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ASSESSMENT OF SOD AND CORRELATION WITH AUDPC,YIELD AND TEST WEIGHT IN RESISTANT AND SUSCEPTIBLE GENOTYPES OF WHEAT DURING INFECTION

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ABSTRACT
The 32 resistant and 2 susceptible lines are inoculated by pathogen viz. *Bipolaris sorokianiana* at the concentration of 10^4 spores per ml of solution in the evening time and materials are assess to quantify superoxide dismutase (SODs) and found that the SODs are increases in both resistant and susceptible lines over a time period but in resistant lines (ranges 0.738 – 0.955 EU/g fresh weight) it increases rapidly and overwhelmed the toxic effects of ROS and causes less damage as compare to susceptible lines viz. Sonalika (0.509 EU/g fresh weight) and CianoT79 (0.5139 EU/g fresh weight) and are negative correlated with AUDPC (r= -0.513), and positively correlated with yields (r= 0.424), test weight (r= 0.487). SODs are high heritability character (69.82%) and good for selection to next generation. SODs increase with the time it shows highest at the time interval of 48 hrs (0.876 EU/g fresh weight) and least at the time interval of 12 hrs (0.707EU/g fresh weight) after the infection. So in this result found that genotypes have high SODs shows high resistance towards the pathogens attack and leads to less damage from the ROS effects which are the causes of biotic stresses in the plants (*B.sorokiniana*).

INTRODUCTION

Wheat (*Triticum spp.)* is a cereal and a temperate crop. The wheat production globally was slightly decreased 674.9 million tonnes in 2012-13 from 704.1 million tonnes in 2011-12 (FAOSTAT), making it, the third most produced cereal after maize and rice. India recorded all time high 94.88 million tonnes of wheat production from an area of 29.90 million hectare during 2012-13 (Wheat annual report from directorate of wheat research Kernal 2012-13). In Uttar Pradesh, the production of wheat is 24.5 million tonnes from 9.2 million ha areas. In world trade wheat is greater than for all other crops combined (Curtis *et al*., 2002). It is grown throughout the world, in a wide variety of climates but major wheat production is concentrated between 30º and 60º N and 40º S latitudes (Nutteson, 1955). It has good nutrition profile with 12.1 percent protein, 1.8 % lipids, 1.8 % ash, 2.0 % reducing sugars, 6.7 % pentosans, 59.2 % starch, 70 % total carbohydrates and provides 314K cal/100 g of food (USDA, 2012). *Bipolaris sorokiniana* (Sacc.) Shoem. *(Helmnthos porium sativum,* telomorph (*Cochliobolus sativum*), the causal agent of spot blotch disease of wheat is a major biotic stress in the warm humid tropic encompassing many countries of the world such as India, Bangladesh, Nepal, Brazil, Argentina and Peru (Kumar *et al*., 2002). The average yield loss caused by leaf blight in South Asia is around 20% (Dubin and Ginkel, 1991), but yield losses between 20% and 80% have been reported by Duveiller and Gilchrist, (1994). Under severe conditions, the yield losses may be as high as 100% (Srivastava *et al*., 1971). *Bipolaris sorokiniana* usually induce visible necrotic symptoms on the leaf, leaf sheath and stem (Chand and Joshi, 2004). The symptoms first appear in lower leaves and gradually spread upward and finally reach theSpikes causing head rot resulting in low weight, shrivelled grains (Kiesling, 1985) with black point symptoms at embryo end of kernel (Chand and Joshi, 2004). It produces oblong, necrotic, dark lesions (up to 20 mm long) which are scattered throughout the leaves. These lesions increased in size and finally coalesce to form a large spot that covers and kills large portions of the leaf, with severely infected leaves senescencing prematurely (Steffenson, 1997). In nature, it reproduces asexually by means of conidia. The conidia are dark brown to black in colour, thick walled with 810 transverse septa. The pathogen is ubiquitous in nature but highly seed borne and transmitted by wheat seed (Rashid and Neergaard, 1996). Conidia on germination produce germ tube, then swells to produce appresorium from which infection hyphae are developed. The infection hyphae enter the host tissue either through stomata or by rupturing the epidermis. The biotic and abiotic stresses are an increase production of reactive oxygen species (ROS) (Polle and Rennenberg, 1993). ROS such as O_2, superoxide radical, H_2O_2, hydrogen peroxide and OH, hydroxyl radical are toxic byproducts of processes such as photosynthetic or respiratory electron transport. Oxidative stresses in various crop plants have been reported in response to salinity (Sairam *et al*., 2005), drought (Sairam *et al*., 2002), high temperature

(Davidson *et al*., 1996) and pollutants (Ranieri *et al.,* 1998). These toxic ROS causes damage to DNA, lipids and chlorophyll etc (Imlay and Linn, 1988). Plants protect cell and sub cellular systems from the cytotoxic effect of the ROS with antioxidant enzymes such as superoxide dismutase (SOD), and others antioxidants it involving both limiting the formation of ROS as well as the formation and removal of O_2 are in balance. However, the defence system, when presented with increased ROS formation under stress conditions, can be overwhelmed. Within a cell, the superoxide dismutase (SOD) constitutes the first line of defence against ROS. SODs are nuclear encoded metalloproteins that catalyses the dismutation of two superoxide molecules to hydrogen peroxide and oxygen and constitute the first defense line against ROS. Metal cofactor, subcellular distribution and sensitivity to H_2O_2 and KCN distinguish the three known isoforms ~ CuZnSOD, FeSOD and MnSOD. In general, plants contains a mitochondrial MnSOD (not inhibited neither by H_2O_2 or KCN) ~ cytosolic and chloroplastic CuZnSOD (sensitive to both inhibitors). Some species also contain FeSOD in the chloroplast which is sensitive to H_2O_2. There are abounded opportunities to identify the innate immunities of plant and SODs is one of them and this innate immunity helps us to screening of resistance genotypes which indirectly helps in reducing environmental hazards from pesticides. The paper deals with the innate immunity resistance and AUDPC, yields and test weight of same genotype and there correlation among them.

MATERIALS AND METHODS

The experiments were conducted during 2012-2013 & 2013-2014 in the Rabi season at the Agricultural Research Farm of Institute of Agricultural Sciences, Banaras Hindu University is situated in the north Gangetic alluvial plain at 25°18′ N latitudes, 83°03′ E longitude and at an altitude of 128.93 metres above the mean sea level. Varanasi region have normal precipitation of 1100 mm and potential evaporation of about 1500 mm with an annual moisture deficit of about 400 mm and moisture deficit index of 20 to 40 per cent. Where the crop was sown in November - December and harvested in April. The experimental field was well drained with uniform topography and assured source of water supply. Preparation of spore suspension and inoculation of the inoculums Spore suspension was prepared on the day of inoculation in the field. Inoculums produced on sorghum grains were mixed in sterile water in a bucket. Then it was stirred so that all spores on sorghum grains should dislodge in water. It was then filtered with plastic net. The concentration of spore suspension was maintained to 10^4 spores′ ml1 with the help of spore count/ 100il. Then 0.05% Tween80 was added to increase the stickiness of the spore suspension. Inoculation of pathogen (*Bipolaris sorokiniana*) was done at 50% flowering stage (GS 65) in the evening hours at 5:00 - 6:00 pm in order to provide sufficient moisture for longer period for spore germination and pathogenesis.

Experimental materials

The experiment consists of total 34lines (Table 1) including two checks and it was sown in 3replications in randomized block design (RBD) during crop season of year 2012 - 2013 & 2013 - 2014. These lines were obtained from CRP project (CGIAR) where purity of each line was maintained by SSD method.

Assessment superoxide dismutase

Superoxide dismutase (SOD) activity was assayed in leaf samples and these samples were collected at the 50% flowering stage at the time interval of at 12 hrs, 24 hrs, and 48 hrs after the inoculation and wrapped in aluminium foils, labelled and immediately dipped in the liquid nitrogen (-196ºC) and transported to central laboratory of Institute of Agricultural Sciences, BHU, and preserve at -80 < "C. Assay was performed adopting the protocol of Dhindsa *et al*., 1981 Leaf sample (100 mg) was crushed with 5 mL of extraction buffer (0.1 M phosphate buffer, pH 7.5 containing 0.5 mM EDTA). The ground sample was centrifuged in cooling centrifuge machine (REMI, C4) at 10000 rpm for 15 minutes. After centrifugation, supernatant was collected, and this supernatant was used as enzyme source. Three mL of the reaction mixture containing 0.1 mL of 1.5 M sodium carbonate, 0.2 mL of 200 mM methionine. 0.1 mL of 2.25 mM NBT, 0.1 mL of 3 mM EDTA, 1.5 mL of 100 mM potassium phosphate buffer, 1 mL of distilled water and 0.1 mL of enzyme extract were taken in test tubes in thee replications from the enzyme sample. Two test tubes without enzyme extract were taken as control. The reaction was started by adding 0.1 mL of riboflavin (60 µM) and placing the test tubes below a light source of two 15W florescent lamp for 15 minutes. Reaction was stopped by switching off the light and covering the test tubes by black cloth. Tubes without enzyme extract developed maximum colour. A nonirradiate complete mixture that did not develop colour serves as a blank. Absorbance was recorded at 560 nm in spectrophotometer (ELICO, SL196).

Calculation

$$\text{Enzyme unit (EU)} = \frac{\text{Enzyme}^*_{\text{light}} - (\text{Enzyme}_{\text{light}} - \text{enzyme}_{\text{Dark}})}{\text{Enzyme}^*_{\text{light}} / 2}$$

Note * indicate the absorbance value of "light without enzyme" shows more colour change.The enzyme unit (EU) was expressed on per gram fresh weight basis as well as on the basis of per mg protein (specific activity).

Area under disease progressive curve (AUDPC)

Disease severity of spot blotch for each genotype was evaluated on five randomly tagged plants in the field and recorded at minimum three time points at 7 to 10 days intervals, using the double digit scale (00–99) of Saari and Prescott (1975) at three different growth stages (GS) viz., GS 65 (half anthesis to half complete), GS 69 (anthesis complete) and GS 77 (late milking) (Zadoks *et al*., 1974) The disease severity (DS) percentage would be based on the formula (Duveiller *et al*., 2005).

% Disease severity = (D1/9) × (D2/9) ×100

The Disease Severity was The AUDPC is calculated using the per cent disease severity estimations corresponding to the disease ratings, as outlined by Roelfs *et al*., (1992).

Test weight of grains

The test weight was estimated from 20 tillers harvested

separately from the experimental plots and grains were threshed and packed in envelopes and taken to laboratory. Counted 1000 seeds, from each genotype including resistance and susceptible lines were weighed to find the test weight.

Grain yield

Grain yield from each genotype 20 tillers selected random from each plot. Threshing of each genotype was done and yield of 20 tillers of each genotypes were computed.

Identification of SOD genes in wheat whole genome sequence

Three classes of SOD enzymes could be encoded by a small gene family. In Arabidopsis, the total of seven SOD genes, including three CuZnSODs, one MnSOD and three FeSODs, have been isolated through the analysis of the large numbers of cDNA and genomic DNA sequences (Kliebenstein *et al*., 1998). Based on the TIGR Gene Indices program in the international bioinformatics website (www.tigr.org), two wheat CuZnSOD genes (tentative consensus (TC) numbers were TC250697 and TC250698) were identified when using superoxide dismutases as the keywords. The BLAST analysis in National Centre for Biotechnology Information (NCBI) using these two wheat CuZnSODs to be the queries showed no wheat CuZnSOD genes with same sequences released, except two homologous genes of SOD1.1 and SOD1.2 (Wu *et al*., 1996). Therefore, they were named TaSOD1.1 (TC250697) and TaSOD1.2 (TC250698).

RESULTS

In this experiment, 34 wheat genotypes (Table 1) were evaluated for SOD activity, Test weight, and yield. Out of these, 32 lines were resistant and 2 lines were susceptible. The activity of SOD was assessed for two years. Effect of time elapsed on SOD activity at 12 hrs, 24 hrs and 48 hrs intervals. SOD was extracted in laboratory from infected and healthy (without inoculation) leaves samples collected from three replicates of experimental Plots.

Analysis of variance (Table 2) reveals the significant (<0.0001) effect of genotypes on SOD activity. Year alone has not influenced the SOD activity while the interaction of year and genotype was significant

Table 3, shows the mean value of SOD unit (EU) per g fresh weight and AUDPC in selected resistant and susceptible wheat genotypes in 2012 -2 013 & 2013 - 2014. There were significant differences in SOD (EU/g fresh weight) among the genotypes. The range of SOD varied from 0.505 to 0.955 EU/g fresh weights among the wheat genotypes. Susceptible genotypes show low (0.505 EU/g fresh weight) SOD activity. The resistance genotypes recorded higher (0.955 EU/g fresh weight) SOD activity. Genotypes showed higher SOD activity

Table 1: Pedigree of genotypes used for SOD

Genotypes	Pedigree
1	ATTILA/3*BCN//BAV92/3/TILHI
2	TILHI/PALMERIN F2004
3	CROC_1/AE.SQUARROSA (205)//KAUZ/3/ENEIDA
4	TILHI/SOKOLL
5	PFAU/MILAN//TROST/3/PBW65/2*SERI.1B
6	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUARROSA (TAUS)/ 4/ WEAVER/ 5/ PASTOR
7	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE.SQUARROSA (498)/5/2*OPATA
8	CHIRYA.3
9	JUPARE C 2001
10	ALTAR 84/AE.SQUARROSA (219)//OPATA/3/WBLL1/FRET2//PASTOR
11	W462//VEE/KOEL/3/PEG//MRL/BUC
12	VORB/4/D67.2/PARANA 66.270//AE.SQUARROSA (320)/3/CUNNINGHAM
13	HD 2967
14	BECARD
15	ATTILA/3*BCN//BAV92/3/TILHI/4/SHA7/VEE#5//ARIV92
16	CNDO/R143//ENTE/MEXI_2/3/AEGILOPSSQUARROSA (TAUS)/4/WEAVER/5/2*KAUZ
17	ASTREB/OAX93.10.1//SOKOLL
18	ALTAR 84/AEGILOPS SQUARROSA (TAUS)//OPATA
19	CMH79A.955/4/AGA/3/4*SN64/CNO67//INIA66/5/NAC/6/RIALTO
20	SW89-5124*2/FASAN
21	BCN/RIALTO
22	CROC_1/AE.SQUARROSA (205)//KAUZ/3/SASIA/4/TROST
23	MILAN/KAUZ/3/URES/JUN//KAUZ/4/CROC_1/AE.SQUARROSA(224)//OPATA
24	NL 750
25	PBW343*2/KUKUNA//PBW343*2/TUKURU/3/PBW343
26	NL748/NL837
27	TILHI
28	UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ/5/MILAN/KAUZ//CHIL/CHUM18/6/UP2338*2/4/SNI/TRAP#1/3/KAUZ*2/TRAP//KAUZ
29	ASTREB/OAX93.10.1//SOKOLL
30	GAN/AE.SQUARROSA (897)//OPATA/3/BERKUT
31	SURUTU-CIAT
32	WESTONIA/4/KRICHAUFF/FINSI/3/URES/PRL//BAV92
33	SONALIKA
34	CIANO T-79

Table 2: ANOVA of year and genotypes

Source	DF	Mean Square	F Value	Pr > F
Year	1	0.0011	0.17	0.6852
Genotype	33	0.0480	6.86	<0001**
Replication	2	0.0483	6.91	0.0014
Year*Genotype	33	0.0238	3.41	<.0001**

** = Significant; Analysis of variance (Table 2) reveals the significant (<0.0001) effect of genotypes on SOD activity. Year alone has not influenced the SOD activity while the interaction of year and genotype was significant.

Table 3: Distribution of SOD and AUDPC in selected resistant and susceptible genotypes 2012 - 2013 & 2013 – 2014

*Geno types	Year 2012 SOD EU/g Fresh Weight of sample	Year 2013 AUDPC	SOD EU/g Fresh Weight of sample	AUDPC	Mean (2012 & 2013) SOD EU/g	AUDPC Fresh Weight of sample
1^R	0.7180	456.23	0.7596	442.90	0.7388	449.56
2^R	0.8231	525.86	0.8540	572.53	0.8385	549.19
3^R	0.8088	467.84	0.7936	464.51	0.8012	466.17
4^R	0.7217	356.70	0.8936	353.40	0.8076	355.04
5^R	0.9247	417.28	0.7208	367.28	0.8227	392.28
6^R	0.7675	473.58	0.9044	496.91	0.8359	485.24
7^R	0.7822	271.30	0.8221	254.63	0.8021	262.96
8^R	0.7730	344.88	0.8550	334.88	0.8140	339.87
9^R	0.7300	452.90	0.7588	442.90	0.7444	447.90
10^R	0.7593	278.86	0.8788	388.89	0.8190	333.87
11^R	0.7451	398.09	0.9507	378.09	0.8479	388.08
12^R	0.8326	417.96	0.8535	421.30	0.8430	419.62
13^R	0.8587	423.83	0.8319	410.49	0.8453	417.16
14^R	0.7553	510.05	0.8390	507.72	0.7971	508.88
15^R	0.7220	460.93	0.9496	550.93	0.8358	505.92
16^R	0.7712	211.67	0.8839	291.67	0.8275	251.66
17^R	1.0736	452.78	0.7848	486.11	0.9292	469.44
18^R	0.9356	398.51	0.9755	464.51	0.9555	431.50
19^R	0.8486	502.78	0.8056	486.11	0.8271	494.44
20^R	0.7264	340.43	0.8215	307.10	0.7739	323.76
21^R	0.7284	449.32	0.8429	529.32	0.7856	489.31
22^R	0.9745	340.62	0.7151	367.28	0.8448	353.95
23^R	0.8110	403.70	0.7110	453.70	0.7609	428.70
24^R	0.7798	478.15	0.7982	648.15	0.7890	563.14
25^R	0.7518	388.89	0.7581	388.89	0.7549	388.88
26^R	0.8896	395.43	0.7941	432.10	0.8418	413.76
27^R	0.7286	417.16	0.7877	410.49	0.7581	413.82
28^R	0.8118	451.85	0.6897	518.52	0.7507	485.18
29^R	0.8067	387.96	0.7977	421.30	0.8021	404.62
30^R	0.8166	280.19	0.7317	226.85	0.7742	253.51
31^R	0.7737	406.91	0.9553	496.91	0.8645	451.91
32^R	0.8661	460.66	0.8236	547.33	0.8448	503.99
33^S	0.5175	1022.15	0.4943	1254.63	0.5059	1138.38
34^S	0.5531	704.84	0.4747	712.96	0.5139	708.90
LSD (0.05) For SOD 0.0955 for AUDPC 56.33						
Mean	0.7932	439.01	0.80	465.94		

*Pedigree of genotypes is given in table 1, Superscript R = resistant lines and S = susceptible lines

Table 4: Correlation among the SOD, Test weight, Yield and AUDPC

	SOD	Test weight	Yields
SOD	1		
Test weight	0.4870(0.0035)	1	
Yields	0.4246(0.0123)	0.6905(<0.0001)	1
AUDPC	-0.5134(<.0019)	-0.2547(0.1460)	-0.1272(0.4734)

Data given in parenthesis is probability; Table 4, shows the correlation among 4 variables viz.: SOD, Test weight, Yield, and AUDPC of 32 resistant and 2 susceptible genotypes. SOD shows the positive and significant correlation with test weight (r = 0.487) and yield (r = 0.424). A negative and significant correlation (r = 0.513) of SOD was recorded with AUDPC. The yield and test weight are also negatively correlated with AUDPC.

Table 5: Mean of AUDPC, SOD, Test weight and Yields in the year 2013

Genotypes	AUDPC	SOD EU/g fresh weight	Test weight (g)	Yield of 20 tillers (g)
1^R	442.90	0.7596	34.00	40.67
2^R	572.53	0.8540	36.00	35.00
3^R	464.51	0.7936	35.00	40.00
4^R	353.40	0.8936	34.67	47.00
5^R	367.28	0.7208	34.67	38.33
6^R	496.91	0.9044	35.00	41.33
7^R	254.63	0.8221	34.00	36.33
8^R	334.88	0.8550	35.00	42.33
9^R	442.90	0.7588	33.67	27.33
10^R	388.89	0.8788	28.67	27.67
11^R	378.09	0.9507	33.33	34.67
12^R	421.30	0.8535	33.00	32.67
13^R	410.49	0.8319	30.67	42.67
14^R	507.72	0.8390	35.33	42.33
15^R	550.93	0.9496	35.67	40.67
16^R	291.67	0.8839	36.33	36.00
17^R	486.11	0.7848	30.67	30.33
18^R	464.51	0.9755	28.00	34.00
19^R	486.11	0.8056	32.33	37.00
20^R	307.10	0.8215	30.67	32.67
21^R	529.32	0.8429	33.67	38.67
22^R	367.28	0.7151	31.33	38.67
23^R	453.70	0.7110	27.33	25.33
24^R	648.15	0.7982	31.00	36.00
25^R	388.89	0.7115	32.33	34.00
26^R	432.10	0.7229	30.67	30.67
27^R	410.49	0.7454	32.67	36.33
28^R	518.52	0.6897	30.33	31.33
29^R	421.30	0.7977	29.00	34.33
30^R	226.85	0.7317	32.33	31.33
31^R	496.91	0.9553	32.67	35.00
32^R	547.33	0.8236	37.33	41.67
33^S	1254.63	0.4943	29.67	33.33
34^S	712.96	0.4747	24.00	24.33

Superscript R = resistant lines and S = susceptible lines; Table 5, shows the mean of AUDPC, SOD, Test weight (g), and Yield of 20 tillers (g) and in resistance and susceptible wheat genotypes. The mean AUDPC ranged 226.85 to 1254.63, SOD 0.474 to 0.975, test weight 24 to 37.33 and yield 24 to 47g among the genotypes.

Table 6: ANOVA of SOD at different time interval

Source	DF	Mean Square	F Value	Pr > F
Time	2	0.7336	30.73	<.0001
Genotype	33	0.1151	4.82	<.0001
Time*Genotype	66	0.0669	2.81	<.0001

Analysis of variance (Table 6) shows significant e (< 0.0001) effect of time interval on the SOD activity. The wheat genotypes were also significant for SOD activity at different time interval. Interaction between time and genotype was also significant.

also had low AUDPC. Both the susceptible genotypes Sonalika and Ciano T79 showed low SOD and higher AUDPC in both the years.

Table 7, shows the mean values of SOD EU/g Fresh weight of wheat leaf samples at three different time intervals *i.e.* 12 hrs, 24 hrs, and 48 hrs after the inoculation of pathogen. SOD mean over the time shows significant difference in its activity. Mean SOD activity (0.876 EU/g fresh weights) of genotypes was the highest at 48 hrs of inoculation of pathogen followed by 24 hrs (0.809 EU/g fresh weights) activity and minimum at 12 hrs (0.707 EU/g fresh weights) activities. In general result shows that the enzyme activity increases with increasing time duration. SOD activity was more in resistance genotypes than the susceptiblegenotypes.The resistance line shows the highest (0.975 EU/g fresh weight) SOD activities and susceptible lines show the low (0.474 EU/g fresh weight) activities. The interaction between genotype and time was significant. Variable expression was also recorded at different time elapsed some genotypes showed maximum SOD activity at 12 and some at 24 hrs and most of them at 48 hrs. This may be occurred due to the other unknown stresses before the pathogen (B. sorokiniana) inoculation (biotic and abiotic stresses) in the plants.In this study, two novel CuZnSOD genes in wheat, referred to TaSOD1.1 and TaSOD1.2, which had high similarities with SOD1.1 and SOD1.2, respectively, were identified from the international bioinformatics website of TIGR (www.tigr.org) based on the gene indices tool using CuZnSOD as the keywords. Based on the sequences of TaSOD1.1 and TaSOD1.2, the two novel wheat CuZnSOD genes were cloned and characterized. From this phylogeny of SOD different varieties of wheat shows the relatedness and variation in the

Table 7: Effect of time intervals on SOD EU/g fresh weight of wheat samples in resistance and susceptible lines

Genotypes	12 hrs	24 hrs	48 hrs	Genotypes(mean)
1^R	0.6685	0.7781	0.8324	0.7597
2^R	0.7415	0.8658	0.9549	0.8541
3^R	0.6773	0.7193	0.9844	0.7937
4^R	0.8561	0.7851	1.0398	0.8937
5^R	0.6512	0.7249	0.7863	0.7208
6^R	0.8949	0.9158	0.9027	0.9045
7^R	0.6810	0.7548	1.0305	0.8221
8^R	0.8965	0.8824	0.8863	0.8851
9^R	0.7499	0.7215	0.8053	0.7589
10^R	0.9326	0.9154	0.7885	0.8788
11^R	0.8184	0.9097	1.1241	0.9507
12^R	0.8105	0.8705	0.8798	0.8536
13^R	0.7572	0.9436	0.9311	0.8773
14^R	0.6987	0.8387	0.9795	0.8390
15^R	1.0050	0.7654	1.0785	0.9496
16^R	0.9403	0.9541	0.9576	0.9540
17^R	0.8439	0.7873	0.7234	0.7849
18^R	1.1289	0.8024	0.9955	0.9756
19^R	0.7448	0.9762	0.7961	0.8057
20^R	0.8099	0.8714	0.8833	0.8815
21^R	0.7944	0.9387	0.7957	0.8429
22^R	0.4470	0.8494	0.8490	0.7152
23^R	0.3559	0.8633	0.9139	0.7110
24^R	0.7629	0.7776	0.8543	0.7983
25^R	0.3717	0.8708	0.9465	0.7116
26^R	0.3742	0.9240	0.9207	0.7229
27^R	0.3448	0.7425	0.9528	0.6788
28^R	0.3679	0.8193	0.8820	0.6898
29^R	0.8431	0.7520	0.7981	0.7977
30^R	0.6980	0.7842	0.8131	0.7318
31^R	1.2503	0.8297	0.7862	0.9554
32^R	0.8265	0.8255	0.8189	0.8236
33^S	0.3419	0.5258	0.6152	0.4943
34^S	0.2899	0.4485	0.6860	0.4748
LSD (0.05) Genotype	0.1436 Time	0.0427		
Time(mean)	0.7078^C	0.8098^B	0.8762^A	

Superscript R = resistant lines and S = susceptible lines

SOD sequence

DISCUSSION

The oxidative burst a rapid transient product of huge amounts of reactive oxygen species (ROS). This is one of the earliest observable aspects of a plants defence strategy. ROS include a variety of short and longlived molecules such as superoxide radicals (O_2), hydroxyl radicals (OH), and hydrogen peroxide (H_2O_2) (Apel and Hirt, 2004). Accumulation of ROS is toxic to plant tissue and may results in DNA, RNA, Lipid and membrane damage and loss of other cellular activities. Under normal conditions, ROS are produced as byproducts of a variety of metabolic pathways (Respiration and photosynthesis etc.) and are detoxified by different antioxidant enzymes present in plants as an effective cellular scavenging mechanism (Apel and Hirt, 2004). However, when challenged by stresses, plants usually produce higher amounts of SODs which act as a part of the innate immunity of plants. In addition to being a toxicant, ROS specially hydrogen peroxide can serve as a secondary messenger in signaling pathways that ultimately alter gene expression including those of various oxidative enzymes that detoxify ROS (Apel and Hirt, 2004). Resistance mechanisms in plants are frequently associated with upregulation or downregulation of antioxidant enzymes such as SOD etc. (Chaman *et al*., 2001). All these enzymes show the first lines defense in the plants specially SOD is highly correlated with resistant plants. In this present study, rapid increased activity of SOD w2as observed in resistant than susceptible genotypes when inoculated with *Bipolaris sorokiniana*. Similar observation of increase in SOD reported by Sheng *et al*. (2008). They recorded higher defense enzyme SOD in resistant inbreed lines than susceptible against *Cercospora zeaemaydis*. The accumulation of ROS, such as H_2O_2 is very important in the plant's responses to pathogen infection ability. In 32 resistant cultivars, high SOD content observed as compare to 2 susceptible checks. This finding further supports that the resistant cultivar was more successful in detoxifying superoxide (O_2) into less harmful substances like hydrogen peroxide (H_2O_2). Conversely, the activity of SOD in susceptible cultivar was observed low as compare to the resistance genotypes. From this it can be deduced that the susceptible cultivar was less effective in detoxifying superoxide accumulation due to the low antioxidant enzymes like SOD, thus potentially experiencing greater damage perhaps due to the toxicity of

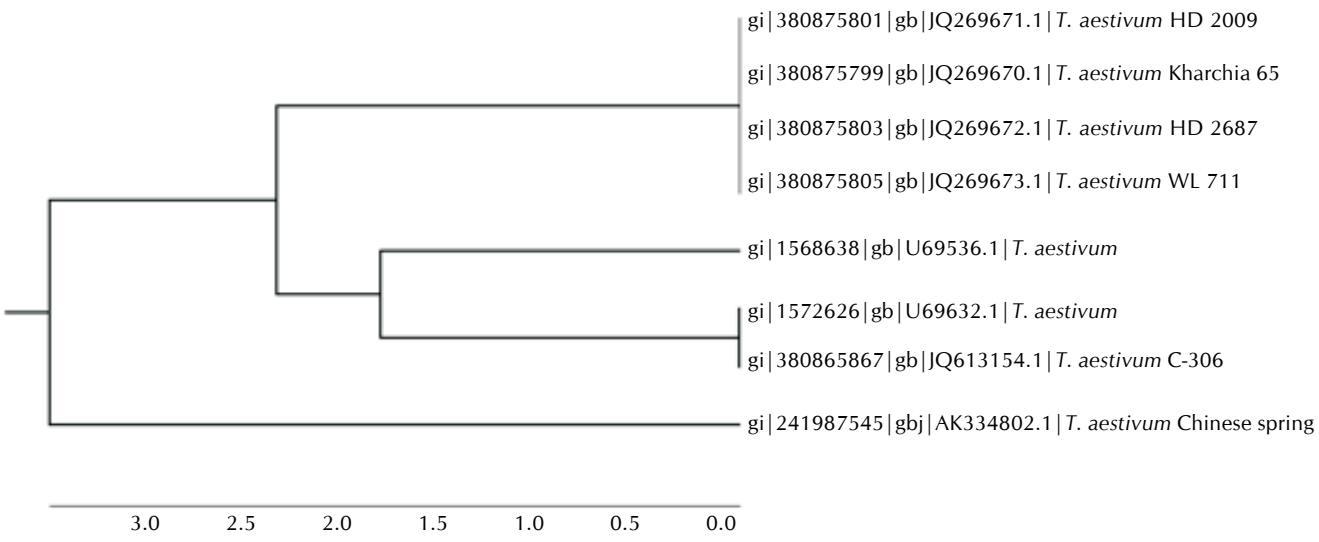

Figure 1:

the reactive oxygen species this can be assessed as a MDA contain in the genotypes. Thus production of ROS increases in both resistant and susceptible genotypes but in case of resistant genotypes the SOD optimize the ROS. However in susceptible genotype the low amount of SOD was not able to control the ROS up to the limit that leads to DNA and, membrane damage of plants. The control of steadystate ROS levels by SOD is an important protective mechanism against cellular oxidative damage, since O_2 Acts as a precursor of more cytotoxic or highly reactive ROS (Mittler *et al*., 2002). Heritability is genetic component of variation that is important since this component is transmitted to the next generations. Estimates of heritability serve as a useful guide to the breeder for the selection of traits. The heritability of SOD for two year was 69.82%.These results concluded that the SOD is a good criterion for selection because it shows the higher heritability. Since, increase of SOD indicator of resistant and susceptibile reaction in the plants for general stresses like biotic and abiotic in the plants due to the different ROS. Three different classes of SOD activity have been identified by the active sitemetal cofactors (Fe, Mn, or Cu and Zn). Typically, MnSOD is mitochondrial, FeSOD is plastidic, and CuZnSOD may be plastidic or cytosolic (Bowler *et al*., 1992). There are also reports on the peroxisomal and extracellular SODs (Bueno *et al*., 1995). The SOD enzymes could be encoded by a small gene family. There were, five wheat SODs genes, including two CuZnSODs (SOD1.1 and SOD1.2) (Wu *et al*., 1996) and two MnSODs (SOD3.1 and SOD3.2) (Wu et al., 1997)~and another one with Gene Bank accession number AF092524) have been isolated.

REFERENCES

Apel, K., Hirt, H. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction, *Annu. Rev. Plant Biol*. **55:** 373-99.

Bowler, C., Montague, M. V. and Inze, D. 1992. Superoxide dismutases and stress tolerance. Annu. Rev. Plant Physiol. *Plant mol. Biol*. **43:** 83-116.

Bueno, P., Varela, J., Gimenez, G. G. and Del, R. L. A. 1995. Peroxisomal copper, zinc superoxide dismutases, characterization of the isoenzyme from watermelon cotyledons. *Plant Physiol*. **108:** 1151-1160.

Chand, R. and Joshi, A . K. 2004. Foliar blight: solved and unsolved problem. In Joshi, A. K., Chand, R., Arun, B., Singh, G., (ed.) A compendium of lectures on wheat improvement in eastern and warmer regions of India: conventional and non conventional approaches. Banaras Hindu University, Varanasi, 221005, India. p. 5869.

Chaman, M., Argandoña, V. H., Cardemil, L., Muñoz, O., Zúñiga, G. E., Corcuera, L. J. 2001. Ethylene production and peroxidase activity in aphidinfested barley, *J. Chemical Ecology*. **27(1):** 53-68.

Curtis, B. C., Rajaram, S., MacPherson, G. H. (ed.), 2002. Bread wheat: improvement and production. *Food and Agriculture Organization, Rome, Italy,* pp. 157-174

Davidson, J. E., Whyte, B., Bissinger, P. H. and Schiestl, R. H. 1996. Oxidative stress is involved in heat induced cell death in Saccharomyces cerevisiae, *Proc. nat. Acad. Sci.* USA. **93:** 5116-5121.

Dhindsa, R. S., Dhindsa, P. P. and Thorpe, T. A. 1981. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot*. **32:** 93-101.

Duveiller, E. and Gilchrist, L. 1994. Productions constraints due to Bipolaris sorokiniana in wheat: current situation and future prospects. In Saunders, D., Hettel, G., (ed.) Proc. wheat in the warmer areas, rice/wheat systems, Nashipur, Dinajpur, Bangladesh, 13-16 Feb. 1993, Mexico, DF, CIMMYT/UNDP. pp. 343-352.

Dubin, H. J. and van Ginkel, M. 1991. The status of wheat diseases and disease research in the warmer areas. In Saunders D.A., Hettel G., (ed.) proc. wheat for non traditional warm areas. *Proc. Int. Conf.*, Foz do Iguazu, Brazil, 29 Jul.3 Aug. 1990, Mexico, D.F., UNDP/ CIMMYT. pp. 125-145.

Duveiller, E., Kandel, Y. R., Sharma, R. C., Shrestha, S. M. 2005. Epidemology of foliar blights (spot blotch and tan spot) of wheat in the plains bordering the Himalayas. *Phytopathology*. **95:** 248.

Imlay, J. A. and Linn, S. 1988. DNA damage and oxygen radical toxicity, *Science*. **240:** 1302-1309.

Kiesling, R. L. 1985. The diseases of barley. In: Rasmusso D.C. (ed.), Barley, *American Society of Agronomy*. pp. 269-312.

Kliebenstein, D. J., Monde, R. A. and Last, R. L. 1998. Superoxide dismutase in Arabidopsis an eclectic enzyme family with disparate regulation and protein localization. *Plant Physiol*. **118:** 637-650.

Kumar, J., Schafer, P. and Huckelhoven, R. 2002. Bipolaris sorokiniana a cereal pathogen of global concern: cytological and molecular approaches towards better control. *Mol Plant Pathol*. **3:** 185-195.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci*. **7:** 405-410.

Nutteson, M. Y. 1955. Wheatclimatic relations and the use of phenology in ascertaining the thermal and photo thermal requirements of wheat. *American Institute of Crop Ecology*. Washington D. C.

Polle, A. M. and Rennenberg, H. 1993. Seasonal variations of glutathione reductase and glutathione in leaves and leaf buds of beech trees {Fagus sylvatica L.), - *Phyton* (Horn, Austria). **32(3):** (99)(102).

Ranieri, A., Castagna, A., Amoroso, S., Nali, Lorenzini, G. and Soldatini, G. F. 1998. Ascorbate levels and ascorbate peroxidise activation in two differently sensitive poplar clones as a result of ozone fumigation. - In: De Kok, L J, Stulen, I. (ed.): Responses of plant metabolism to air pollution and global change. *Backhuys Publishers,* Leiden. pp. 435-438.

Rashid, A. Q. M. B. and Neergaard, E. 1996. Histopathological behaviour of seedborne Bipolaris sorokiniana in wheat seedling ~ *Bangladesh J. Plant pathology*. **1&2:** 58.

Roelfs, A. P., Espino H. J. and Marshall, D. 1992. Barley stripe rust in Texas. *Plant Dis*. **76:** 538.

Saari, E. E., Prescot, J. M. 1975. A scale for appraising the foliar intensity of wheat disease, *Plant Dis*. *Rep*. **59:** 377-380.

Sairam, R. K., Rao, K. V. and Srivastava, G. C. 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration, *Plant Sci*. **163:** 1037-1046.

Sairam, R. K., Srivastava, G. C., Agarwal, S. and Meena, R. C. 2005. Differences in antioxidant activity inresponse to salinity stress in tolerant and susceptible wheat genotypes. *Biol*. *Plant*. **49:** 85-91.

SAS Institute 2010. SAS user's guide: statistics. Version 9.1, Cary, North Carolina, USA.

Sheng, X. C., Qin, X. S., Qiang, H. H. and Chen, G. Y. J. 2008. Influence of Cercospora zeaemaydis toxin on defence enzyme activities and its function in the induced resistance of corn, *J. Northwest* A & F *University*. *Natural Science edition*. **36:** 175-180.

Srivastava, O. P., Luthra, J. K. and Narula, P. N. 1971. Inheritance of seedling resistance to leaf blight of wheat, *Indian J. Genet. & Plant Breeding*. **45:** 941-943.

Steffenson, B. J., Hayes, P. M. and Kleinhofs, A. 1997. Genetics of seedling and adult plant resistance to net blotch (Pyrenophora teres f. sp. teres) and spot blotch (Cochliobolus sativus) in barley, *Theor*. *Appl*. *Genet*. **92:** 552-558.

Wu, G., Robertson, A., Wilen, R. and Gusta, L. 1997. Plant electronic genes register PGR 97024, isolation and characterization of two cDNAs (Accession No. U72212 (SOD3.1) and U73172 (SOD3.2)) encoding mitochondrial manganese superoxide dismutases in wheat, *Plant Physiol*. **113:** 660-664.

Wu, G., Wilen, R., Robertson, A. and Gusta, L. 1996. The electronic plant genes register PGR 96126. Molecular cloning of two wheat (Triticum aestivum) genes (Accession No. U69536 (SOD1.1) and U69632 (SOD1.2)) encoding chloroplast CuZn superoxide dismutases. *Plant Physiol*. **112:** 17-36.

Zadoks, J. C., Chang, T. T. and Konjak, C. F. 1974. A decimal code for the growth stages of cereals, *Weed Res*.