

Genetic Evaluation of Spring Wheat (*Triticum aestivum* L.) Recombinant Inbred Lines for Spot Blotch (*Bipolaris Sorokiniana*) Resistance and Yield Components under Natural Conditions for South Asia

N. Meena¹, V. K. Mishra¹, D. K. Baranwal^{1,2*}, A. K. Singh¹, V. P. Rai¹, R. Prasad¹, B. Arun¹, and R. Chand³

ABSTRACT

The objectives of the present study were to evaluate spring wheat recombinant inbred lines (RILs) of diverse origin by estimating genetic parameters viz., variability, character association, cluster analysis, and principal component analysis (PCA) for spot blotch resistance and yield components at BHU Agricultural Research Farm during 2010-2011. Grain yield per plot was significantly and positively associated with biomass, 1,000-grain weight, harvest index, chlorophyll content, and grains per spike at genotypic level. The line 65 exhibited lowest mean of AUDPC value (632) indicating its potential as resistant parent. Cluster analysis grouped all the 324 spring wheat lines into 19 clusters using Ward's method. Extreme divergence was observed among clusters. By using D^2 -statistics, the highest inter cluster distance (584.72) was found between Clusters VIII and XIX. Cluster VIII recorded highest mean values for chlorophyll content, peduncle length, biomass, grains per spike, 1000-grain weight and grain yield. The major contributing trait towards genetic divergence was found to be AUDPC (60.36%). First 5 principal components (PC1, PC2, PC3, PC4 and PC5) accounted for proportionate values of 20.66, 17.96, 15.07, 8.28, and 7.38%, respectively, contributing 69.35% of the total variability. The second PCs had high positive PC value for plant height, biomass, and 1,000-grain weight. The breeding objectives of the present experiment was to identify genetically diverse wheat spot blotch resistant RILs for developing high yielding spot blotch resistant cultivars especially adopted to south Asia in future breeding programs.

Keywords: AUDPC, Cluster analysis, D2 analysis, PCA.

INTRODUCTION

Spot blotch disease caused by *Bipolaris sorokiniana* (sacc.) shoem syn. *Drechslera sorokiniana* (Sacc.) Subrm and Jain (syn. *Helminthosporium sativum*, teleomorph *Cochliobolus sativus*), is the most prominent disease of warmer, humid, and late-sown wheat growing regions of South Asia affecting livelihood of millions of farmers

(Saari, 1998; Joshi *et al.*, 2002). At present, spot blotch resistant potential in high yielding wheat varieties is poor and needs rigorous investigation, especially for warmer humid regions of South Asia (Sharma *et al.*, 2004; Joshi *et al.*, 2007). Several factors viz., time of sowing, sites, and moisture have adverse impact on crop yield. Severe infestation of spot blotch results in substantial yield losses ranging between 20-

¹Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi -221005, India.

*Corresponding author; e-mail: dkbbhu@gmail.com

² Department of Plant Breeding and Genetics, Bihar Agricultural University, Sabour-813210, India.

³ Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, India.



100% in South Asia by blighting of leaves and premature senescence (Duveiller and Gilchrist, 1994).

Continuous breeding results in narrow genetic diversity of the elite wheat germplasm pool and leads to problems relevant to biotic stresses, abiotic stresses, as well as adaptation (Zhang *et al.*, 2005). Maximum genetic dissimilarity among parents is essential to exploit transgressive segregation (Joshi *et al.*, 2004). Selection of genetically diverse parents upon hybridization results in higher heterosis in progenies. Therefore, there is an urgent need to exploit the existing genetic variability in wheat for evolving high yielding varieties that have wide adoptability and are highly productive under a changing climatic scenario (Baranwal *et al.*, 2012). Cluster and PC analyses are principal genetic diversity analysis tools having relative differences with each other. The cluster analysis is a robust approach for assessing family relationships (Mellingers, 1972). The objective of the present experiment was to identify genetically diverse spot blotch resistant wheat RILs for developing high yielding spot blotch resistant cultivars for South Asia.

MATERIALS AND METHODS

The present experiment was undertaken at Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University (BHU), Varanasi, India, during Rabi season of 2010-11. A collection of 324 lines including 307 RILs (F8 generation) of 21 diverse crosses, seventeen distinct parents including one check i.e., Sonalika, and a highly susceptible cultivar to spot blotch disease was evaluated. Varanasi region is considered as hot spot for screening and evaluation against spot blotch disease. The genotypic set was developed by using promising spring wheat parental lines introduced from CIMMYT, Mexico and South Asia regional office, CIMMYT, Kathmandu (Nepal) including Chinese material, and other

germplasm collections. The experimental materials were sown in randomized block design with 3 replications (Table 1). The RILs were developed as per the methods described by Singh and Rajaram (1992) and Joshi *et al.* (2004). The experimental site is located in South- Eastern part of the Varanasi city at 25° 26' North latitude and 82° 99' East longitude at an elevation of 75.5 m above the mean sea level. Date of sowing was 31st of December 2010 and artificial inoculation was conducted during 23 February (4:30 pm). Growth observations were recorded for 15 yield components through random sampling method. Data on five plants of each line was averaged and mean data was used for statistical analysis. Agronomic practices recommended for irrigated and normal fertile soil (120 kg N; 60 kg P₂O₅ and 40 kg K₂O ha⁻¹) were followed to raise a good crop.

Area under Disease Progress Curve (AUDPC)

Spot blotch disease was recorded at different growth stages (Zadoks *et al.*, 1974). Disease severity (%) was recorded at different stages to calculate AUDPC (Van der Plank, 1963; Roelfs *et al.* 1992) using the following formula:

$$AUDPC = \sum_{i=1}^n \left[\left\{ \frac{Y_i + Y_{(i+1)}}{2} \right\} (t_{i+1} - t_i) \right]$$

Where, Y_i is the disease level at time t_i and $t_{(i+1)} - t_i$ the time (days) between two disease scores and n is the number of dates on which spot blotch was recorded.

Statistical Analysis

Character association was calculated following Robinson *et al.* (1951). Genetic divergence among different lines was assessed based on the estimated *inter-se* genetic distances among the lines using D^2 -statistics of Mahalanobis (1928), which is one of the most effective tools to measure

Table 1. Collection of 324 spring wheat lines representing source of spot blotch resistance.

Line No.	Lines / Parents	Crosses/Details
1 – 9	Spot blotch RILs – 1 (9)	Yangmai-6×Monసు kunjer
10 – 25	Spot blotch RILs – 2 (16)	Monalds×Bonly
26 – 52	Spot blotch RILs – 3 (27)	Yangmai-6×Monalds
53 – 69	Spot blotch RILs – 4 (17)	Monalds×Songhi
70 – 86	Spot blotch RILs – 5 (17)	Songhi-4×IA 814-467
87 – 103	Spot blotch RILs – 6 (17)	Chirya 7×Tink
104 – 131	Spot blotch RILs – 7 (28)	Yangmai-6×Chirya-7
132 – 153	Spot blotch RILs – 8 (22)	Yangmai-6×Tink
154 – 168	Spot blotch RILs – 9 (15)	Songhi-4×BR-8-471
169 – 178	Spot blotch RILs – 10 (10)	Songhi-4×Chirya-7
179 – 198	Spot blotch RILs – 11 (20)	Monalds×Yangmai-6
199 – 210	Spot blotch RILs – 12 (12)	Yangmai-6×Ning-8027
211 – 224	Spot blotch RILs – 13 (14)	Songhi-4×Bonly
225 – 240	Spot blotch RILs – 14 (16)	IA-814-867×Tink
241 – 255	Spot blotch RILs – 15 (15)	Suzo-8×Monalds
256 – 275	Spot blotch RILs – 16 (20)	Monalds×Chirya-7
276 – 284	Spot blotch RILs – 17 (9)	Monalds×Ning-8127
285 – 304	Spot blotch RILs – 18 (20)	Songhi-4×Ning-8119
305	Spot blotch RILs – 19 (1)	Chirya-7×Longmai-10
306	Spot blotch RILs – 20 (1)	Songhi-4×Monసు Kunjer
307	Spot blotch RILs – 21 (1)	Monald×Ning- 8119
308	Parent - 1	Sonalika (Highly susceptible variety)
309	Parent - 2	HUW-234
310	Parent - 3	HUW-468
311	Parent - 4	HUW-510
312	Parent - 5	Sonalika
313	Parent - 6	IA-814-877
314	Parent - 7	Chirya-7
315	Parent - 8	Ning-8119
316	Parent - 9	Monalds
317	Parent - 10	Monసు Kunjer
318	Parent - 11	Tink
319	Parent - 12	Bonaly
320	Parent - 13	Songhi-4
321	Parent - 14	Suzo-8
322	Parent - 15	songhi-4
323	Parent - 16	Yangmai-6
324	Parent - 17	Lok-1

the genetic distance between lines as measured by allelic frequencies at a sample of loci. After arranging the D^2 -values of all combinations of one genotype with the others in ascending order of magnitudes, the lines were grouped into a number of clusters by Ward's method described by Rao (1952). The inter- and intra-cluster distances were calculated and their relationships were diagrammatically represented. The Statistical Analyses Software (SAS) and STATISTICA ver.10 were utilized for statistical analysis.

RESULTS AND DISCUSSION

In the present experiment, 324 spring wheat lines of diverse origin were analyzed for genetic parameters viz., character association, cluster analysis, and principal component analysis (PCA) for spot blotch resistant and yield components. The best ten lines based on the mean performance of few promising traits in desirable direction are represented in Table 2. The lines 239 and 308 showed lesser days to 50% flowering

**Table 2.** Promising ten lines for each of the promising traits under study.

Traits	1	2	3	4	5	6	7	8	9	10
DF (Early) ^a	Line 239 (60.33) ^b	Line 308 (60.33)	Line 240 (61.33)	Line 110 (62.00)	Line 243 (62.33)	Line 287 (62.33)	Line 215 (63.00)	Line 237 (63.00)	Line 282 (63.33)	Line 288 (63.33)
DPM1 (Early) ^b	Line 300 (93.33)	Line 302 (93.33)	Line 110 (94.00)	Line 261 (94.33)	Line 264 (94.33)	Line 270 (94.33)	Line 276 (94.33)	Line 280 (94.33)	Line 299 (94.33)	Line 111 (95.00)
AUDPC (Low) ^c	Line 65 (632.00)	Line 1 (644.32)	Line 41 (682.61)	Line 44 (744.34)	Line 91 (744.34)	Line 97 (744.34)	Line 98 (761.63)	Line 108 (762.86)	Line 92 (765.95)	Line 107 (778.91)
PH (cm) (Dwarf) ^d	Line 173 (48.33)	Line 171 (51.33)	Line 174 (55.83)	Line 172 (56.33)	Line 128 (58.33)	Line 176 (61.83)	Line 177 (61.83)	Line 175 (62.33)	Line 224 (63.33)	Line 109 (64.33)
BM (g) (High) ^e	Line 54 (232.50)	Line 107 (232.50)	Line 96 (217.50)	Line 46 (207.50)	Line 97 (207.50)	Line 108 (207.50)	Line 188 (203.33)	Line 9 (202.50)	Line 65 (192.50)	Line 249 (189.17)
GY (g) (High) ^f	Line 108 (69.60)	Line 41 (69.10)	Line 304 (66.17)	Line 107 (66.00)	Line 43 (65.60)	Line 258 (64.87)	Line 46 (64.60)	Line 78 (64.50)	Line 263 (63.97)	Line 54 (63.90)
TGW (g) (High) ^g	Line 12 (41.34)	Line 54 (40.74)	Line 34 (38.94)	Line 46 (38.94)	Line 296 (37.68)	Line 25 (37.54)	Line 175 (37.38)	Line 13 (36.94)	Line 207 (36.18)	Line 22 (36.14)
HI (%) (High) ^h	Line 78 (55.88)	Line 76 (54.08)	Line 41 (48.18)	Line 81 (47.68)	Line 43 (45.38)	Line 26 (45.18)	Line 85 (45.18)	Line 146 (42.65)	Line 47 (42.18)	Line 170 (41.95)

^a Days to 50% Flowering (Days); ^b Days to Physiological Maturity (Days); ^c Area Under Disease Progress Curve; ^d Plant Height (cm); ^e BioMass (g); ^f Grain Yield (g); ^g Thousand Grain Weight (g), and ^h Harvest Index (%). Bracketed values indicate the mean performance of the corresponding lines and bold line numbers are common for more than one trait.

(60.33 days) indicating early maturity. The line 65 exhibited lowest *AUDPC* value (632) indicating a resistant parent in consonance with Sharma *et al.* (1997). Low yield of few lines was indicating high susceptibility to spot blotch disease (Phadnawis *et al.*, 2002). Highest 1,000-grain weight was observed in the line 12 (41.34 g). The highest harvest index was observed in line 78 (55.88%) (Table 2). The observations suggested vast differences among the RILs in terms of spot blotch resistant and yield components.

Grain yield per plot was significantly and positively associated with biomass, 1000-grain weight, harvest index, chlorophyll content, and grains per spike at genotypic level (Table 3) as reported by Khodarahmpour *et al.* (2011) and Olfati *et al.* (2010). It suggests that the characters should be included in phenotyping for genetic improvement for wheat genotypes. Negatively significant correlation was observed between yield and *AUDPC*, indicating that spot blotch was the major problem for wheat yield at phenotypic level (Table 5) as reported by Gilchrist and Pfeiffer (1991), but non-significant (0.23) at

genotypic level, indicating major role of environment for the disease incidence. *AUDPC* showed negative and significant association with biomass, sheath length, days to physical maturity, chlorophyll content, days to physiological maturity, and days to 50% flowering at genotypic level (Table 3).

Genetic Divergence Analysis

All the 324 wheat lines were grouped into 19 clusters through Ward's method (Table 4; Figure 1). By using D^2 -statistics of Mahalanobis (1928), the D values (cluster distance) of this genotypic collection (square of D value represents D^2 value) are represented in Table 5 (Figure 2). In this method, highest intra cluster distance was found for Cluster VIII (53.07) followed by XIV. Similarly, lowest intra cluster distances have been obtained for Cluster I (32.16). The highest inter cluster distance (584.72) through this method has been found between Clusters VIII and XIX (Table 5).

Table 3. Character association between yield and its components for the 324 spring wheat lines.^a

Characters	CL	DPM1	DPM2	AUDPC	SL	PL	EL	PH	BM	GY	TGW	GPS	HI	SCM
DF	r_p 0.22*	0.67**	0.72**	-0.47**	0.13*	-0.23*	-0.04	-0.04	0.00	-0.30**	-0.30**	0.05	-0.38**	0.08
	r_g 0.19*	0.79**	0.62**	-0.35**	0.68**	-0.12*	0.09	0.14*	0.24*	-0.35**	-0.46**	0.97**	-0.67**	0.34**
CL	r_p 0.20*	0.17*	-0.36**	0.10	-0.08	0.01	-0.04	0.26**	0.18*	0.20*	0.20*	0.17*	-0.05	0.06
	r_g 0.03	0.21*	-0.42**	0.43**	0.12*	0.29**	0.20*	0.54**	0.56**	0.23*	0.23*	0.55**	-0.36**	-0.16*
DPM1	r_p 0.90**	-0.34**	0.09	-0.13*	-0.01	0.32**	0.31**	-0.09	0.39**	-0.05	-0.14*	0.92**	-0.16*	0.16*
	r_g 0.66**	-0.37**	0.92**	0.28**	0.10	0.31**	0.01	-0.11*	-0.11*	-0.14*	0.14*	0.92**	-0.21*	0.27**
DPM2	r_p -0.33**	0.10	-0.12*	-0.02	0.32**	-0.09	0.34**	-0.09	0.34**	-0.10	-0.07	-0.07	-0.22*	0.17*
	r_g -0.50**	0.92**	0.39**	0.53**	0.44**	0.16	-0.27**	0.16	-0.27**	-0.25*	-0.63**	-0.63**	-0.51**	0.11*
AUDPC	r_p -0.17*	-0.16*	-0.04	-0.01	-0.32**	-0.45**	-0.11*	-0.10	-0.32**	-0.15*	-0.15*	-0.11*	-0.10	-0.09
	r_g -0.77**	0.53**	-0.22*	-0.06	-0.82**	-0.23*	0.02	-0.47**	-0.82**	0.02	-0.47**	0.78**	0.78**	-0.75**
SL	r_p 0.54**	0.31**	0.56**	0.26**	0.08	0.06	0.06	0.15*	0.26**	0.08	0.06	0.15*	-0.17*	0.10
	r_g -0.60**	-0.19*	0.10	0.38**	-0.50**	0.10	-0.57**	-0.91**	0.38**	-0.50**	0.10	-0.57**	-0.91**	0.00
PL	r_p 0.26**	0.77**	0.11*	0.00	0.04	0.04	0.04	0.00	0.11*	0.00	0.04	0.00	-0.07	0.10
	r_g -0.22*	0.78**	0.38**	-0.42**	0.02	-0.83**	-0.42**	0.02	0.38**	-0.42**	0.02	-0.83**	-0.72**	0.29**
EL	r_p 0.34**	0.13*	0.07	0.07	0.09	0.09	0.09	0.07	0.13*	0.07	0.09	-0.06	-0.05	0.14*
	r_g 0.22*	-0.01	-0.06	-0.06	-0.01	-0.06	0.11*	-0.06	-0.01	-0.06	0.11*	0.43**	-0.14*	0.28**
PH	r_p 0.23*	0.23*	0.01	0.14*	0.17*	0.17*	0.17*	0.01	0.23*	0.01	0.14*	-0.01	-0.19*	0.14*
	r_g 0.41**	-0.14**	0.59**	0.34**	0.64**	0.64**	0.34**	0.59**	-0.14**	0.59**	0.34**	0.32**	-0.45*	0.35**
BM	r_p 0.85**	0.66**	0.23*	0.23*	0.11	0.11	0.11	0.11	0.85**	0.66**	0.23*	-0.43**	0.56**	0.07
	r_g 0.66**	0.66**	0.66**	0.66**	0.66**	0.66**	0.66**	0.66**	0.66**	0.66**	0.66**	0.66**	0.66**	0.66**
GY	r_p 0.78**	0.46**	0.46**	0.46**	0.46**	0.46**	0.46**	0.46**	0.78**	0.46**	0.46**	0.46**	0.66**	-0.04
	r_g -0.09	0.41**	0.41**	0.41**	0.41**	0.41**	0.41**	0.41**	-0.09	0.41**	0.41**	-0.09	0.66**	0.07
TGW	r_p -0.59**	0.51**	0.51**	0.51**	0.51**	0.51**	0.51**	0.51**	-0.59**	0.51**	0.51**	-0.59**	0.66**	-0.04
	r_g -0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	0.66**	0.07
GPS	r_p 0.14*	-0.78**	0.01	0.01	0.01	0.01	0.01	0.01	0.14*	-0.78**	0.01	0.01	0.14*	0.01
	r_g 0.14*	-0.78**	0.01	0.01	0.01	0.01	0.01	0.01	0.14*	-0.78**	0.01	0.01	0.14*	0.01
HI	r_p 0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	r_g 0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04	0.04

*** Significant at 5 and 1% level of significance.

^a r_p : Phenotypic correlation coefficient; r_g = Genotypic correlation coefficient, (SCM): Seed Colour Mean, and Abbreviations: As in Table 2.



Table 4. Ward's clustering pattern (D²-statistics) representing 19 clusters for 324 spring wheat lines.

Cluster	Cluster members	Total no. of lines
I	1 2 3 4 5 14 102 111 121 157 201 203 204 246 248 289 317	17
II	9 10 12 25 27 29 32 66 87 88 90 112 180 189 191 194 197 210 244 254 255 261 263 272 284 305	26
III	11 70 122 124 148 171 173 175 176 177 179 190 193 219 225 226 230 233 262 316 323	21
IV	8 16 20 34 35 45 55 62 93 95 99 100 104 105 127 142 147 149 188 249 264 267 275	22
V	19 24 132 133 134 136 137 143 153 174 181 183 186 218 265	14
VI	7 36 46 60 63 89 94 96 101 106 145 150 268	12
VII	6 21 44 129 146 151 198 205	8
VIII	13 41 54 65 91 92 97 98 107 108 144	11
IX	15 38 76 81 85 109 128 135 152 156 163 168 184 216 227 270 295 319	17
X	18 30 42 48 49 117 118 119 120 164 182 185 192 199 202 209 234 245 250 253 266 274 276 278 280 283 285 286 306 314	30
XI	86 113 139 158 235 236 251 256 257 303 307 310 315	13
XII	17 22 23 28 33 40 47 67 68 77 123 131 138 141 159 170 178 217 220 229 231 259 260 279 318	25
XIII	43 103 125 126 130 140 208 240 247 269 273 277 281 321	14
XIV	26 31 72 73 74 79 115 116 169 172 206 207 214 223 271 291 292 293 298 302	20
XV	50 53 61 64 69 78 84 110 114 224 228 239 241 242 252 258 282 313	17
XVI	37 39 51 52 56 57 80 154 162 187 195 196 222 232 237 296 297 299 300 301 309	25
XVII	58 71 82 155 160 161 166 211 213 221 287 288 294 311 312 324	16
XVIII	59 75 83 200 212 215 243 290 304 320	11
XIX	165 167 238 308 322	5

Table 5. Average intra and inter-cluster D values (Ward's method) among 19 clusters of 324 spring wheat lines.^a

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX
I	32.18	46.79	55.99	49.73	61.43	83.24	99.70	142.02	135.00	100.93	128.12	80.37	72.01	213.68	171.38	263.97	372.96	319.06	453.01
II		39.30	61.70	71.19	88.57	109.02	128.97	168.48	110.57	75.78	100.50	60.57	49.80	187.22	143.89	235.78	344.90	290.75	424.33
III			46.52	81.27	73.69	114.91	120.07	169.69	120.41	95.04	126.61	70.32	83.04	197.69	161.25	251.49	357.79	305.30	439.59
IV				37.06	59.88	56.23	77.97	111.28	167.23	131.42	156.02	111.85	95.95	245.36	201.90	294.40	404.17	349.68	483.34
V					38.49	70.43	64.89	116.29	175.45	145.25	175.45	120.17	119.75	254.90	215.77	308.13	415.77	362.69	497.11
VI						37.24	55.25	74.86	208.58	172.45	196.28	152.64	135.28	287.15	243.19	335.85	445.78	391.24	524.51
VII							37.46	72.86	224.96	191.86	219.98	168.06	159.78	304.80	263.70	356.95	465.77	412.01	546.42
VIII								53.07	268.54	232.70	256.54	212.03	194.99	347.14	303.52	396.14	506.14	451.44	584.72
IX									41.37	53.99	58.58	68.44	90.63	92.08	60.61	141.39	246.65	194.16	327.75
X										33.73	48.78	44.48	54.80	122.94	80.85	170.38	278.76	224.84	358.57
XI											35.17	72.81	71.24	107.58	62.51	146.33	255.06	200.67	332.43
XII												33.96	53.42	143.74	104.91	195.10	302.68	249.28	383.69
XIII													33.72	162.04	116.60	206.92	316.57	261.93	394.71
XIV														51.52	66.01	75.32	170.39	120.52	250.86
XV															39.73	102.02	208.19	154.71	287.11
XVI																41.61	118.81	69.54	195.09
XVII																	47.11	71.05	95.63
XVIII																		44.19	142.47
XIX																			49.72

^a Figures in diagonal indicate intra-cluster distance.

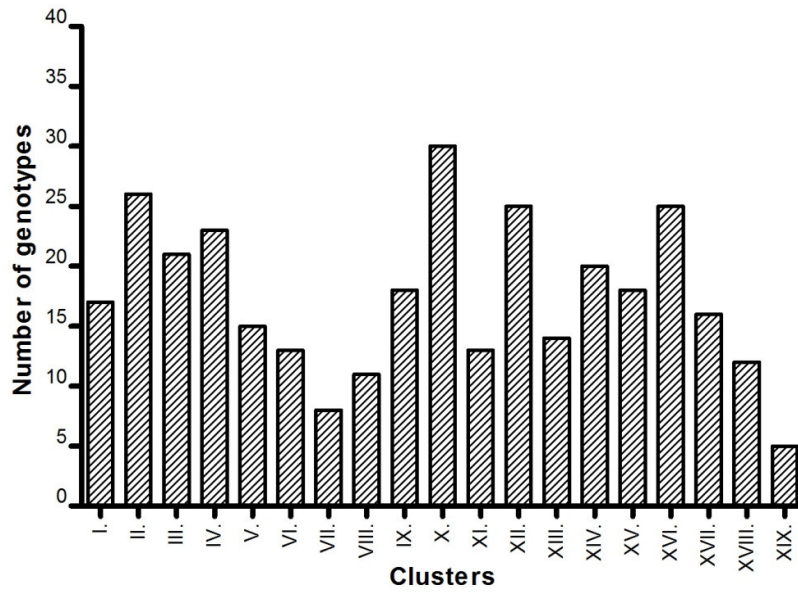


Figure 1. Graphical representation of 324 spring wheat lines into 19 clusters and number of respective genotypes within each cluster.

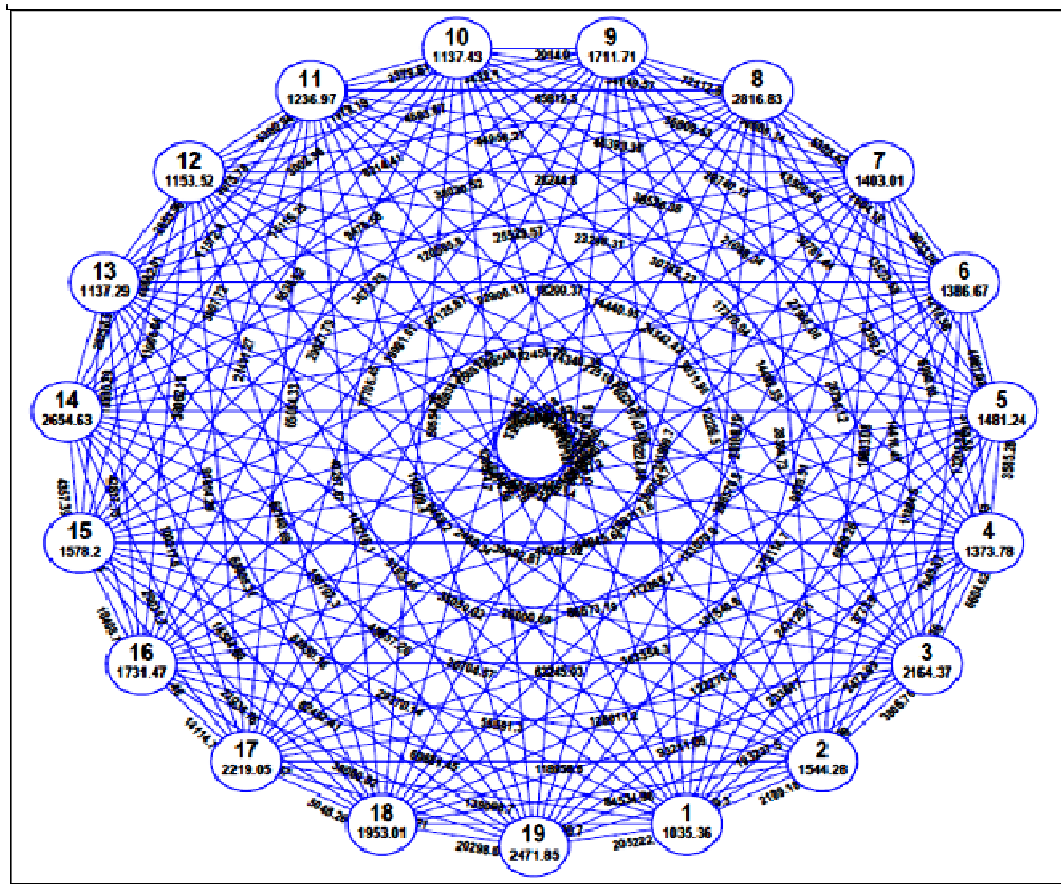


Figure 2. Ward's clustering pattern representing 19 clusters of D^2 -statistics for 324 spring wheat lines.

Hybridization between these cluster members revealed transgressive segregation for effective selection. Clusters VI, VII, VIII, XI, XII and XIX exhibited highest cluster mean values for most of the traits. Highest mean values for ear length and plant height are found in Cluster VI (Table 6). Cluster VII expressed highest values for days to 50% flowering, days to physical maturity, and seed colour mean. Cluster VIII recorded highest mean values for chlorophyll content, peduncle length, biomass, grains per spike, 1,000-grain weight, and grain yield as reported by Khodadadi *et al.* (2011). Highest cluster mean values for sheath length, harvest index and AUDPC have been exhibited by Clusters XI, XII and XIX, respectively (Table 6). The major contributing traits towards genetic divergence was found to be AUDPC (60.36%) followed by biomass (5.96%), plant height (0.58%) and grain yield (0.54%) similar to that reported by Goel *et al.* (2005) (Table 6). In the present experiment, AUDPC and biomass played major role in clustering wheat lines (Table 6; Figure 3-A).

Principal Components Analysis

PC analysis revealed the largest contributor to the total variation at each axis of differentiation. Seven PCs (PC1 to PC7) were considered from the original data explaining 79.85% of the total variation (Table.7) similar to that reported by Hailegiorgis *et al.* (2011),

Ravishanker *et al.* (2013), and Caliskan and Bayazit (2013). Out of the total 7 PCs, 5 principal components (PC1, PC2, PC3, PC4 and PC5) accounted with proportionate variance values of 20.66, 17.96, 15.07, 8.28, and 7.38%, respectively, and contributed 69.33 % of the cumulative variation having Eigen value greater than one (Table 7). Two dimensional ordinations of 324 spring wheat lines on PC axes 1 and 2 are represented for separation of the lines which reveal existence of extreme variability in the present wheat genotypic set (Figure 3-B). The first principal component has high positive component value for days to 50% flowering, chlorophyll content, days to physical maturity, and days to physiological maturity. PC1 has negative component value for AUDPC, 1,000-grain weight, grain yield, and harvest index. The second principal component had high positive component value for plant height, biomass, peduncle length, and sheath length and high negative component value for AUDPC. The abovementioned traits having high positive or negative component value reveal more genetic diversity and they play tremendous role in representing the clusters. The third principal component had high positive component value for grain yield, 1,000-grain weight, and harvest index and high negative component value for AUDPC, sheath length and peduncle length (Table 7) similar to that reported by Hailegiorgis *et al.* (2011). The projection of component traits on PC1 and PC2 revealed

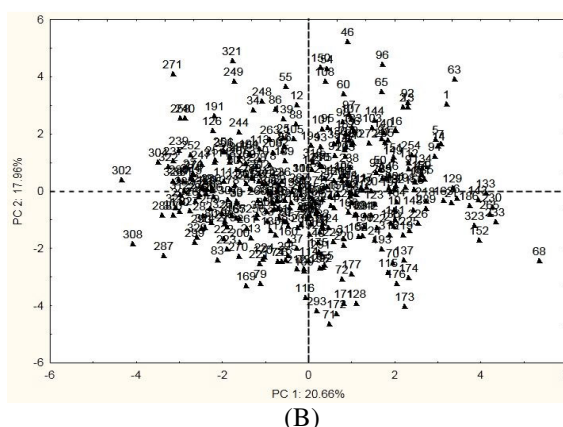
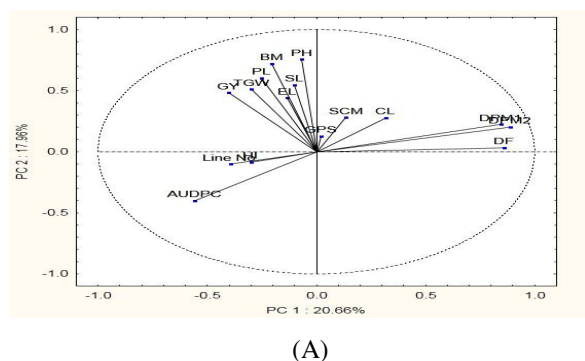


Figure 3- A) Principal component biplot for yield components in the spring wheat lines , B) Scattered diagram of the first two principal components for yield components in the spring wheat lines.

Table 6. Cluster mean values (Ward's method) and contribution of traits toward divergence for the studied traits in spring wheat lines.^a

Cluster	DF	CL	DPM1	DPM2	AUDPC	SL	PL	EL	PH	BM	GY	TGW	GPS	HI	SCM
I.	73.94	45.62	101.82	107.23	998.55	20.03	34.08	10.35	93.10	144.95	39.31	26.09	36.19	27.37	104.33
II.	71.12	46.96	99.73	105.34	1063.43	19.88	33.41	10.28	88.54	154.39	43.23	28.34	39.94	28.29	104.12
III.	75.86	47.91	102.00	107.13	1019.44	18.23	27.87	9.37	73.52	105.95	30.66	24.46	35.83	29.13	99.99
IV.	70.94	47.24	99.03	105.04	943.67	19.43	32.95	10.50	89.20	167.10	46.10	29.31	39.76	28.23	100.02
V.	79.00	46.62	102.27	108.37	899.44	19.75	31.23	9.20	84.50	114.67	29.43	23.20	40.76	26.30	104.55
VI.	75.00	47.87	102.77	108.07	869.72	19.80	34.62	11.10	97.79	178.14	45.86	30.47	41.41	25.38	103.01
VII.	79.13	46.40	103.50	108.64	809.39	19.07	31.68	9.59	83.90	138.65	40.71	26.29	38.09	28.95	106.05
VIII.	73.15	48.03	103.24	108.00	752.48	19.66	36.03	9.51	95.56	187.58	54.36	31.57	45.20	29.44	100.44
IX.	71.93	45.82	99.54	105.28	1251.17	18.16	32.39	9.59	80.94	119.40	33.55	23.10	39.83	30.48	100.08
X.	70.43	44.39	98.93	104.24	1193.83	19.39	32.88	10.05	84.95	144.25	43.32	27.45	39.08	30.09	102.90
XI.	68.90	45.98	98.13	103.02	1258.90	20.46	34.26	10.09	89.26	173.85	46.01	27.91	38.83	26.69	104.23
XII.	71.01	45.46	100.13	105.27	1136.84	18.50	31.42	9.48	81.13	125.47	38.79	25.94	39.87	31.82	99.72
XIII.	68.69	46.77	98.33	103.71	1132.81	19.77	34.13	10.81	90.90	173.27	50.78	31.32	37.49	29.62	101.31
XIV.	69.77	42.93	98.07	103.30	1411.80	18.28	31.39	8.96	79.33	114.38	34.73	24.93	37.54	30.58	96.27
XV.	68.48	44.03	97.98	103.56	1341.92	18.58	34.26	10.39	87.28	145.09	42.10	26.10	40.93	29.59	102.99
XVI.	68.20	44.21	97.39	103.17	1536.34	18.90	34.45	9.74	86.36	145.33	39.66	26.71	38.55	27.73	100.41
XVII.	68.08	43.73	97.71	103.96	1754.28	19.15	34.66	9.89	84.46	123.85	33.25	24.52	34.66	26.96	100.91
XVIII.	65.67	40.54	97.53	102.99	1645.82	18.88	33.69	10.47	84.44	140.21	40.07	24.40	41.22	29.51	100.10
XIX.	68.40	43.30	98.40	103.49	1937.86	19.84	35.86	10.01	92.23	154.83	36.59	25.36	38.06	24.24	103.62
% contribution of traits towards divergence	0.01	0.02	0.00	0.00	60.36	0.00	0.01	0.00	0.58	5.96	0.54	0.02	0.27	0.04	0.03

^a Abbreviations: As in Table 2

**Table 7.** Principal Component analysis (PCA) for spot blotch resistant and yield components in the spring wheat lines.

Traits	PC1	PC2	PC3	PC4	PC5	PC6	PC7
DF	0.47	0.02	-0.11	0.14	0.15	-0.06	0.13
CL	0.17	0.16	0.17	0.37	0.03	0.16	-0.26
DPM1	0.46	0.13	0.08	-0.10	0.10	-0.16	0.13
DPM2	0.49	0.12	0.08	-0.14	-0.01	-0.09	0.12
AUDPC	-0.31	-0.24	-0.19	-0.11	-0.02	0.06	-0.02
SL	-0.06	0.32	-0.34	0.18	0.27	-0.13	0.32
PL	-0.14	0.35	-0.29	-0.26	-0.27	0.16	0.20
EL	-0.07	0.26	-0.18	-0.09	0.32	-0.07	0.06
PH	-0.04	0.45	-0.25	-0.23	-0.22	0.03	0.07
BM	-0.11	0.43	0.11	0.24	-0.15	-0.18	-0.38
GY	-0.22	0.28	0.43	0.09	0.09	-0.05	0.17
TGW	-0.16	0.30	0.40	-0.09	0.09	-0.20	-0.15
GPS	0.01	0.07	0.00	0.61	-0.32	0.44	0.34
HI	-0.16	-0.05	0.45	-0.16	0.21	0.16	0.57
SCM	0.07	0.16	-0.01	-0.19	0.46	0.74	-0.31
Eigen value	3.31	2.87	2.41	1.32	1.18	0.85	0.83
Cumulative Eigen value	3.31	6.18	8.59	9.91	11.09	11.95	12.78
Individual variance (%)	20.66	17.96	15.07	8.28	7.38	5.34	5.18
Cumulative variance (%)	20.66	38.61	53.68	61.96	69.33	74.67	79.85

^a Abbreviations: As in Table 2.

that the ear length, 1,000-grain weight, biomass, and harvest index were positively associated with grain yield (Figure 3-A).

CONCLUSIONS

The present experiment was conducted to identify spot blotch resistant RILs with better yield potential for south Asia. Based on character association study, grain yield had strong positive association with chlorophyll content, 1,000-grain weight, biomass and harvest index and negative association with the disease. The present experimental materials revealed extreme genetic variability. The materials were classified under 19 clusters and its major proportion of variance depicted by principle components. The promising RILs will be evaluated under multi-location trial for their location suitability. Genetically diverse promising RILs will be exploited as potent donor against spot blotch disease.

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REFERENCES

1. Baranwal, D. K., Mishra, V. K., Vishwakarma, M. K., Yadav, P. S. and Arun, B. 2012. Studies on Genetic Variability, Correlation and Path Analysis for Yield and Yield Contributing Traits in Wheat (*T. aestivum* L. em Thell.). *Plant Arch.*, **12**: 99-104.
2. Caliskan, O. and Bayazit, S. 2013. Morphological and Chemical Diversity of Pomegranate Accessions Grown in Eastern Mediterranean Region of Turkey. *J. Agr. Sci. Tech.*, **15**: 1449-1460.
3. Duveiller, E. and Gilchrist, L. 1994. Productions Constraints Due to *Bipolaris*

- sorokiniana* in Wheat: Current Situation and Future Prospects. In: "Wheat in Heat-stressed Environments: Irrigated, Dry Areas and Rice-Wheat Farming Systems", (Eds.): Saunders, D. A. and Hettel, G. P.. ISBN-13: 9789686127874, UNDP/ARC/BARI/CIMMYT, DF, Mexico, PP: 343-352.
4. Gilchrist, L. I. and Pfeiffer, W. H. 1991. Resistance to *Helminthosporium sativum* in Bread Wheat: Relationship of Infected Plant Parts and Their Association of Agronomic Traits. In: "Wheat for the Non-traditional Warm Areas", (Ed.): Saunders, D. A. CIMMYT, El Batán, DF, Mexico, PP.1-549.
 5. Goel, P., Pal, S. S. and Jaiswal, J. P. 2005. Genetic Analysis for Spot Blotch (*Bipolaris sorokiniana* Sacc. in Borok. Shoem.) Reaction in Wheat [*Triticum aestivum* (L.) Em. Thell.]. *Indian J. Genet. Plant Breed.*, **65**: 305 -306.
 6. Hailegiorgis, D., Mesfin, M. and Genet, T. 2011. Genetic Divergence Analysis on Some Bread Wheat Genotypes Grown in Ethiopia. *J. Central Eur. Agric.*, **12**: 344 -352.
 7. Joshi, A. K., Chand, R. and Arun, B. 2002. Relationship of Plant Height and Days to Maturity with Resistance to Spot Blotch in Wheat. *Euphytica*, **123**: 221 -228.
 8. Joshi, A. K., Kumar, S. Chand, R. and Ortiz-Ferrara, G. 2004. Inheritance of Resistance to Spot Blotch Caused by *Bipolaris sorokiniana* in Spring Wheat. *Plant Breed.*, **123**: 213 -219.
 9. Joshi, A. K., Ortiz-Ferrara, G. Crossa, J. Singh, G. and Alvarado, G. *et al.*, 2007. Associations of Environments in South Asia Based on Spot Blotch Disease of Wheat Caused by *Cochliobolus sativus*. *Crop Sci.*, **47**: 1071 - 1081.
 10. Khodadadi, M., Fotokian M. H. and Miransari, M. 2011. Genetic Diversity of Wheat (*Triticum aestivum* L.) Genotypes Based on Cluster and Principal Component Analyses for Breeding Strategies. *Aust. J. Crop Sci.*, **5**: 17 -24.
 11. Khodarahmpour, Z., Choukan, R., Bihanta, M. R. and Hervan, E. M. 2011. Determination of the Best Heat Stress Tolerance Indices in Maize (*Zea mays* L.) Inbred Lines and Hybrids under Khuzestan Province Conditions. *J. Agr. Sci. Tech.*, **13**: 111-121.
 12. Mahalanobis, P. C. 1928. A Statistical Study at Chinese Head Measurement. *J. Asiatic Soc. Bengal*, **25**: 301 -377.
 13. Mellingers, J. S., 1972. Measures of Genetic Similarity and Genetic Distance. VII. In: "Studies in Genetics", (Ed.): Wheeler, M. R. Publication No. 7213, University of Texas, Austin, PP. 145-153.
 14. Olfati, J. A., Peyvast, G., Shabani, H. and Nosratie-Rad, Z. 2010. An Estimation of Individual Leaf Area in Cabbage and Broccoli Using Non-destructive Methods. *J. Agr. Sci. Tech.*, **12**: 627-632.
 15. Phadnawis, B. N., Khatod, J. P., Vitkare, D. G., Shivankar, R. S. and Nagone, A. H. 2002. Genetic Variability and Correlation Coefficient Studies in Durum Wheat. *Ann. Plant Physiol.*, **6(2)**: 115-118.
 16. Rao, C. R. 1952. *Advanced Statistical Methods in Biometrical Research*. John Wiley and Sons, New York, PP. 361-367.
 17. Ravishanker, Kumar, S., Baranwal, D. K., Chatterjee, A. and Solankey, S. S. 2013. Genetic Diversity Based on Cluster and Principal Component Analyses for Yield and Quality Attributes in Ginger (*Zingiber officinale* Roscoe). *Int. J. Plant Breed. Gene.*, DOI:10.3923/ijpbg.
 18. Robinson, H. F., Comstock, R. E. and Harvey, P. H. 1955. Genotypic and Phenotypic Correlation's in Wheat and Their Implications in Selection. *Agrono. Jour.*, **43**: 282-287.
 19. Roelfs, A. P., Singh, R. P. and Saari, E. E. 1992. *Rust Diseases of Wheat: Concepts and Methods of Disease Management*. CIMMYT, Mexico, USA, PP: 37-38.
 20. Saari, E. E. 1998. Leaf Blight Diseases and Associated Soil Borne Fungal Pathogens of Wheat in North and South East Asia. In: "Helminthosporium Blights of Wheat: Spot Blotch and Tan Spot", (Eds.): Duveiller, E., Dubin, H. J., Reeves, J. and McNab, A. CIMMYT, Mexico, USA, PP: 37-51.
 21. Sharma, R. C., Dubin, H. J., Bhatta, M. R. and Devkota, R. N. 1997. Selection for Spot Blotch Resistance in Four Spring Wheat Populations. *Crop Sci.*, **37(2)**: 432-435.
 22. Sharma R.C., Duveiller, E., Ahmed, F., Arun, B., Bhandari, D., Bhatta, M.R., Chand, R., Chaurasiya, P.C.P., Gharti, B., Hossain, M.H., Joshi, A.K., Mahto, B.N., Malaker, P.K., Reza, M.A., Rahman, M., Samad, M.A., Shaheed, M.A., Siddique, A.B., Singh, A.K., Singh, K.P., Singh, R.N. and Singh. S.P., 2004. Helminthosporium Leaf Blight Resistance and Agronomic Performance of Wheat Genotypes Across Warm Regions of South Asia. *Plant Breed.*, **123**: 520 -524.
 23. Singh, R. P. and Rajaram, S. 1992. Genetics of Adult-plant Resistance of Leaf Rust in



- 'Frontana' and Three CIMMYT Wheats. *Genome.*, **35**: 24-31.
24. Van der Plank, J. E. 1963. *Plant Diseases: Epidemics and Control*. 1st Edition, ISBN: 0127114505, Academic Press, New York, 349 PP.
25. Zadoks, J. C., Chang, T. T. and Konzak, C. F. 1974. A Decimal Code for the Growth Stages of Cereals. *Weed Res.*, **14**: 415-421.
26. Zhang, P., Dreisigacker, S., Melchinger, A.E., Reif, J.C., Ginkel, M. V., Kazi, A., Hoisington, D. and Warburton, M.L. 2005. Quantifying Novel Sequence Variation and Selective Advantage in Synthetic Hexaploid Wheats and Their Backcross-derived Lines Using SSR Markers. *Mol. Breed.*, **15**: 1-10.

ارزیابی ژنتیکی رگه های خویش آمیخته و نو ترکیب گندم بهاره (*Triticum aestivum* L.) برای مقاومت به Spot Blotch (*Bipolaris Sorokiniana*) و ارزیابی اجزای عملکرد گندم در شرایط طبیعی جنوب آسیا

ن. منا، و. ک. میشر، د. ک. بارانوال، ا. ک. سینک، و. پ. رای، ر. پراساد، ب. آرون و ر. چاند

چکیده

هدف این پژوهش ارزیابی ژنتیکی رگه های خویش آمیخته و نو ترکیب گندم بهاره (RILs) با مبداء های متنوع بود و برای این منظور پارامترهای ژنتیکی شامل تغییرپذیری، همراهی شاخص ها، تجزیه خوشه ای، و تجزیه به مولفه های اصلی (PCA) برای مقاومت به Spot Blotch، و اجزای عملکرد در مزرعه تحقیقاتی دانشگاه هندوی بناراس طی ۱۱-۲۰۱۰ ارزیابی شد. عملکرد دانه در هر کرت به گونه ای معنی دار و مثبت با زیست توده، وزن هزار دانه، شاخص برداشت، محتوی کلروفیل، و تعداد دانه در خوشه در سطح ژنوتیپی همراهی (رابطه) داشت. رگه ۶۵ کمترین مقدار میانگین "مساحت زیرمنحنی پیشرفت مرض" (AUDPC) را که برابر ۶۳۲ بود نشان داد و این نشانگر استعداد و پتانسیل این رگه به عنوان والد مقاوم بود. تجزیه خوشه ای داده ها با استفاده از روش Ward، همه ۳۲۴ رگه گندم را در ۱۹ خوشه دسته بندی کرد. واگرایی شدیدی بین خوشه ها مشاهده شد. با استفاده از آماره D^2 بیشترین فاصله بین خوشه ای (۵۸۴/۷۲) بین خوشه VIII و XIX به دست آمد. بیشترین میانگین برای کلروفیل II و طول ساق گل، زیست توده، دانه در خوشه، وزن هزار دانه و عملکرد دانه برای خوشه VIII ثبت شد. صفت اصلی که به واگرایی ژنتیکی کمک می کرد AUDPC بود (60.36%). پنج مولفه اصلی (PC1, PC2, PC3, PC4, PC5) ارزش متناسبی برابر ۲۰/۶۶٪، ۱۵/۰۷٪، ۱۷/۹۶٪، ۸/۲۸٪، ۷/۳۸٪ داشتند و مجموعاً ۶۹/۳۵٪ از تغییرات را توجیه میکردند. مولفه های اصلی دوم مقادیر مثبت بالایی برای طول گیاه، زیست توده و وزن هزار دانه داشتند. هدف بهنژادی در این پژوهش شناسایی رگه های گندم مقاوم به Spot Blotch با تنوع ژنتیکی متنوع بود تا بتوان در برنامه های آینده بهنژادی کولتیوارهای پر محصول که به ویژه با جنوب آسیا سازگارند به وجود آورد.