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Production and evaluation of bread wheat doubled haploid lines with resistance to stem rust (*Puccinia graminis* f. sp. *tritici*)

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ABSTRACT

Bakhtiar, F., Ghazvini, H., Aghaee Sarbarzeh, M. and Afshari, F. 2017. Production and evaluation of bread wheat doubled haploid lines with resistance to stem rust (*Puccinia graminis* f. sp. *tritici*). **Crop Breeding Journal 7 (1 & 2): 37-47.**

Stem rust, caused by *Puccinia graminis* f. sp. *tritici*, is a devastating wheat disease, which can cause serious grain yield reduction. Doubled haploid (DH) technique reduces the time required for the development of new varieties by up to almost five years. In this study, three F1 wheat hybrids including W1: Ghods*3/MV17, W2: Flanders/3*Ghods and W3: Hybrid de Bersee/3*Ghods along with three maize genotypes, H1: KSC 108, H2: SC 301 and H3: SC 704, along with a mixture of pollen grains of these three genotypes (H4) were used to generate DH lines. During DH lines production some characteristics, such as percentage of seed set, haploid embryo and haploid plantlets, were studied. In total, 150 DH lines resulting from all crosses, along with their parents and three check cultivars, Parsi, Mihan and Bolani, were evaluated for their infection type to *Pgt* races PTMNC, TTSTK and TTKSK. Results indicated that differences between check cultivars for coefficient of infection induced with race PTMNC at the adult plant stage was significant. Also, for infection type at the seedling stage, differences between check cultivars inoculated with race TTSTK were significant (P<0.01), but non-significant reactions were observed for races PTMNC and TTKSK. Cluster analysis based on infection type and coefficient of infection showed that DH lines were classified into two major groups of susceptible and resistant when induced with all three races. In general, out of 150 DH lines that were evaluated at the adult plant stage with race PTMNC, 41 lines were resistant, and 109 lines were susceptible, while more than 50% of resistant lines belonged to the W3 population.

Key words: Wheat, Doubled haploid, Race, Stem rust

INTRODUCTION

Haploid individuals are sporophytes with a gametic chromosome number (Ouyang *et al*., 1973; Riley, 1974) and doubled haploids (DHs) can instantly be produced by doubling these haploid chromosome complements. Each group of wheat forms its own respective haploids. The haploids from einkorn (*T. monococcum* L., 2n = 2x = 14), emmer (*T. turgidum* L., 2n = 4x = 28), and dinkel (*T. aestivum* L., 2n = 6x = 42) possess n = x = 7, n =2x = 14, and n = 3x = 21 chromosomes with genomic constitution of A, AB, and ABD, respectively (Quisenberry and Reitz, 1967; Fehr, 1993; Folling and Olesen, 2002). Haploids can originate spontaneously in nature or as a result of various induction techniques. Spontaneous development of haploid plants has been known since Blakeslee *et al*. (1922) first described it in *Datura stramonium* L.; however, spontaneous occurrence is rare and therefore of limited practical value.

Bread wheat (*Triticum aestivum* L.) haploids were also produced by anther culture (Ouyang *et al*., 1973; Picard and De Buyser, 1973), isolated microspore culture (Wei, 1982), and by using wide hybridization with wild barley *Hordeum bulbosum* L. (Barclay, 1975), maize, (*Zea mays* L.); (Laurie and Bennett 1986, 1989; Suenaga and Nakajima, 1989; Inagaki and Tahir, 1990 and Laurie and Reymondie, 1991) pearl millet [Pennisetum glaucum (L.) R. Br.] (Inagaki and Mujeeb-Kazi, 1995), teosinte (Z. mays L. spp. mexicana) (Ushiyama *et al*., 1991) and sorghum [Sorghum bicolor (L.) Moeneh] (Ohkawa *et al*., 1992; Inagaki and Mujeeb-Kazi, 1995). The two systems of anther culture and wheat × maize crossing are the most commonly used induction methods in wheat.

The crossability of bread wheat × *H. bulbosum* depends on the wheat allelic composition for the *Kr* genes responsible for the incompatibility between these two species (Sitch and Snape, 1987). Production of wheat haploids through wheat × maize crossing was reported as successful with no development of albino plants (Sadasivaiah *et al*., 1999; Ushiyama *et al*., 2007) and insensitivity of

maize to the action of cross-incompatibility genes. Several reports demonstrated the success of DH plant production using maize pollen on bread wheat (Suenaga and Nakajima, 1989; Amrani *et al*., 1993; Niroula *et al*., 2007) but relatively few durum wheat genotypes show such crossability with maize (Almouslem *et al*., 1998; Inagaki *et al*., 1998; David *et al*., 1999; Garcia-Lilamas *et al*., 2004).

Stem or black rust of wheat is caused by fungus *Puccinia graminis* Pers. f. sp.*tritici* Eriks. & E. Henn. Historically it is known to cause severe devastation periodically and was the most feared disease in various countries on all continents where wheat is grown.

Stem rust was historically a major problem in Africa, the Middle East, Asia (except Central Asia), Australia, New Zealand, Europe and the Americas (both North and South). The last major stem rust epidemic occurred in Ethiopia during 1993 and 1994 (Shank, 1994).

In 1998, severe stem rust infections were observed on wheat in Uganda**,** and a race, designated as Ug99 with virulence on plants with the *Sr31* gene, was detected (Pretorius *et al*., 2000). Race Ug99 was subsequently detected in Kenya and Ethiopia in 2005 (Wanyera *et al*., 2006), and in Sudan and Yemen in 2006. A new variant of this race with virulence to *Sr24* was detected in Kenya in 2006 (Jin *et al*., 2007)

The occurrence of Ug99 in Yemen was considered particularly significant as it provided strong evidence that Ug99 was moving toward the important wheat areas of the Middle East and Asia. The subsequent confirmation of Ug99 (race TTKSK) in Iran**,** announced by the Food and Agriculture Organization of the United Nations in Iran in March 2008, supported these predictions. Positive Iranian Ug99 isolates were actually collected in 2007 from two sites**,** Broujerd and Hamedan, in western Iran but underwent extensive testing to confirm the race (Nazari *et al*., 2009). Detection of Ug99 in Iran in 2007 was followed by drought conditions, and no reports of Ug99 were received from Iran in 2008**.** However, in 2009, Ug99 was found in the southern Iranian province of Khuzestan where spring wheat is grown and growing conditions are favorable. Given the regular northeasterly airflows out of Yemen (Singh *et al*., 2008), the possibility that this was a new incursion from Yemen is considered likely. Alternatively, Ug99 may have been introduced into Khuzestan in 2007 but remained undetected and migrated to the northwest, where facultative and winter wheat are grown and mature approximately two months later. There is no evidence that the Ug99 lineage has become well established in Iran, and no crop losses have been reported so far. Also, to date it is not known if the new race has been spread beyond Iran.

Reynolds and Borlaug (2006) estimated that the potential area under the risk from Ug99 along the natural migration path in North Africa, the Middle East and Asia (excluding China) might amount to 50 million ha of wheat; that is about 25% of the world's wheat area and accounts for an estimated 19% of global wheat production of about 117 million tons.

The main objective of this research was to produce bread wheat lines with desirable agronomic traits and resistance to stem rust diseases using the doubled haploid method.

MATERIALS AND METHODS

Three F1 wheat hybrids (W1: Ghods*3/MV17, W2: Flanders/3*Ghods and W3: Hybrid de Bersee/3*Ghods) along with three maize genotypes (H1: KSC 108, H2: SC 301, H3: SC 704) and a mixture of all three genotypes (H4) were used as plant materials to produce doubled haploid lines. The F1 hybrids were produced in the cropping season 2005-2006 with the goal of increasing rust resistance among wheat germplasm in the Cereal Research Department, SPII, Karaj. F1 plants were pollinated with pollens of each maize genotype separately and also with a mixture of pollens from all three pollinators in the 2010-2011 and 2011-2012 cropping seasons. Twenty spikes from each cross were used to calculate some florets characteristics such as percentage of seed set, embryo formation and haploid plantlet. In addition, to evaluate the effect of wheat and maize genotypes and their interactions, six spikes (replications) from each cross were selected randomly to conduct analysis of variance for the above-mentioned traits using a factorial experiment where wheat hybrids and maize genotypes were considered as two factors.

Nomenclature of isolates and disease evaluation at the seedling stage were carried out in greenhouse of the Cereal Research Department, SPII, Karaj in 2012-2015 while disease evaluation at the adult plant stage was done under field condition in Kelardasht station in 2015.

Wheat × maize system:

The plant materials were studied under controlled conditions in the greenhouse. Greenhouse regimes were 25/12^{o}C (day/night), 16h photoperiod, and 45-65% relative humidity. Wheat spikes were emasculated before anthesis and covered with plastic bags. Two to three days later, the spikes were pollinated with fresh maize pollen. Pollinated spikes were then detached from the peduncular node and

placed in a baker with an aqueous solution of 100 mg L^{-1} 2, 4-D, 40 gr L^{-1} sucrose and 8ml L^{-1} sulfurous acid (6% SO_2). They are then transferred to a solution containing only sucrose and sulfurous acid and cultured until embryo rescue.

Fourteen to twenty days after pollination, excised embryos were cultured on strength MS (Murashige and Skoog 1962) medium supplemented with 20 g L^{-1} sucrose and 6 g L^{-1} agarose in test tubes. Then, embryos were incubated for 3-5 weeks at 20-25°C and 16h day length. In general, seedlings were ready to transfer after that period and need to be hardened for one week in a growth chamber under the same environmental regime.

At the 3-5 tiller stages, the colchicine treatment procedure of Inagaki (2003) was applied. According to this method, the roots of the haploid seedling were pruned leaving a zone of 2-3 cm and submerged in a 0.1% colchicine solution supplemented with 2% dimethyl sulfoxide (DMSO) and 0.05% Tween-20 at 20°C for 5 h. After this treatment the plants were then taken out of the colchicine and washed with running tap water overnight and transplanted into pots with 2:1:1 soil:sand:peat-moss mixture in a greenhouse and covered with plastic bags for 1 day. Before anthesis, fertile heads were covered with bags to avoid outcrossing. The plants were left to grow until maturity**.** Seeds from each plant were harvested separately and kept as individual DH lines.

Stem rust evaluation:

Pathogen isolates and seedling assessment

Pgt races, PTMNC and TTSTK collected in the Kelardasht region in northern Iran and race TTKSK identified in Dasht-e-Azadegan in Khuzestan province were used for seedling assessments of test genotypes at SPII, Karaj, Iran. Race identities were based on the North American race nomenclature system (Jin *et al.,* 2007). Prior to inoculation of test genotypes, differential lines and susceptible cultivar McNair were grown in peat moss at 20°C for 10 days. Urediniospores of races that were stored at -80°C were first heat-shocked at 42°C for 3 minutes and then urediniospore suspensions in light industrial mineral oil (Soltrol, 170; Phillips Petroleum Co., the Woodlands, TX) were atomized over seedlings at first-leaf stage by using a small pressure inoculator. Inoculated plants were incubated in a humid chamber at 18±2°C in darkness for 16h followed by 8h fluorescent light, and then maintained in a greenhouse at 22±2°C with 16h light/8h darkness. Two weeks after inoculation, seedlings were rated for their infection type (IT) following a 0; (flecks) and 0 to 4 scale (Stakman *et al*., 1962; McIntosh *et al.,* 1995). Plants with ITs of 0; and 0 to 2, or combinations were classified as resistant, while genotypes with ITs of 3 or greater were classified as susceptible.

Adult-plant assessment under field condition

In order to evaluate wheat genotypes in adult plant stage in field condition, a DH lines assessment was conducted in a field trial in the Kelardasht research station located in the northern Iran in cropping season 2014-2015. Each DH line was planted in 1-m rows with 30 cm spacing between rows under natural infection conditions. In order to provide enough inoculums for the uniform spread of disease on all genotypes, susceptible cultivar McNair was planted as a spreader after each 20 rows. To identify the predominant race of *puccinia graminis* in Kelardasht station, 10 leaf samples with juicy stem rust pustules were collected from infected plants for further evaluation of race nomenclature. Infection responses were recorded as resistant (R)**,** moderately resistant (MR), moderately susceptible (MS), and susceptible (S) (Roelfs *et al*., 1992) along with a percentage severity rating using the modified Cobb scale (Peterson *et al***.**, 1948). Field response was recorded at the soft-dough stage when disease severity on susceptible cultivars was 100%.

RESULTS AND DISCUSSION

Some important characteristics, such as percentage of seed set, embryo formation and haploid plantlets were studied during the DH lines production. Results for evaluated characteristics have been summarized in Table 1.

Seed set percentage was varied from 57.5% in cross W3× H2 to 90% in cross W2× H3 (Table 1). The average of seed set percentage for all populations was 73.9 % that indicates high success in this step. Percentage of seed set in our study was equal to the results of seed production in a study carried out by Suenaga *et al*. (1991). Additionally, in research conducted by Sirohi and Khanna (2003), average seed production of 85.4% was reported. Embryo formation ranged from 0% in W3× H1 to 36.6% in W3× H4. The average of embryo formation for all three populations was 9.16% (Table 1). The low percentage of embryo formation is common and also has been reported by other researchers. In a study conducted by Sirohi and Khanna (2003), the percentage of embryo formation was reported to be between 2.2 and 9.35 percent. Laurie and Bennett (1986) reported the percentage of embryo formation ranged from 13.9 to 22.5%. In research conducted by Lizarazu and Mujeeb-Kazi (1990) and Inagaki and Mujeeb-Kazi (1994) the

Table 1. Percentage of seed set, embryo formation and haploid plantlets in three populations evaluated in this study.

Evaluated traits	Cross											
	W1:Ghods*3/MV17				W2:Flanders/3*Ghods				W3:Hybrid de Bersee/*3Ghods			
	H1	H2	H3	H4*	H1	H2	H3	H4	H1	H2	H3	H4
Florets Pollinated	540	440	440	508	600	240	240	337	100	280	200	258
Number of seed set	378	277	374	452	527	166	216	231	67	161	135	186
% of seed set	70.0	63.0	85.0	88.9	87.8	69.2	90.0	68.5	67.0	57.5	67.5	72.1
Number of embryo formation	19	20	30	45	36	14	20	25	0	9	3	68
% of embryo formation	5.0	7.2	8.0	10.0	6.8	8.4	9.3	10.8	0.0	5.6	2.2	36.6
Number of haploid plantlet	14	17	24	36	23	8	14	16	0	4	2	48
% of haploid plantlet	73.7	85.0	80.0	79.6	63.9	57.1	70.0	64.0	0.0	44.4	66.7	70.6
Sum of DH plants		75				45				30		
% of DH plant formation		82.4				73.8				55.5		

*** H4: Mixture of pollen from H1, H2 and H3 maize genotypes**

production of embryos in conventional and detached-tiller culture methods, respectively, (12%, 28% and 20. 5%, 19.4%) have been reported. A higher embryo recovery (28.7%) by detached spike culture method, was reported by Lizarazu and Mujeeb-Kazi (1990).

Embryo germination can be enhanced with improved embryo culture procedures or by boosting embryo development on the crossed spikes. Keeping embryos on their attached spikes on the maternal plants for 20 days after pollination can promote better development of the embryos and thus increases the number of haploid seedlings. Prior to and after pollination other factors, such as environmental conditions, the vigor of donor plants, 2,4-D treatment and pollen grain viability, may affect embryo formation. Inagaki (1997) showed that the freshness of corn pollen grains is effective in haploid embryogenesis production of wheat. The embryo production rate of 20.4% efficiency increased by 2.8% when fresh, rather than stored, pollen grains were used.

In two different research studies conducted by Suenaga and Nakajima (1989) and Niroula *et al*. (2007), the use of 2,4-D hormone after pollination was effective in production of haploid embryos. Niroula *et al*. (2007) reported that auxin has a positive effect on embryo formation in crosses between wheat and maize.

Analysis of variances showed that the effect of wheat genotypes on seed production was significant ($P<0.01$) (Table 2). However, the effects of maize genotypes and interactions between wheat and maize genotypes were not significant. Also, the effect of wheat and maize genotypes and their impact on embryo formation and haploid plantlet production was not significant.

Stem rust evaluation

Pathogen isolates and seedling assessment

Based on the reaction of standard differential lines, two races of the Kelardasht region and a race of Dasht-e-Azadegan of Khuzestan province were designated as TTSTK, PTMNC and TTKSK, respectively. Infection responses of differential lines possessing genes specific to each race are shown in Table 3. All three races were virulent on differential lines that have *Sr,5,9e,7b,11,6,8a,9g,9a,10,McN* genes, but none of races had virulence on the differential line with the *Sr24* gene (line LcSr24Ag). Two races collected from Kelardasht had virulence on the differential line possessing the *Sr36* gene (W2691SrTt-1) but the *Pgt* race from Dashte-Azadegan was avirulent on this differential line. The formula of pathogenic/non-pathogenic races of *Puccinia graminis* f. sp. *tritici* in greenhouses are summarized in Table 4. After the designation of these three races, they were used to evaluate DH populations and their parents for stem rust resistance.

Table 2. Analysis of variance effect of wheat and maize genotypes on seed set, embryo formation and haploid plantlet.

SOV	df	MS		
		Seed set	Embryo formation	Haploid Plantlet
Wheat	2	51.5^{**}	0.88^{ns}	0.13^{ns}
Maize	3	1.5^{ns}	0.37^{ns}	0.32^{ns}
Wheat × Maize	6	1.06^{ns}	0.36^{ns}	0.22^{ns}
Error	60	3.394	1.883	1.15
Total	72			

ns,* and ** Not significant, significant at 5% and 1% probability levels, respectively.

In a study by Patpour *et al*. (2014) on the virulence of predominant Iranian stem rust races, they reported that those races were virulent on all known designated *Sr* genes except the *Sr24* gene.

The results of infection responses of DH lines, induced by three *Pgt* races PTMNC, TTSTK and TTKSK, are illustrated in Table 5. From the 75 DH lines derived from Ghods*3/MV17 (DH-26 population) that were evaluated against race PTMNC at the adult stage, 4 lines (5.33%) were resistant and 71 lines (94.66%) were susceptible. Evaluation of these DH lines at the seedling stage showed that 2 lines (2.67%) were susceptible, 47 lines (62.67%) were moderate resistant to moderate susceptible, 22 lines (29.33%) were resistant to moderate resistant and 4 lines (5.33%) were immune.

Evaluation of infection responses of DH lines at the seedling stage against race TTKSK showed that 16 lines (21.33%) were susceptible, 43 lines (57.33%) were moderate resistant to moderate susceptible, 13 lines (17.33%) were resistant to moderate resistant and 2 lines (2.66%) were immune to resistant.

Based on the resistance of the three DH lines to race PTMNC (DH-7, DH-90 and DH-91) in this population at both the seedling and adult plant stages, it can be concluded that the adult plant resistance of these lines might have been induced by a seedling resistance gene or genes.

Cluster analysis using Ward's method for infection type and coefficient of infection to race PTMNC and infection type for races TTSTK and TTKSK grouped DH lines derived from Ghods*3/MV17 into two distinct groups of resistant and susceptible (Figure 1). Based on this grouping, 27 DH lines as well as cultivars Parsi, Mihan and MV17 were located in the resistance cluster whereas 48 DH lines and susceptible check Bolani as well as

Table 3. Seedling reaction of standard differential lines to infection induced by three *Pgt* races collected from kelardasht (2 races) and Dashte-Azadegan using rating scale of Stockman *et al* (1962) and Macintosh *et al* (1995)

Differential lines																					
Name	ISr5-Ra	Cns_T_mono_deriv	Vernsatine	ISr7b-Ra	IS11-Ra	ISr6-Ra	ISr8a-Ra	CnSr9g	W2691SrTt-1	W2691Sr9b	BtSr30Wst	Combination VII	ISr9a-Ra	ISr9d-Ra	W2691Sr10	CnsSrTmp	LcSr24Ag	Sr31/6*LMPG	Trident	McNair 701
Gene	Sr5	Sr21	Sr9e	Sr7b	Sr11	Sr6	Sr8a	Sr9g	Sr36	Sr9b	Sr30	Sr17	Sr9a	Sr9d	Sr10	SrTmp	Sr24	Sr31	Sr38	SrMcN
Minimum infection type expected	0;0;	1;2-	;1^{+}	2	;2-;2+3-	0;0;	2	2-	0;0;;X	22^{+}	2	0;;1	2-;23	;2-	;1^{+}	2-	2	;1^{+}	1	;1
Pgt races	Observed infection type																			
Dasht-Azadegan	4	4	4	3	4	4	4	4	0;	4	4	4	4	4	4	2+3-	2CN	4	4	4
Klardasht 1	4	4	4	4	3?	4	4	4	4	4	4	X+?	4	4	4	4	;2CN	4	3^{+}	4
Klardasht 2	3	;2C	3	3	3	4	3^{+}	4	4	;2^{+}	X	4	3^{+}	2^{+}	4	;XC	;1^{+}	;2C	;2^{+}	3^{+}

Table 4. Formula of pathogenic / non-pathogenic races of stem rust diseases *Puccinia graminis* f. sp. *tritici* in green house.

Isolate	Race	Formula of non-pathogenic/pathogenic
94-15	PTMNC	Sr21,9b,30,9d,Tmp,24,31,38/5,9e,7b,11,6,8a,9g,36,17,9a,10,McN
92-10	TTSTK	Sr17,24/5,21,9e,7b,11,6,8a,9g,36,9b,30,9a,9d,10,Tmp,31,38,McN
88-4	TTKSK	Sr,24,36,Tmp/5,21,9e,7b,11,6,8a,9g,9b,30,17,9a,9d,10,31,38,McN

Table 5. Reaction of DH lines induced by three *Pgt* races PTMCN, TTSTK and TTSTK at the seedling and adult plant stages.

Race	PTMNC						TTSTK				TTKSK			
Population	Adult Plant		Seedling				Seedling				Seedling			
	0-MR	MS-S	0 ; 1	2	3	4	0 ; 1	2	3	4	0 ; 1	2	3	4
W1	4	71	4	22	47	2	0	5	53	16	2	13	43	16
%	5.3	94.7	5.3	29.3	62.7	2.7	0	6.7	70.7	21.3	2.7	17.3	57.3	21.3
W2	14	31	3	15	26	1	0	4	24	14	1	7	26	8
%	31.1	68.9	6.7	33.3	57.8	2.2	0	8.9	53.3	31.1	2.2	15.6	57.7	17.8
W3	23	7	19	5	6	0	2	3	19	6	0	4	12	14
%	67.7	23.3	63.3	16.7	20	0	6.7	10	63.3	20	0	13.3	40	46.7
Total	41	109	26	42	79	3	2	12	96	36	3	24	81	38
Total %	27.3	72.7	17.3	28	52.7	2	1.3	8	64	24	2	16	54	25.3

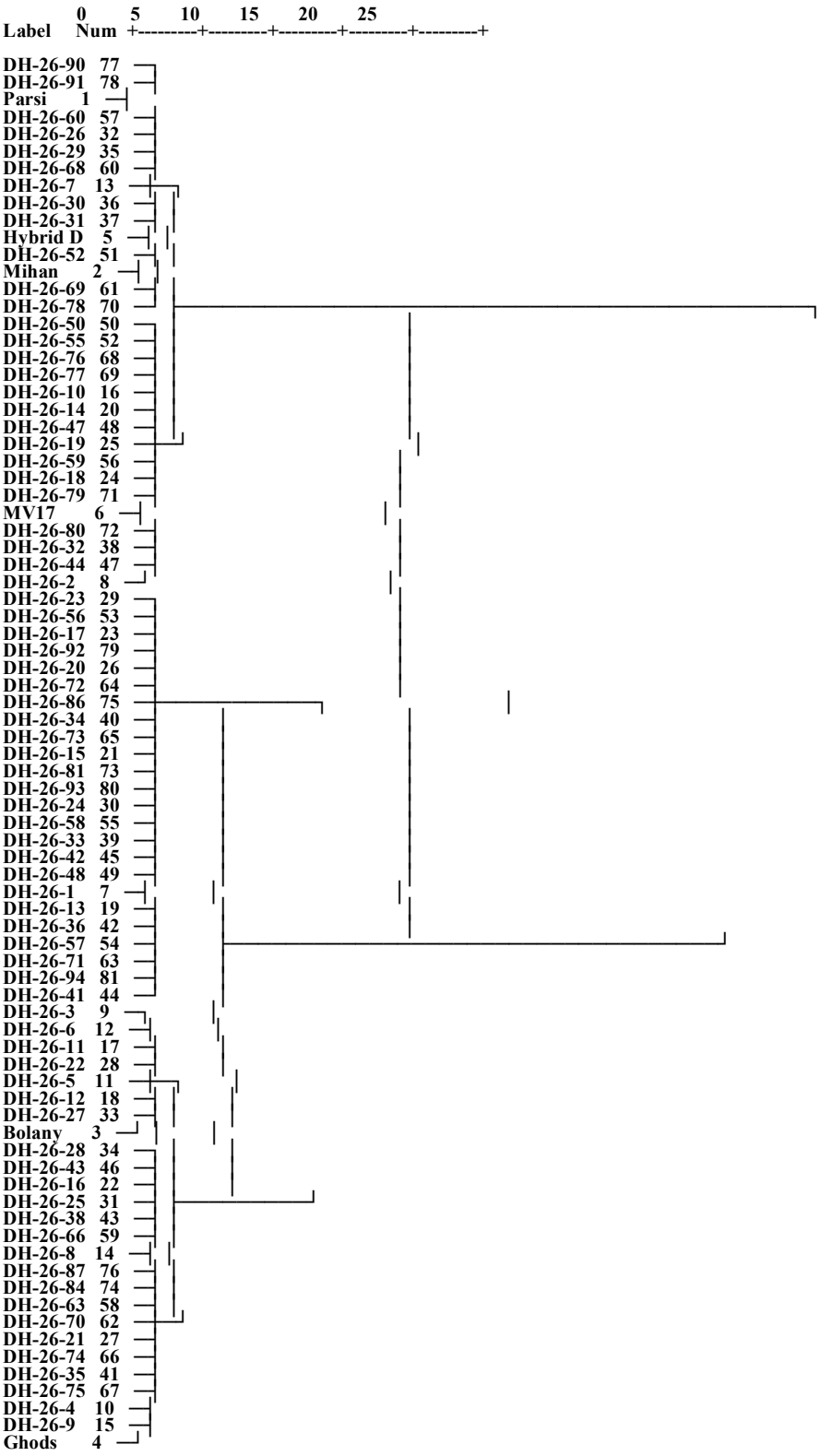

Fig. 1. Grouping of DH lines of population Ghods*3/MV17 based on coefficient of infection (CI) and infection type (IT), induced by *Pgt* races PTMNC, TTSTK and TTKSK by cluster analysis using ward's method

susceptible parent cultivar Ghods were located in the susceptible cluster. In total, based on this grouping, 36% of the DH lines were resistant and 64% of them were susceptible.

From the 45 DH lines derived from Flanders/3*Ghods (DH-27 population) that were evaluated against race PTMNC at the adult stage, 14 lines (31.11%) were resistant and 31 lines (68.88%) were susceptible. Evaluation of these DH lines at the seedling stage showed that 1 line (2.22%) was

susceptible, 26 lines (57.78%) were moderate resistant to moderate susceptible, 15 lines (33.33%) were resistant to moderate resistant and 3 lines (6.67%) were immune.

Evaluation of infection responses of DH lines at the seedling stage against race TTSTK showed that 14 lines (31.11%) were susceptible, 24 lines (53.33%) were moderate resistant to moderate susceptible and 4 lines (8.88%) were resistant to moderate.

Based on the resistance of three DH lines to race PTMNC (DH-10, DH-50 and DH-60) in this population at both the seedling and adult plant stages, it can be concluded that the adult plant resistance of these lines might have been induced by a seedling with a resistant gene or genes.

Evaluation of the infection responses of DH lines at the seedling stage against race TTKSK showed that 8 lines (17.77%) were susceptible, 26 lines (57.77%) were moderate resistant to moderate susceptible, 7 lines (15.55%) were resistant to moderate resistant and 1 line (2.2%) were immune to resistant. Cluster analysis using Ward's method for infection type and coefficient of infection to race PTMNC and infection type for races TTSTK and TTKSK grouped DH lines derived from Flanders/3*Ghods into two distinct groups of resistant and susceptible (Figure 2). Based on this grouping 26 DH lines as well as cultivars Parsi, Mihan and MV17 were located in the resistant cluster whereas 19 DH lines and susceptible check Bolani as well as susceptible parent cultivar Ghods were located in the susceptible cluster. In total, based on this grouping, 57.8% of DH lines were resistant and 42.2 % of them were susceptible.

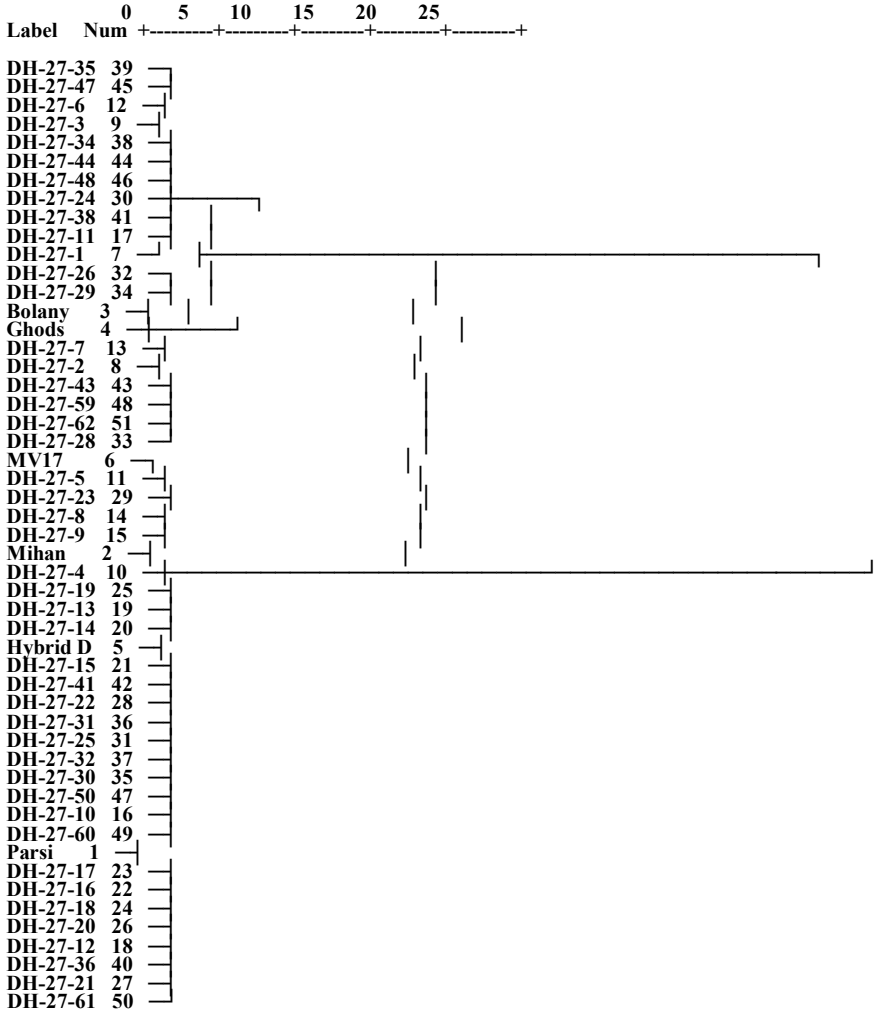

Fig. 2. Grouping of DH lines of population Flanders/3*Ghods based on coefficient of infection (CI) and infection type (IT), induced by *Pgt* races PTMNC, TTSTK and TTKSK by cluster analysis using ward's method.

From the 30 DH lines derived from Hybrid de Bersee/*3Ghods (DH-28 population) that were evaluated against race PTMNC at the adult stage, 23 lines (67.66%) were resistant and 7 lines (23.33%) were susceptible. Evaluation of these DH lines at the seedling stage showed that 6 lines (20%) were

moderately resistant to moderately susceptible, 5 lines (16.67%) were resistant and 19 lines (63.33%) were immune to resistant. Evaluation of infection responses of DH lines at the seedling stage against race TTSTK showed that 6 lines (20%) were susceptible, 19 lines (63.33%) were moderate resistant to moderate susceptible, 3 lines (10%) were resistant and 2 lines (6.66%) were resistant to moderate resistant. Evaluation of infection responses of DH lines at the seedling stage against race TTKSK showed that 14 lines (46.66%) were susceptible, 12 lines (40%) were moderate resistant to moderate susceptible and 4 lines (13.33%) were resistant to moderate resistant.

In this population, 11 DH lines with a reaction that was moderately resistant to moderately susceptible in the seedling stage, have an immune reaction in the adult stage. This population also has greater numbers of immune and resistance lines than other populations.

Cluster analysis using Ward's method for infection type and coefficient of infection to race PTMNC and infection type for races TTSTK and TTKSK grouped DH lines derived from Hybrid de Bersee/*3Ghods into two distinct groups of resistant and susceptible (Figure 3). Based on this grouping, 27 DH lines as well as cultivars Parsi, Mihan, Hybrid de Bersee and MV17 were located in the resistance cluster whereas 3 DH lines and susceptible check Bolani as well as susceptible parent cultivar Ghods were located in the susceptible cluster. In total, based on this grouping, 90% of the DH lines were resistant and 10% of them were susceptible.

The reactions of 62 Iranian commercial bread wheat cultivars induced by *Puccinia graminis* races including TTSTC, TTTTF, TRTFC, TTKSK (Ug99), TTTTC and TKTTC at the seedling and adult plant stages were tested (Patpour *et al*., 2014). Among these wheat cultivars, 39 (63%) were identified without any effective resistance against all tested races, whereas 13 (21%) were resistant to one or more races but susceptible to others, and 10 (16%) cultivars including Rasool, Falat, Golestan, Atrak, Shiroodi, Dez, Zagros, Bahar, Gonbad and MV17 were susceptible to TTKSK (Ug99) and resistant to other races (Patpour *et al*., 2014). From 150 DH lines that were evaluated against race PTMNC in the adult plant stage in this study, 41 lines were resistant and 109 lines were susceptible. Evaluation of the DH populations at the seedling stage showed that a large number of DH lines were susceptible to races TTSTK (123 lines) and TTKSK (119 lines) while more than 68 lines were resistant to race PTMNC.

The results of this study showed that more than 50% of resistant DH lines belong to DH-28

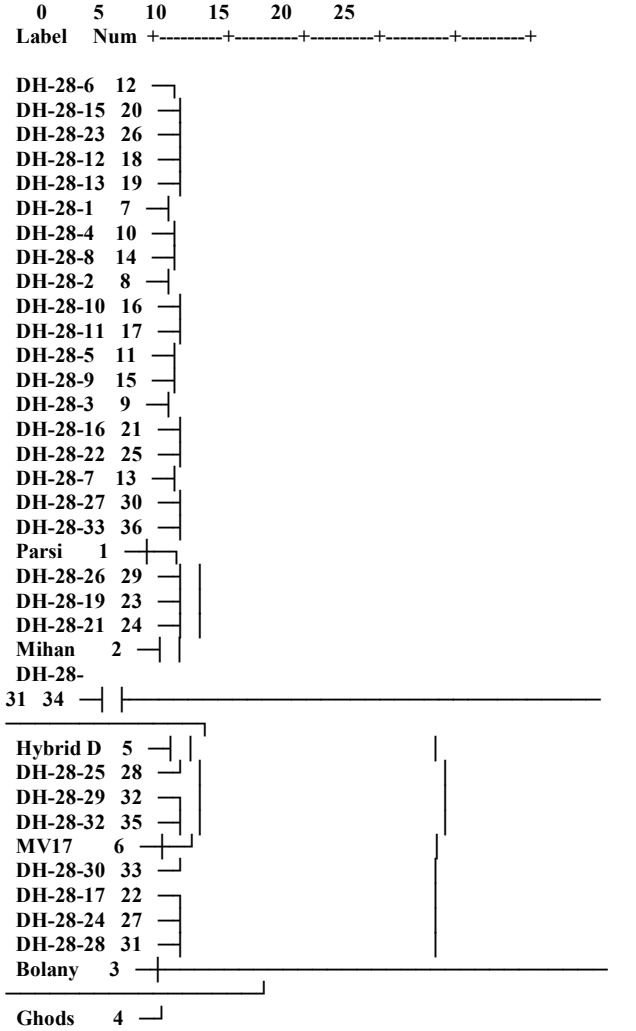

Fig. 3. Grouping of DH lines of population Hybrid de Bersee/*3Ghods based on coefficient of infection (CI) and infection type (IT), induced by *Pgt* races PTMNC, TTSTK and TTKSK by cluster analysis using ward's method.

population with pedigree of Hybrid de Bersee/*3Ghods. Based on previous studies, the frequency of stem rust resistance genes that are effective against Ug99 races is low in Iranian wheat germplasm (Mohammadi *et al*., 2013; Patpour *et al*., 2014; Mehrabi *et al*., 2014). Our results showed that by selecting appropriate parents of a cross, haploid breeding can accelerate efficiency of the selection for stem rust resistance by increasing the frequency of the favorite genes in the breeding populations to improve advanced breeding lines with superior stem rust resistance.

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