred lines by under field hybridizations or in vitro transformation methods (Zeven 1998).

Evaluation of the interrelationship of traits in crop germplasm is important for clarifying population structure and modelling selection criteria for increasing plant productions. Simple statistical analyses are not able to clarify cause and effect relations of important traits contributed to yield and other important trait's variation. Alternatively, the knowledge of genetic diversity is a useful tool in gene-bank management and plant breeding programs (Dwevedi and Lal 2009). Information of genetic parameters is also important in modelling selection approaches for improving crop structures (Khan and Qasim 2004). Estimating heritability of traits and the extent of genetic variation can be used in prediction of genetic advances in a crop population (Ali *et al.* 2008). Genotypic and environmental factors are necessary in breeding programs.

Estimation of genetic variation and its contribution in phenotypic variance and the heritability of the traits are of importance to achieve significant genetic improvement in chickpea production using different genotypes (Arshad, Bakhsh, and Ghafoor 2004). Therefore, given the importance of genetic variation and resolution of population structure for crop improvement, the main objectives of this study were to investigate the interrelationships of biochemical traits and the extent of genetic variation in wheat landrace varieties.

MATERIALS AND METHODS

This study was conducted at the Biotechnology Laboratory of Agriculture and Natural Resources Research Center, Kermanshah province, Iran in the 2013-2014 growing season. Twenty Iranian landraces of bread wheat (*Triticum aestivum* L.) were cultivated in a randomized complete block design with three replications. These seeds of landraces (Table 1) were obtained from Seed and Plant Improvement Institute, Karaj, Iran.

Table 1: The codes and name of twenty Iranian landraces of bread wheat.

WC-5053	1	WC-4640	6	WC-47360	11	WC-47367	16
WC-4931	2	WC-4823	7	WC-47636	12	WC-5047	17
WC-47381	3	WC-4780	8	WC-4566	13	WC-476191	18
WC-47399	4	WC-46617-11	9	WC-47628	14	WC-47341	19
WC-47456	5	WC-4530	10	WC-4584	15	Pishtaz	20

Each experimental plot consisted of four one-meter long rows spacing 25 cm. Weeds were pulled out manually and no fertilizer was applied. Plants were irrigated seven times during the growing period.

Measuring biochemical traits. Proline measurement. Free proline was extracted from fresh leaves according to the method of Bates, Waldren, and Teare (1973). Leaf samples (0.5 g) were homogenized in 10 mL of 3% (w/v) aqueous sulphosalicylic acid and the solution filtered using a Whatman No. 2 ?lter paper. Two mLs of solution was then mixed with 2 mLs acid ninhydrin and 2 mLs glacial acetic acid in a test tube, and incubated at 100°C water bath for 1 h. The reaction was terminated by placing the mixture in an ice bath. Free proline of solution was finely extracted with 4 mLs toluene. The absorbance was recorded at 520 nm and proline concentration was determined as μmol g^{-1} fresh weight using a standard curve.

Measurement of total protein and antioxidant enzymes activity. Leaf samples were frozen in liquid nitrogen and kept refrigerated at -80°C. Frozen leaves were ground to fine powder with a mortar and pestle in liquid nitrogen and were extracted with ice-cold 0.1 M Tris-HCl buffer (pH 7.5) containing 5% (w/v) sucrose and 0.1% 2-mercaptoethanol (3:1 buffer volume/fresh weight). The homogenate was centrifuged at 12000 × g for 20 min at 4°C and the supernatant was used to measure protein content and enzymes activity. Enzyme extraction was carried out at 4°C.

The protein content was estimated according to the method of Bradford (1976), using bovine serum albumin (BSA) as a standard and observance of 595 nm.

Superoxide dismutase (SOD) inhibits the photochemical reduction of nitrobluetetrazolium (NBT) (Beauchamp and Fridovich 1971), this ability used to determine its activity (DHINDSA, PLUMB-DHINDSA, and THORPE 1981). For SOD assay, the reaction mixture contained 50 mM K-phosphate buffer (pH 7.8), 13 mM methionine, 75 μM NBT, 0.1 μM EDTA, 4 μM riboflavin and extracted enzyme. The reaction started by adding riboflavin after which the tubes were placed under two 15 W fluorescent lamps for 15 min. A complete reaction mixture lacking enzyme, which gave the maximal colour, considered as control. A non-irradiated complete reaction mixture was used as a blank. One unit of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT as monitored at 560 nm (Giannopolitis and Ries 1977).

Peroxidase (POD) activity was assayed (Polle *et al.* 1994) at 436 nm by its ability to convert guaiacol to tetraguaiacol (ε = 26.6 mM cm^{-1}). The reaction mixture contained 100 mM K-phosphate buffer (pH 7.0), 20.1 mMguaiacol, 10 mM H_2O_2 and enzyme extract. The increase in absorbance was recorded by adding H_2O_2 at 436 nm for 5 min. The activity of Catalase (CAT) was determined by monitoring the disappearance of H_2O_2 at 240 nm (ε = 40 mM cm^{-1}) according to the method of (Aebi 1984). The reaction mixture contained 50 mM K-phosphate buffer (pH 7.0), 33 mM H_2O_2 and enzyme extract.

Malondialdehyde content. The content of malonaldehyde (MDA) was measured according to Dhindsa *et al.* (1981) with some modifications. Leaf samples (0.15 g) were homogenized in 4 ml of 10% TCA and centrifuged at 10 000 g for 15 min. The supernatant (1 ml) was mixed with 1 ml of 0.6% thiobarbituric acid, heated at 95°C for 30 min and then was quickly cooled down on ice. After centrifugation at 10 000 g for 10 min, absorbance of the supernatant was measured with a spectrophotometer at 450, 532 and 600 nm, respectively (Schmedes and Hølmer 1989).

Hydrogen peroxide (H_2O_2)

The concentration of hydrogen peroxide (H_2O_2) was measured based on the Velikova, Yordanov and Edreva (2000) method. Leaves were homogenized in an ice bath with 0.1% (w/v) TCA. The extract was centrifuged at 12,000 × g for 15min, and 0.5 ml of 10Mm potassium phosphate buffer (pH 7.0) and 1.0ml of 1.0M KI were added to 0.5 ml of the supernatant, and the absorbance was read at 390 nm. The concentration of H_2O_2 was given on a standard curve.

Biometrical and statistical analysis. Phenotypic (CVp) and genotypic (CVg) coefficients of variation and heritability (h2) of the traits were calculated using the following formulas (Mackay 2001):

$$Cv_p = \frac{\sqrt{Vp}}{\mu} \times 100 \quad , \quad Cv_g = \frac{\sqrt{Vg}}{\mu} \times 100 \quad ,$$

$$h^2 = \frac{Vg}{Vp} \times 100$$

Where, V_P, V_g and μ are phenotypic variance, genotypic variance and mean of the traits, respectively. An analysis of variances was performed using SAS software and trait's means were compared based on Least Significant Difference (LSD). Cluster analysis (SAS 9.1, SAS Institute) was used to classify genotypes based on the value of the traits in both conditions.

RESULTS AND DISCUSSION

Genotypic and phenotypic coefficients of variation, heritability, genotypic and phenotypic variance, and also other descriptive statistics of biochemical measured traits for is presented in Table 2. The highest genotypic and phenotypic coefficients of variation were obtained for superoxide dismutase (CVg = 181.6% and CVp = 236%) while the lowest ones were observed for hydrogen peroxide content (CVg = 3.66% and CVp = 5.75%). Genotypic and phenotypic coefficients of variation were high for peroxidase and ascorbic peroxidase (more than 100%). This is showing that the overall variation related to antioxidant activity of the wheat landraces of Iran are high and breeding programs can started in order to using of this variation. The observed variation for Malondialdehyde and hydrogen peroxide content were low reversely. The highest heritability was estimated for proline (h2 = 76.75%). The lowest estimated heritability was observed for hydrogen peroxide content (h2 = 43.67%). High heritability for all measured parameter were observed among the landrace population meaning that screening program for selecting landraces having higher content of antioxidant and lower content of oxidant could be carried out.

Table 2: Heritability in broad sense, genotypic, phenotypic and some basic parameters of measured variables.

Variable	Minimum	Maximum	Mean	SE Mean	StdDev	Vg	Vp	h^2%	GCV%	PCV%
Proline	3.10	9.42	5.42	0.42	1.87	8.78	9.08	76.75	161.96	167.40
Car	3.36	7.47	5.96	0.23	1.02	2.67	2.87	73.09	44.78	48.10
POD	48.82	86.77	65.18	2.04	9.12	207.35	219.43	74.49	118.11	136.65
APX	127.76	172.32	143.94	2.61	11.69	340.01	373.15	71.12	136.22	159.25
CAT	34.01	43.56	39.26	0.52	2.34	14.72	17.32	65.00	37.50	44.11
SOD	275.92	455.70	345.72	9.06	40.52	4085.02	4275.44	75.55	181.60	236.68
H2O2	2.66	3.44	3.17	0.04	0.19	0.12	0.18	43.67	3.66	5.75
MDA	5.18	7.30	6.35	0.14	0.62	1.30	1.53	65.19	20.36	23.90

Vg, Genotypic variance; Vp, Phenotypic variance; H, heritability in broad sense; GCV, Genotypic coefficient of variation; PCV, Phenotypic coefficient of variation; StdDev, Standard deviation of variables; SE, Standard error.

One of the most important effects of stresses on plants is generation of reactive oxygen species (ROS) such as -O1, O_2^-, OH. and H_2O_2 that are extremely reactive in nature and they can interact with a number of other molecules and metabolites such as DNA, pigments, proteins, lipids, and other essential cellular molecules which lead to a series of destructive processes (Saed-Moocheshi *et al.* 2014).

Plants' response to generation of ROS is producing ROS scavengers that are consisting of enzymatic and non-enzymatic scavengers (Saed-Moucheshi, Shekoofa, and Pessarakli 2013). Important antioxidant enzyme are consist of superoxide dismutase, catalase, peroxidase, and ascorbic peroxidase (Saed-Moucheshi *et al.* 2014). Exceeding the production of reactive oxidative species (ROS) cause occurring oxidative stress and in this condition the capacity of cellular antioxidant defenses to remove these toxic species is lower than ROS production (Hossain *et al.* 2015). Because of important roles of oxidant and antioxidant, high variation among landraces of this experiment and their high heritability is a suitable for using in the breeding programs in order gain high antioxidant content and low oxidant content new genotype and cultivars.

Genotypic and phenotypic correlation coefficients between the measured biochemical parameters are presented in Table 3. Highest positive phenotypic correlation was observed between proline and peroxidase activity, while highest negative phenotypic correlation were obtained for peroxidase activity and hydrogen peroxide content showing that higher activity of peroxidase caused lower content of hydrogen peroxide. Phenotypic correlation hydrogen peroxide content with proline and carotenoid content were positive, but these correlation coefficients for malondialdehyde were negative.

Table 3: Genotypic (above the main diagonal) and phenotypic (under the main diagonal) correlation.

Variable	Proline	Car	POD	APX	CAT	SOD	H_2O_2	MDA
Proline	1	-0.13	0.50	-0.16	-0.28	-0.41	-0.39	-0.33
Car	-0.17	1	-0.19	-0.25	0.04	0.17	-0.16	-0.29
POD	0.53	-0.24	1	0.22	-0.11	0.21	-0.24	-0.18
APX	-0.10	-0.32	0.28	1	0.42	-0.13	-0.05	-0.36
CAT	-0.15	-0.05	0.16	0.56	1	0.20	-0.34	-0.14
SOD	0.07	-0.22	0.04	0.56	0.60	1	-0.44	-0.21
H_2O_2	0.11	0.24	-0.41	-0.31	-0.31	-0.29	1	0.08
MDA	-0.13	-0.07	-0.15	-0.20	-0.13	-0.07	0.24	1

The phenotypic correlation between hydrogen peroxide content and malondialdehyde content was positive. Similar to phenotypic coefficient of correlation, highest positive genotypic correlation was measured for relationship between proline and peroxidase. Highest negative genotypic correlation was obtained for relationship between superoxide dismutase and hydrogen peroxide content. Genotypic correlation of hydrogen peroxide and malondialdehyde content with all other variables were negative. Phenotypic correlation between hydrogen peroxide content with proline and with carotenoid content were positive but these correlation were genotypicaly negative indicating that genetically and naturally, proline and carotenoid content are working for detoxification of hydrogen peroxide but environment affects this relationship. So that, screening genotypes for higher content of proline and carotenoid content must performed precisely to gain higher detoxification of ROSs.

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