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European Journal of Experimental Biology, 2012, 2 (5):1486-1491

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Evaluation of Resistance of some Wheat Doubled Haploid Lines to Virulence Pathotype, the Causal Agent of Wheat Leaf Rust

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ABSTRACT

Brown rust of leaf rust that causes by Puccinia recondite f. sp. tritici is one of the main diseases of wheat in north, south and west of Iran by considerable damages. In this research, resistance of 64 wheat double haploid lines were studied in cereal rust greenhouse of Karaj seed and plant improvement institute, and resistance of seeding by one race of brown rust from Ahvaz was evaluated. Brown rust resistance components, latent period (Number of days from inoculation till appearance of the first pustule), infection type (9-12 days from inoculation), and pustule density in greenhouse was measured. The results showed that there is a significant difference among lines from all four traits in P=%1 lines 2, 6, 29, 31, 32, 35, 36, 37, 43, 48, 49, 50, 51, 53, 55, 57, 58 had the lowest infection type, long latent period and low pustule size and density. In total, 17 lines from 64 studied lines showed resistance in seeding period, 47 lines were sensitive.

Keywords: Brown rust, Resistance components, Seeding resistance, Wheat.

INTRODUCTION

Puccinia triticina (syn: *P. recondite*) f. sp. *tritici* is pathogen of wheat brown rust in all region of wheat and it is considerable than yellow and black rust in world [4]. The pathogen of wheat brown rust was reported in 1325 in Iran [6]. It is important disease after yellow rust by high expansion In addition to its epidemic and damages, this disease reduces yield considerably in late of wheat germination period. Suffered grains are dried up, small and poor and the weight of product is reduced 90% [2]. The damage of brown rust has be estimated 4/11 million/ton in 1973-1975 [16]. Cultivation of resistance cultivars is the best way for control. Resistance as host genetics characteristics is used for production of resistant cultivars by specialists. Investigation of wheat lines and cultivars tolerance against brown rust is necessary for preservation of current cultivars and introduction of new cultivars resistant lines can be used in improvement plans as resources. Genetics resistance reduces or eliminates toxins consumption need and it does not have known environmental effect and it is cost effective, since resistance is transferred in next generations and because of production of new biotypes or breeds of pathogen resistant cultivar reduces its resistance after several cultivation [17], so continuous determination of photogenes in management of using resistance genes and employing effective combination prevent pathogen by genetics diversity. Resistance components are wed in determination of

mechanism and manner of heritance of resistance, so that each component or all components determine studied population genetics parameters relative resistance is accompanied by increase latent period, reduction of infection abundance and pustule size. Latent period is main element in relative resistance in cereal rust since rusts are multi cycles by increase in latent period the speed of epidemic is reduced [18]. The simple define for Latent period is number of days form plant inoculation till appearance of the first pustule on leaf. Roelfs at el. [17] defined it as infection period until appearance of 50% of pustules, the short latent period shows high sensitivity of plant. Pustule density is average of pustules in infected leaf surface; it depends on level of host acceptance or pathogen pathogenic. Cultivars by less pustule density are resistant [17]. Pustule size is average pustule size on leaf. Small size indicates low infection type and high host resistance [17]. Infection type is reciprocal effect between host and pathogen. There uniform infection types for black and brown rust [17]. The aim of this article is to evaluate resistance of some wheat double haploid lines based on resistance components in embryonic stage in order to determine resistance lines and use them in improvement plans.

MATERIALS AND METHODS

64 wheat double haploid lines in 1387-1388 were examined in cereal research center of seed, seed and plant improvement institute in completely randomly blocks in three replications by control cultivar (Bolan sensitive cultivar) in order to evaluate their resistance against brown rust isolate from Ahvaz in greenhouse.

Five seeds from each treatment were cultivated in a pot involving soil and pit mass as a one replication. After growth of the first leaf distilled water involving Tween-20 droplet in litter was sprayed and mixture of spore and cleaning powder by 1:4 ratio was obtained after inoculation post were covered by plastic CR moisture with distilled watery.

All pots were placed in dark room for 24 hours by 20 ± 1°C and humidity of 100%. Then they transferred to a greenhouse by 21 ± 3°C, humidity of 50% and 16 h light and 8 h dark, latent period was noted. Infection type was noted based on 0-4 scales nine-twelve days after inoculation [12].

Infection types of 0-2 were considered as resistance (R) type, infection types of 3-4 were identified as sensitive (S) types. Latent period, pustule size and density were measured. Latent period was measured as number of days from inoculation till appearance of the first pustule on leaf from. 5th day so all embryos were observed every day and in case of observation of the first pustule on leaf, stem was marked by colored wire (each color indicated special dote). In plants without pustule number 12 was considered for analysis. After measuring latent period and infection type, infected leaves were cut in length of 2-3 cm in each pot and they were transferred to lacto phenol solution in order to fixed cut leaves and pustules were counted 3-5 times in three replications in leaf area unit. Obtained numbers were converted to number of pustules in cm^2. In order to measure pustule, length and width 3-5 numbers from each sample were measured by magnification of 40 and the pustule area was calculated by pustule size=bis diameter × small diameter × π /4 [10, 20].

RESULTS AND DISCUSSION

64 double haploid Lines by their parents and control cultivar were cultivated in completely randomly plan. In greenhouse experiment, Ahvaz brown rust isolated by following formulae was used. A virulence/virulence formulae: (Lr2a, Lr9, Lr17, Lr19, Lr28, Lr29/Lr1, Lr2c, Lr2b, Lr3, Lr3bg, Lr3ka, Lr10, Lr11, Lr12, Lr13, Lr14a, Lr14b, Lr15, Lr16, Lr20, Lr21, Lr23, Lr24, Lr25, Lr26, Lr27, Lr30, Lr32, Lr33, Lr35, Lr37. Table 1 shows results of analysis. After analysis of variance each traits of latent period, infection type, pustule size and density were significant. In other words there was significant difference in all experiment lines.

Table 1: Analysis of variance of unbalanced completely randomized design for different traits to race of Ahvaz.

		MS			
S.O.V	**df**	**Latent period**	**Infection type**	**Pustule size**	**Pustule density**
Genotype	63	5.68**	2.50**	1089**	103.60**
Error	128	0.25	0.061	12.37	1.69

***: Significant at α=0.01*

Table 2: Comparison of different traits in doubled haploid wheat lines in greenhouse conditions to race of Ahvaz.

NO.	Line name	Infection type	Latent period	Pustule size	Pustule density
1	PWS-N-3	3B	8FG	13.97I	57.73ABCDEFGH
2	PWS-N-5	0F	12A	0L	0N
3	PWS-N-7	3B	9CDEF	17.73ABCDEFG	60.17ABCDE
4	PWS-N-8	3B	8.6DEF	16.30CDEFGHI	51.17EFGHI
5	PWS-N-9	3B	9.3CDE	15.47CDEFGHI	58.10ABCDEFG
6	PWS-N-11	0F	12A	0L	0N
7	PWS-N-12	3B	8FG	18.37ABCDEF	56.33ABCDEFGH
8	PWS-N-13	3B	8.6DEF	16.33CDEFGHI	53.93CDEFGHI
9	PWS-N-15	3B	9CDEF	17.33ABCDEFGHI	56.60ABCDEFGH
10	PWS-N-17	3B	8.6DEF	16.93BCDEFGHI	57.90ABCDEFG
11	PWS-N-18	3B	8.3EF	18.30ABCDEF	57.67ABCDEFGH
12	PWS-N-19	3B	9.3CDE	18.33ABCDEF	53.50CDEFGHI
13	PWS-N-23	3.3B	8.3EF	14.97FGHI	60.37ABCDE
14	PWS-N-24	4A	7G	16.53BCDEFGHI	49.33GHI
15	PWS-N-25	3B	10BC	18.17ABCDEF	50.17FGHI
16	PWS-N-26	3B	9.3CDE	17.67ABCDEFG	57.67ABCDEFGH
17	PWS-N-29	3B	8FG	15.73CDEFGHI	60.77ABCD
18	PWS-N-30	3B	8FG	14.10HI	56.63ABCDEFGH
19	PWS-N-31	3B	8.3EF	15.90CDEFGHI	54CDEFGHI
20	PWS-N-33	3B	9CDEF	18.43ABCDE	51.33EFGHI
21	PWS-N-34	3B	10BC	17.77ABCDEFG	56BCDEFGH
22	PWS-N-36	3B	9.3CDEF	15.60CDEFGHI	57.60ABCDEFGH
23	PWS-N-40	3B	8FG	16.60BCDEFGHI	53.50CDEFGHI
24	PWS-N-42	3B	8FG	18.73ABC	49.33GHI
25	PWS-N-43	3B	8.6DEF	17.47ABCDEFGH	57.67ABCDEFGH
26	PWS-N-47	3B	9CDEF	16.63BCDEFGHI	56.83ABCDEFGH
27	PWS-N-48	3B	9CDEF	16.40CDEFGHI	59.13ABCDEF
28	PWS-N-49	3B	8.6DEF	14.50GHI	59.17ABCDEF
29	PWS-N-51	1DE	12A	2.03L	12.13LM
30	PWS-N-53	3B	8.6DEF	17.73ABCDEFG	49.50GHI
31	PWS-N-54	2C	11AB	7.90JK	23.60J
32	PWS-N-55	2C	11AB	8.36J	20.63JK
33	PWS-N-56	3B	8FG	16.57BCDEFGHI	60.40ABCDE
34	PWS-N-57	3B	9.3CDE	18.50ABCD	46.53I
35	DH-141	2C	11AB	6.33JK	20.67JK
36	DH-142	2C	11AB	8.16J	21.80J
37	DH-143	0F	12A	0L	0N
38	DH-144	3B	8.6DEF	15.17DEFGHI	54.73CDEFGHI
39	DH-147	3B	10BC	17.53ABCDEFG	57.67ABCDEFGH
40	DH-148	3B	8FG	15.57CDEFGHI	56.03BCDEFGH
41	DH-149	3B	8FG	16.37CDEFGHI	56.73ABCDEFGH
42	DH-150	3B	9CDEF	17.43ABCDEFGH	59.10ABCDEF
43	DH-151	1DE	12A	1.76L	12.80KLM
44	DH-152	3B	9.6CD	15.63CDEFGHI	55CDEFGHI
45	DH-153	3B	8.6DEF	17.87ABCDEFG	49.10GHI
46	DH-154	3B	8FG	15EFGHI	54.70CDEFGHI
47	DH-155	3B	9CDEF	19.83AB	49.17GHI
48	DH-156	1.3D	11.3A	4.8K	17.03 JKL
49	DH-159	0.66E	11.6A	1.9L	8.16M
50	DH-160	2C	11AB	8.2J	25.07J
51	DH-161	2C	11AB	5.96JK	24.13J
52	DH-162	3B	9.3CDE	18.27ABCDEF	60.40ABCDE
53	DH-163	2C	11AB	7.73JK	23.83J
54	DH-164	3B	9CDEF	18.30ABCDEF	60.80ABCD
55	DH-165	2C	11AB	6.63JK	24.50J
56	DH-166	3B	8.3EF	16.27CDEFGHI	52.53DEFGHI
57	DH-167	0F	12A	0L	0N
58	DH-168	2C	11AB	6.9JK	22.47J
59	DH-171	3B	9.6CD	16.16CDEFGHI	48.50HI
60	DH-172	3B	9CDEF	18.43ABCDE	50.83FGHI
61	DH-173	3.3B	7.6FG	14.93FGHI	59ABCDEF
62	DH-174	3B	9.6CD	18.57BCDEFGHI	51.23EFGHI
63	DH-176	3.3B	8.3EF	16.57BCDEFGHI	62.27ABC
64	DH-177	3.3B	8FG	16.50BCDEFGHI	64.77AB
65	susceptible	4A	7G	20.33A	65.33A

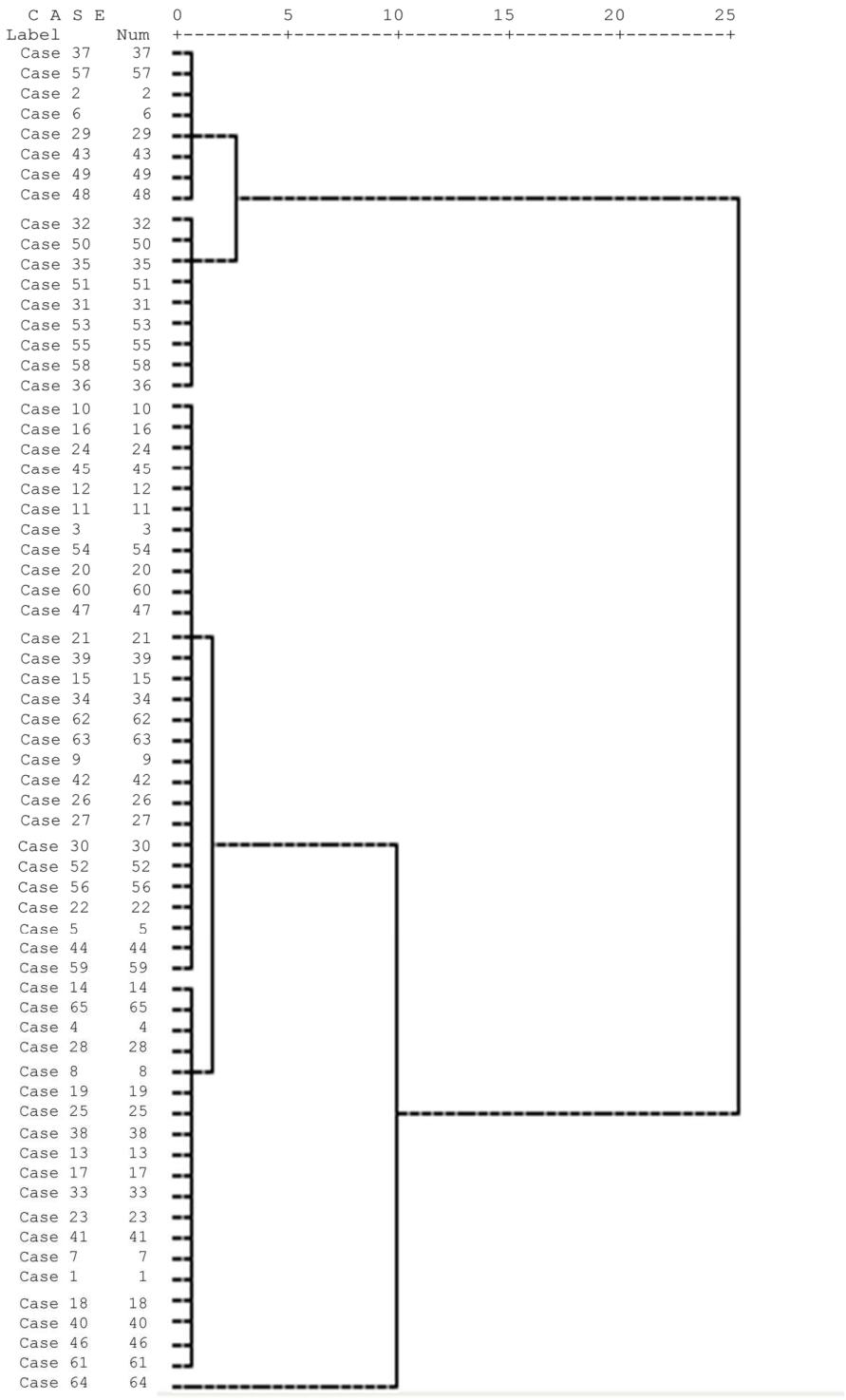

Figure 1: Dendrogram of wheat doubled haploid lines based on their resistance to Ahvaz race of *Puccinia recondite*.

According to table 2 lines 2, 6, 29, 31, 32, 35, 36, 37, 43, 48, 49, 50, 51, 53, 55, 57, and 58 were resistant in embryonic stage indicate that there are resistance genes against brown rust. Seeding resistance genes and adult-plant resistance genes are brown rust resistance genes [13]. Resistance from first leaf till end stage of growth is called

seeding resistance [5, 9]. It can be sensitivity, immunity or moderate resistance [3]. This resistance is monogenic by considerable effect [8, 11]. Ash and Brown [1] suggested seeding infection as reducing of yield and 1000 grains weight. One cultivar can be sensitive to pathogen in seeding stage, but it can be resisted in maturity stage.

Maturity stage resistance is important in control of reduction in yield and disease and it is cost-effective [7]. Mc Intosh [13] believes that seeding and adult plant resistance genes are effective in enhancement of different cultivars resistance. The results of analysis of variance showed that there is a significant difference among lines in latent period, infection type, pustules size and density P=%1. Latent period is one of the slow rusting components used in study of epidemic [15, 19]. There is a negative correlation between latent period and infection type [20]. The results showed that in line 14 the first pustules appeared 7 days after inoculation that is the lowest number for latent period among lines (Table 2). Lines 2, 6, 29, 31, 32, 35, 36, 37, 43, 48, 49, 50, 51, 53, 57 and 58 had resistance infection type by latent period of 11-12 days. Greenhouse condition affects on latent period in addition to genotype selection of genotypes for long latent period is important in regions by short rust season since there is no chance for pathogen [20]. Latent period has genetics diversity [9] and its length depends on plant growth stage and leaf age.

If plant is in ear formation period, stamen leaf has long latent period and this period is reduced in lower leaves [14, 20]. In order to measure this trait it should be tested in greenhouse by controlled condition or some defined spore on plant. Big pustules indicate high infection type and less host resistance [17]. The results showed that lines 64, 63, 62, 61, 60, 59, 56, 54, 52, 47, 46, 45, 44, 42, 41, 40, 39, 38, 34, 33, 30, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 5, 4, 3, 1 have high infection type and low pustule size.

In resistance cultivars pustules size was low number of produced spores in each pustule is one of the main resistance component [20]. Since, its measurement is difficult pustule size is used. Also, these had more pustule density. Pustule density depends on host acceptance or pathogen infection capacity, cultivars by less pustule density are resistant [17].

In order to measure and determine genetic intervals nearness or farness, relativity and patterning of genetic diversity in brown rust resistance components clustering method was used. Euclidian coefficient determines genetic intervals of genotypes the far interval leads to far clusters. In this experiment, lines were measured based on latent period, infection type, pustules size and density in different clusters. All lines were divided into 4 groups based on figure 1by less sensitivity from left to right. Lines 58, 57, 55, 53, 51, 50, 49, 48, 43, 37, 36, 35, 32, 31, 29, 6, 2 are resistance and lines 64, 63, 62, 61, 60, 59, 56, 54, 52, 47, 46, 45, 44, 42, 41, 40, 39, 38, 34, 33, 30, 28, 27, 26, 25, 24, 23, 22, 21, 20, 19, 18, 17, 16, 15, 14, 13, 12, 11, 10, 9, 8, 7, 5, 4, 3, 1 are sensitive lines. According to dendrogram (Figure 1), resistant are selected among experiment samples.

CONCLUSSION

In conclusion, in cluster analysis lines were divided into two groups based on these four traits and resistance lines were selected accordingly. It is recommended in order to uniformity of inoculation results, defined number of spores in controlled condition of greenhouse the resistance components investigated. According to data lines 58, 57, 55, 53, 51, 50, 49, 48, 43, 37, 36, 35, 32, 31, 29, 6, 2 by less infection type, long latent period and asymptote lines were as resistance resource in improvement plans these lines have more chance for introduction as resistant cultivar against brown rust in future.

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