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Full Length Research Paper

Wheat yellow rust resistance improvement in wheat and maize cross progenies using double haploid method

Tayebeh Bakhshi[1]*, Reza Bozorgipour[2], Khodadad Mostafavi[3] and Hamed Haddad Kashani[4]

[1]Departeman of Agronomy and Plant Biotechnology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]Seed and Plant Improvement Institute, Karaj, Iran.
[3]Department of Agronomy and Plant Breeding, Karaj Branch, Islamic Azad University, Karaj, Iran.
[4]Anatomical Sciences Research Center, Kashan University of Medical Sciences, Kashan, Iran.

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This research was conducted to produce doubled haploid lines of wheat through chromosome elimination (wheat × maize crosses) technique. Three wheat hybrids: DH-29: MILAN / SH7 // SHIROODI, DH-30: INIA // SW89.3064 / STAR / 3 / MILAN / SHA7 and DH-32: NANJING8201 / KAUZ // MILAN / SHA7 were pollinated with maize pollen from two genotypes H1: KSC108, 403 and combination of pollen of them, to produce doubled haploid lines. Fertilization frequencies ranged from 87.35% in H1 genotype of maize to 92.96% in 403 genotype of maize (mean 90.15%) was significantly different among the maize genotypes. Embryo formation in the dissected seeds ranged from 93.59% in DH-29 genotype of wheat to 27.18% in DH-32 genotype. Out of the 517 cultured embryos (mean 27.18%), 80.69% germinated and 69.93% of them developed into plants. After colchicines treatment, 53.06% of plants survived and their seeds were harvested. Significant variation was found among wheat and maize progenies in the frequency of haploid plants. Analysis of variance showed significant differences among genotypes, for all traits. Overall, these results suggest that the doubled haploid system could be used as an effective means to produce desirable resistant lines in the shortest time.

Key words: Wheat × maize cross, doubled haploids, yellow rust resistance.

INTRODUCTION

In breeding among plant varieties is the valuable technique in plant genetics and plant improvement programs. In some cross-incompatibility of the chromosome leads to omission of the male chromosome in the beginning of the growth that after saving of the embryo and transfer to artificial culture, the seedling is produced. Haploid is a common word used for identification of an organism with gamete chromosome (half of natural chromosomes for each specie). According to this fact that haploid plant is formed from embryonic sag gamete cell or from germinated seed, it can be independent saprophyte by chromosomes equal to own gametophyte (Jain et al., 1996). This system was reported by Kasha and Kao (1970) in cross of *Hordeum bulbosume* × *Hordeum vulgare* and then by using this technique the haploid was produced in wheat (Stich dna Snape, 1986). Wheat haploid was produced in 1986 to 1987 by inbreeding with corn (Laurie dna Bennet, 1986, 1987). Recently the best method is crossing of wheat with corn since other methods like culture of stamen and technique of bulbosume have disadvantages like genetic dependency; it seems that this method is not affected by wheat genotype. Yellow rust is the main disease in Iran, west and central Asia. Epidemics of this disease caused considerable damages about loss of 1.5 million ton wheat in 1993 and 1995. So access to resistance resources of different genes of yellow rust and cultivation of the resistance cultivars is the best way to control the disease and reduction of the damages (Johnson, 1993). Haploid improvement method is the best way for production of the resistance cultivars against yellow rust disease, since accelerates improvement plants (Snape, 1989).

*Corresponding author. E-mail: adelehbakhshi@yahoo.com.

Table 1. Comparison of the studied traits in cross of the wheat genotypes and maize genotypes H1.

Studied traits	Number (% based on germinated floret)			
	20 × H1	21 × H1	23 × H1	χ^2
Germinated floret	129(100)	132(100)	182(100)	-
Produce seed	108(83.72)	124(93.93)	155(85.16)	4.89^{ns}
Produced embryo	24(18.60)	34(25.75)	36(19.78)	12.33*
Germinated embryo	14(10.85)	33(25)	6(3.29)	10.19*
Haploid seedling	4(3.10)	31(23.48)	5(2.74)	10.37*
Double haploid seedling	4(3.10)	31(23.48)	4(2.19)	12.82*

*, ns, significant at 5% level and not significant, respectively.

MATERIALS AND METHODS

Plant materials

In this research three genotypes of wheat was used from DH-29, DH-30, DH-32 as a mother of parent. The variety 'Bolani' was used as control for evaluation of susceptibility to rust resistance in seedling stage. The maize genotypes used as pollinators were 403, H1 and combination of pollen of them.

Emasculation and pollination

Seeds of the parent genotypes were sown in 3-inch pots. After germination, the pots were transferred to greenhouse. Emasculation and pollination were carried out in greenhouse according to the method of Laurie and Bennet (1986).

Treatment of spikes with 2, 4-D

Twenty four hours after pollination, 5 ml of a solution of 2, 4-D (10 mg/l) was injected into the stems of the donor plants and a drop of the same solution was also placed into each pollinated floret. Treatment of spikes with 2, 4-D enabled the embryos to remain alive until they were large enough to survive the shock of the conventional embryo rescue procedures (Laurie and Bennet, 1986, 1987).

Embryo rescue

The spikes were transferred to laboratory 16 to 18 days after pollination, and the embryos were excised under stereomicroscope in a laminar flow hood. The excised embryos were transferred to vials containing MS (Murashige and Skoog, 1962) basal medium supplemented with 20 g sucrose/L and 8 g agar /L (Zhang et al., 1996; Inagaki, 1997). Embryos that developed into plantlets with 10 cm long stems were transferred to small pots. Ten days after transfer to the pots, chromosomes were counted in root tips.

Colchicine treatment

Roots of the haploid plants with 3 to 4 tillers were treated with 0.05% w/v colchicine solution for 5.5 h. The roots were washed after treatment and the plants replanted in pots (Mujeeb-kazi et al., 1995).

Test for yellow rust resistance at seedling stage

Sixty four DH lines and the 'Bolani' as a control were tested using race 6E150A^+ in a completely randomized design with unequal replications (base replications were 3) and treatments were used wheat DHs as well as parents. Observations were recorded on infection type (IT), latent period (LP), pustule size (PS), and pustule density (PD) after inoculation of seedlings with urediospores (Knott, 1989; Mc Neal et al., 1971; Cromey, 1992; Torabi et al., 1995).

Statistical analysis

Observations were recorded on percentage of seed set, embryo formation, haploid plantlets and DH obtained. To evaluate the effect of wheat and maize genotypes on haploid production, two-way analysis of variance and mean separation was carried out by the MSTAT-C statistical package. Appropriate data transformation was applied when it was needed. Duncan analysis was carried out by SPSS software.

RESULTS AND DISCUSSION

Study of the studied traits in crossing of wheat genotypes and maize genotype H1

As shown in Table 1, each three genotypes of the wheat with maize H1 were crossed and the traits were noted. In DH-29×H1, DH-30×H1, DH-32×H1 among four traits of the embryo: the germinated embryo, haploid, seedling and double haploid plant and a significant difference was not observed. By fixing of father and mother genotype effect on germination. It is probable that some part of this deference relates to environment. There is no meaningful difference among production of the haploid seedling, and double haploid plant.

Investigation of the studied traits in cross of wheat genotypes and maize genotype 403

According to Table 2, three wheat genotypes of DH-29, DH-30, DH-32 were germinated with corn genotype 403. After χ^2, it was identified that there is a significant difference in three traits of the produced seed and double haploid seedling in 95% and there is a significant difference in double haploid plant in 99%. Investigation of the studied traits in cross of wheat genotypes and combination of pollen of maize genotypes (403, H1)

Table 2. Comparison of the studied traits in cross of the wheat genotypes and maize genotypes 403.

Studied traits	Number and percentage of germinated floret			
	20 × 403	21 × 403	23 × 403	χ^2
Germinated floret	310(100)	347(100)	239 (100)	-
Produced seed	288(92.90)	320(92.21)	225(94.14)	8.11*
Produced embryo	21(6.77)	152(43.80)	65(27.19)	5.18^{ns}
Germinated embryo	21(6.77)	142(40.92)	60(25.10)	3.35^{ns}
Haploid seedling	20(6.45)	123(35.44)	58(24.26)	7.02*
Double haploid seedling	9(2.90)	62(17.80)	27(11.29)	21.62**

**, ns: significant at 1% level and not significant, respectively.

Table 3. Comparison of the studied traits in cross of wheat genotypes and combination of pollen of maize genotypes (403, H1).

Studied traits	Number and percentage of germinated floret			
	20 × combination	21 × combination	23 × combination	χ^2
Germinated floret	182 (100)	302 (100)	192 (100)	-
Produced seed	155 (85.16)	287 (95.03)	168 (87.5)	8.11*
Produced embryo	42 (23.07)	92 (30.46)	51 (26.56)	5.18^{ns}
Germinated embryo	39 (21.42)	85 (28.14)	48(25)	3.35^{ns}
Haploid seedling	39 (21.42)	82 (27.15)	46 (23.95)	7.02*
Double haploid seedling	14 (7.69)	56 (8.60)	27 (14.06)	21.62**

*, ns: significant at 5% level and not significant, respectively.

Table 4. The effect of maize genotype on studied traits in cross of different wheat genotypes.

Corn genotype (%)	Germinated floret (%)	Produced seed (%)	Produced embryo (%)	Germinated embryo (%)	Haploid seedling (%)	Double haploid seedling (%)
	676	610(90.23)	185(30.32)	172(92.97)	172(92.97)	97(90.65)
403	896	833(92.96)	238(28.57)	223(93.69)	223(93.69)	98(48.75)
H1	443	387(87.35)	94(24.28)	43(45.74)	43(45.74)	39(43)
total	2015	1830(89.94)	5.7(27.72)	438(77.44)	438(77.44)	234(60.8)
χ^2	-	15.6**	2.47^{ns}	9.68*	9.68*	0.72^{ns}

*, **, ns: significant at 5 and 1% level and not significant, respectively.

Three wheat genotypes of DH-29, DH-30, DH-32 were germinated with combination of pollen of maize genotypes (403, H1). There is a significant difference in three traits of the produced seed, double haploid seedling and elbuod tnalp diolpah in 99% (Table 3).

According to the aforementioned results and Constance of the father it can be inferred that mother affects produced seed, double haploid seedling and double haploid plants.

The effect of father genotype

The result of Table 4, show that the percentage of the produced seed by using combination of the maize seeds of H1 and 403 are as follows: 90.23, 87.35 and 92.96%. After calculation of the χ^2 and statistics comparison, there was a significant difference among different genotypes of the maize in produced seed in 99% and the father effects on this trend. The percentage of the produced seed in different genotypes of corn was variable in 76.4, 63.2 and 93.1% (Matzk and Mahn, 1994). There was no farther effect on embryo since there is no significant difference among produced embryo.

The percentage of the production of the embryo in hybrids of H1, 403 by average of 77.46% is 30.32, 24.28 and 28.57% respectively. Matzk and Mahn (1994) used different genotypes of corn in germination and in each experiment the maximum and minimum percentage related to the different parents. Cultivar DT108 had the

Table 5. The effects of the wheat genotype on studied traits in cross with different maize genotypes.

Corn genotype (%)	Germinated floret (%)	Produced seed (%)	Produced embryo (%)	Germinated embryo (%)	Haploid seedling (%)	Double haploid seedling (%)
20	621	551(88.72)	87(15.78)	64(73.56)	63(98.43)	27(42.85)
21	781	731(93.59)	278(38.03)	260(93.52)	236(90.76)	149(63.13)
23	613	548(89.39)	52(27.73)	114(75)	109(95.61)	58(53.21)
Total	2015	1830(90.56)	517(27.18)	438(80.69)	408(69.93)	234(53.06)
χ^2	-	6.42^{ns}	10.37*	4.27*	4.21^{ns}	3.51^{ns}

*, ns: significant at 5% level and not significant, respectively.

Table 6. Balanced randomly design variance analysis of different traits relative to $6E150A^+$

S.O.V	df	MS			
		Latent period	Infection type	Pustule size	Pustule density
Genotype	63	78**	23.69**	10.86**	8.40**
Error	128	9.75	2.92	1.27	0.84

**: significant at 1% level.

highest percentage in experiment (2) and it had the lowest percentage in experiment (3). This difference is as a result of seasonal and greenhouse climate changes. Suenaga et al. (1991) experimented Japanese and American 47 wheat verities and 55 maize varieties and concluded that the percentage of the formation of the embryo is the same and maize genotype did not affect the embryo. There is no relationship among percentage of formation of the embryo, length of the stem, length of the tassel or number of the branch. It can be selected easily hybrid F1 of maize among these genotypes by the highest percentage of formation of the embryo (Suenaga, 1991). According to Table 4, father genotype affects significantly on growth and there is a significant difference in embryo reproduction among different maize genotypes by cross with wheat. The minimum and maximum percentage is 45.74% in H1 and 93.69% in 403 by the average embryo growth of 69.71%.

Zhang et al. (1996) obtained 58.25% maize genotype effects on embryo reproduction. According to Table 4, when different genotypes of the wheat are crossed by maize seeds genotypes 403 and H1, 90.65, 48.75 and 43.27% produced double haploid plants that there is no meaningful difference. In other words, double abundance of the haploid, does not depend on corn genotype. Among studied genotypes, 403 is the best genotype for production of the double haploid plants. This finding is in agreement with Moradi results that in cross of different wheat genotypes with maize seeds from H1 and 403, 40.91, 53.12% was obtained without significant difference.

The effect of mother genotype

According to Table 5, three wheat genotypes were used by germination of the 2015 florets. From this amount, the percentage of produced seeds in DH-29, DH-30, DH-32 were 88.72, 93.59 and 89.39% respectively. There is no significant difference. Mother does not affect this trend. Father had significant effect on produced seed. It can increased haploid production by selection of the father genotype. But from formation of the embryo view point 15.78% in genotype DH-29, 38/03% in DH-30 and 22.73% in DH-32 were observed. The significant difference was obtained after χ^2 test. The regeneration trait is affected by mother genotype and the deference is as a result of wheat genotype.

Table 5 shows that there is no significant difference in production of the seedling. The average of the haploid seedling is 94.93% it means that averagely in each three wheat genotype from 100 germinated embryos only 94.93% was transferred to pot and the other 5.07% was destroyed because of fungal infection and improper environmental condition.

The percentage of double haploid seedling in genotypes DH-29, DH-30, DH-32 was 42.85, 63.13 and 53.21% respectively. There is no significant difference, mother does not affect this trend. Generally, among three mother genotypes, DH-30 is better in formation of the embryo and reproduction traits.

Evaluation of the resistance of wheat double haploid lines against yellow rust

In this research 64 lines were produced besides the parents and one control cultivar (sensitive Bolani cultivar) in balanced randomly block design and the results are reported in Table 6. The result of the analysis of variance in 64 double haploid lines produced relative to $6E150A^+$

Table 7. Comparison of different traits in doubled haploid wheat lines in greenhouse conditions to race 6E150A^{+}

Number of line	Name of line	Infection type	Latent period	Pustule size	Pustule density
1	PWS-N-3	7 ABC	12 CD	4.3 ABCDEFG	3.8 ABCDE
2	PWS-N-5	0 F	25 A	0 I	0 I
3	PWS-N-7	2 DEF	21.6 AB	0.66 I	0.50 HI
4	PWS-N-8	0 F	25 A	0 I	0 I
5	PWS-N-9	1.6 DEF	22 AB	0.93 HI	0.66 HI
6	PWS-N-11	0 F	25 A	0 I	0 I
7	PWS-N-12	0 F	25 A	0 I	0 I
8	PWS-N-13	1.6 DEF	22 AB	0.96 HI	0.63 HI
9	PWS-N-15	0 F	25 A	0 I	0 I
10	PWS-N-17	7 ABC	12 CD	4.8 ABC	3.7 ABCDEF
11	PWS-N-18	2 DEF	20.3 AB	1.5 GHI	1.4 GHI
12	PWS-N-19	7.3 AB	11.6 CD	4.5 ABCDEF	3.5 ABCDEFG
13	PWS-N-23	7.6 A	11.3 D	4.7 ABCD	4.4 ABC
14	PWS-N-24	7 ABC	12 CD	4.5 ABCDEF	3.9 ABCD
15	PWS-N-25	0 F	25 A	0 I	0 I
16	PWS-N-26	7.6 A	11.3 D	5.2 AB	4.4 ABC
17	PWS-N-29	2.6 CDEF	19.6 ABC	1.6 FGHI	1.2 HI
18	PWS-N-30	1 EF	22.6 AB	0.73 HI	0.66 HI
19	PWS-N-31	2.6 CDEF	19.6 ABC	1.9 DEFGHI	1.5 FGHI
20	PWS-N-33	0 F	25 A	0 I	0 I
21	PWS-N-34	3 BCDEF	19.3 ABCD	1.9 DEFGHI	1.1 HI

which shows that there is a significant difference among latent period, infection type, pustule size and pustule density. In other words there is a difference among all experimented lines and they have diverse resistance. This diversity has been shown in Table 7 by comparison of average of multi scope Duncan test.

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