International Journal of Plant Production 3 (3), July 2009
ISSN: 1735-6814 (Print), 1735-8043 (Online)
This is a refereed journal and all articles are professionally screened and reviewed.

GUASNR
www.ijpp.info

Development of yellow rust resistant doubled haploid lines of wheat through wheat × maize Crosses

P. Moradi[a,*], A. Haghnazari[b], R. Bozorgipour[c], B. Sharma[d]

[a]*Research Center of Agriculture and Natural Resources, Zanjan, Iran*
[b]*Department of Agronomy & Plant Breeding, College of Agriculture, Zanjan University, 313, Zanjan, Iran*
[c]*Seed and Plant Improvement Institute, Karaj, Iran*
[d]*Division of Genetics, Indian Agricultural Research Institute, 110012 New Delhi, India*
[*]corresponding author; Email: parvizmoradi@gmail.com

Received 17 November 2008; Accepted after revision 24 May 2009; Published online 10 July

Abstract

In order to produce doubled haploid lines of bread wheat resistant to strip or yellow rust, three F_1 wheat hybrids were crossed using pollens of three maize hybrids. Out of 1071 pollinated florets, success in seed set ranged from 63.1% to 93.3% (mean 78%). Differences in seed set among the crosses were not significant. Embryo formation in the seeds developed on different crosses also varied from 17.2% to 60.7% (mean 27.4%). Embryos continued to develop up to 16 days when 2, 4-D was applied after pollination. One hundred three out of 211 embryos cultured, of which 42.7% developed into plants. Colchicine treatment resulted in diploidization and seed production on 56% of plants. Significant variation was observed among wheat genotypes in the frequency of DH plants (overall success). There were no significant differences among crosses dependent on various maize genotypes, indicating non-influence of maize genotypes. Response to yellow rust of 51 DH lines was assessed along with two parental cultivars, 'MV17' (a Hungarian cultivar resistant to the yellow rust pathotypes prevalent in Iran), and 'Falat' (a susceptible cultivar) and the check variety 'Bolani' at seedling stage in greenhouse. Resistance was evaluated in terms of infection type, latent period, pustule size, and pustule density using the most virulent race of yellow rust in North- west of Iran, 166E134A+. Analysis of variance showed significant differences among the DH genotypes for all the resistance parameters. The results demonstrated the high effectiveness of the method to produce yellow rust resistant homozygous wheat genotypes in short time by doubling of haploid lines generated through pollination of wheat F_1 plants with maize pollens.

Keywords: wheat × maize hybridization; doubled haploids; embryo rescue; yellow rust resistance

Introduction

Stripe or yellow rust, caused by *Puccinia striiformis* West. f. sp. *tritici*, is one of the most important diseases of bread wheat (*Triticum aestivum* L.) in temperate areas of the world, particularly those with cool, maritime climates such as northern Europe and north-western America. This disease is also common at high altitudes in some subtropical

regions, including parts of East Africa, Central and South America, and the Indian subcontinent. The disease has also been recorded in the warm and arid areas of Egypt, Iran, and Turkey (Imtiaz et al., 2003). In Iran, it is the most important wheat disease. According to Torabi et al., (1995), this disease caused a loss of 1.5 million tons in wheat production of Iran in 1993.

Although chemical control can be very effective and is the preferred means of control in some areas, resistance remains a major objective for many breeding programs, especially in lower yielding regions and developing countries. Resistance to rust diseases may comprise genes effective at the seedling and adult growth stage, genes effective at the post-seedling and adult stages only, or combinations of both types. Genes effective at the seedling stage tend to contribute greater effects on phenotype. Seedling-effective resistance genes are present in cultivars and breeding populations in different parts of the world (Eriksen et al., 2004).

Creating sources of resistance to different pathotypes of yellow rust and developing new resistant cultivars could be the most urgent task to protect the wheat crop from this devastating disease. Among various approaches (backcross, pedigree method, bulk method, multiline breeding and so on), haploidy breeding is the most appropriate biotechnological way to produce cultivars resistant against yellow rust because of its advantages; speed, and selection efficiency (Snape, 1989; Moeini, 1997). Production of haploid wheat plants through interspecific hybridization and subsequent production of dihaploids (doubled haploids) can fix desirable characters in a single generation. Utilizing this technique, attempts have been made to obtain variation in resistance to yellow rust without drastically altering positive agronomic characteristics of the original genotypes. This is one of the most useful techniques in gene transfer experiments (Mehta and Angra, 2000).

By this technique, chromosomes of the male gamete are eliminated in the developing embryo due to cytoplasm–chromosome incompatibilities, and haploid plants can be produced through embryo rescue (Laurie and Bennet, 1989; Laurie and Bennet, 1990; Berzonsky et al., 2003; Mochida et al., 2004). Kasha and Kao (1970) first applied this procedure in *H. vulgare* × *H. bulbosum* crosses. Using the *bulbosum* technique, Barclay (1975) produced bread wheat haploids. Subsequently, Laurie and Bennet (1986, 1987) reported production of wheat haploids by crossing with maize. This method is being used in a large scale as it is believed to be applicable to all wheat genotypes while other methods, such as anther culture is genotype dependent and microspore culture needs complex medium. Furthermore, the *bulbosum* technique is used only for genotypes which have recessive alleles of *kr1* and *kr2* at the crossability loci on chromosomes 5A and 5B (Sitch and Snape, 1986; Laurie and Bennet, 1987; Singh et al., 2004; Bakos et al., 2005) and the use of anther culture is limited by an overall low level of haploid production and frequent albinism in regenerants. The wheat × maize hybridization technique is less genotype-dependent with no albinism; and the ease with which it can be applied makes it more efficient than anther culture for the production of haploids in common wheat (Sadasivaiah et al., 2001).

`Here we report the results of study to produce yellow rust resistant doubled haploid lines of wheat through pollination of wheat F_1 plants with maize pollens.

Materials and Methods

Plant materials

Three F1 hybrids of wheat (*Triticum aestivum* L.) were: 'Falat'×'MV17', 'MV17'× 'Falat', and 'Sabalan*2' × 'Kal-Blo"s"'. 'Falat' (Kvz/Buho/Kal/Bb), a commercial variety of Iran, is susceptible to yellow rust. 'MV17' (Salvia/MVTE/ZG4431) is a Hungarian cultivar resistant to the yellow rust pathotypes prevalent in Iran. Seeds of the hybrid 'Sabalan*2' × 'Kal-Blo"s"' were obtained from the Dryland Research Institute of Iran (Sabalan*2 and Kal-Blo"s" were susceptible and resistant genotypes to yellow rust, respectively). The variety 'Bolani' was used as control for evaluation of susceptibility to rust resistance in seedling stage. The maize genotypes used as pollinators were single cross hybrids 'KSC 108', 'KSC 301', and 'KSC 704' obtained from Yugoslavia.

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). Totally 1071 florets were pollinated, including 644 florets of 'Falat' × 'MV17', 246 of 'MV17' × 'Falat', and 181 of 'Sabalan*2' × 'Kal-Blo"s"'.

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; Laurie and Bennet, 1987).

Embryo rescue

The spikes were transferred to laboratory 16–18 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/lit and 8 g agar /lit (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. One hundred eleven embryos were rescued in the FM, 46 in MF, and 54 in KS crosses

Colchicine treatment

Roots of the haploid plants with 3–4 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

Fifty one DH lines, two parental cultivars ('MV17' and 'Falat') and the 'Bolani' as a control were tested using pathogen race 166E134A+ 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).

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. Cluster analysis was carried out by JMP ver. 3.1.2.

Results

Seed set and caryopsis

In total 1071 wheat florets crossed with the pollens of maize bore caryopses at 15 days after pollination that were smaller than those obtained by self pollination. The caryopses were filled with an aqueous solution instead of the solid endosperm normally found in the wheat, and some contained immature embryos. As shown in table 1, seed set (No. of grains obtained out of florets pollinated) varied from 63.1% to 93.3% with the average of 78.1%. In other words 78.1% of pollinated florets formed caryopses with the lowest number in 'KSC704'× 'FM' cross and the highest one in the cross 'KSC301'× 'SK', respectively.

Table1. Frequency of seed set (% of pollinated florets) in each of the crosses.

Genotypes	'FALAT' × 'MV17'	'MV17' × 'FALAT'	'Sabalan*2' ×'Kal-Blo s'	Total
KSC 108	185 (86.6)	70 (85)	55 (85.9)	310 (86.1)
KSC 301	126 (81.2)	47 (69.1)	38 (93.3)	211 (78.7)
KSC 704	174 (63.1)	65 (68.3)	51 (70.1)	290 (65.7)
Total	485 (75.3)	182 (74)	144 (79.6)	811(75.7)

Embryo formation

Lack of endosperm in the caryopses harvested served as the initial criterion for identifying haploid embryos. There small embryos were observed floating in watery embryo sacs, from where they were rescued onto the MS medium. A total of 211 embryos (26% of caryopses) were rescued and transferred to vials containing support medium. The obtained embryos grew well and formed normal shoots and roots. As shown in table 2 embryo formation (No. of embryos obtained from seeds) ranged from 17.2% in the 'MF' × 'KSC301' cross to 60.7% in 'SK' × 'KSC704' with an average of 27.4%.

Table 2. Frequency of embryo formation (% of seed set) in each of the crosses.

Genotypes	'FALAT' × 'MV17'	'MV17' × 'FALAT'	'Sabalan*2' ×'Kal-Blo s'	Total
KSC 108	43 (25.7)	18 (33.8)	20 (18.2)	81 (26.1)
KSC 301	24 (23.2)	10 (17.2)	12 (25)	46 (21.8)
KSC 704	44 (19.7)	18 (25.2)	22 (60.7)	84 (29)
Total	111 (22.9)	46 (25.7)	54 (37.5)	211(26)

Regeneration of haploid plantlets

One hundred three out of 211 embryos cultured of which 42.7% were developed in to haploid plants. The haploid status of plants (n=21) was confirmed by counting of somatic chromosomes of randomly selected plants (Figure 1). Haploid plants were produced from all wheat×maize crosses, indicating that fertilization was successful in all cross combinations. The frequency of haploid production (No. of haploid plantlets regenerated out of embryos germinated in culture) ranged from 22% in the ('MV17'×'Falat') × 'KSC108' cross to 60% in ('Sabalan*2' × 'Kal-Blo"s"') × 'KSC301' cross (average 43.3%) (Table 3). This parameter actually indicates efficiency of embryo rescue operation.

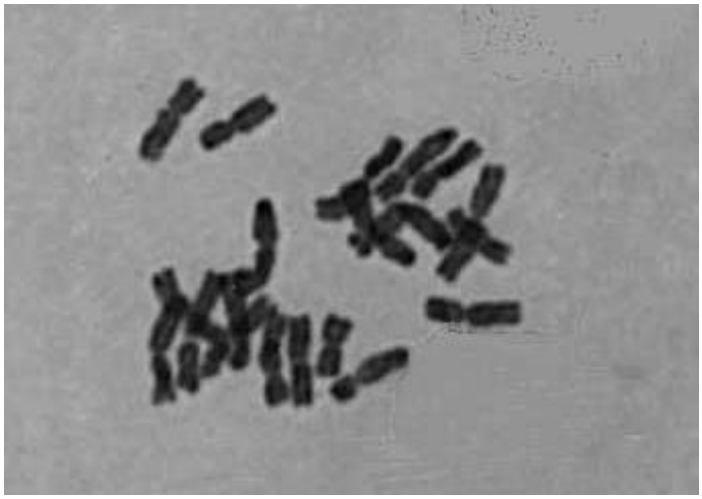

Figure 1. The haploid status of plantlets (n=21) confirmed by counting of somatic chromosomes of randomly selected plantlets.

Table 3. Frequency of haploid plantlets (% of rescued embryos) in each of the crosses.

Genotypes	'FALAT' × 'MV17'	'MV17' × 'FALAT'	'Sabalan*2' ×'Kal-Blo s'	Total
KSC 108	16 (37.2)	4 (22)	12 (60)	32 (39.5)
KSC 301	11 (45.8)	3 (30)	8 (60)	22 (47.8)
KSC 704	18 (41)	5 (27.8)	13 (59)	36 (42.9)
Total	45 (40.5)	12 (26.1)	33 (61.1)	90(42.7)
Mean	41.3%	26.6%	59.67%	

Doubled haploid production

Regenerated plants were treated with colchicines and diploid plants were obtained. Colchicine treatment resulted in depolarization and seed production of 56% of plants. The seeds of doubled haploids showed normal chromosomes of common wheat. In total, of 90 colchicine treated haploid plants, 51 doubled haploid plants (4.8% of pollinated florets) were obtained. Table 4 shows the frequency of doubled haploid plants obtained from different cross combinations. The lowest number of DH plants obtained from the cross 'MV17 × Falat' ×'KSC 704' (1%) and the highest one was observed in the cross 'Sabalan*2' ×'Kal-Blo s' ×'KSC 704' (11.1%).

Table 4. Frequency of DH lines (% of pollinated florets) in each of the crosses.

Genotypes	'FALAT' × 'MV17'	'MV17' 'FALAT'	'Sabalan*2' ×'Kal-Blo s'	Total
KSC 108	11 (5.1)	2 (2.4)	6 (9.4)	19 (4.7)
KSC 301	6 (3.9)	1 (1.5)	3 (6.7)	10 (3.4)
KSC 704	13 (4.7)	1 (1)	8 (11.1)	22 (4.3)
Total	30 (4.7)	4 (2.2)	17 (9.3)	51 (4.8)
Mean	4.6%	1.6%	9.1%	

Effect of maize and wheat genotypes

In general, according to the analysis of variance, there were no significant differences among maize genotypes used as male parent (Table 5). This means that the genotypic composition of pollinator has not affected the amount of seed set, embryo formation, haploid and DH production.

The grain or embryo development traits were mostly not influenced by the hybrid wheat genotypes used as female parent (Table 5). Significant differences were observed among wheat hybrids only for haploid regeneration and DH production ($P<0.01$). After mean separation with least significant difference (LSD), hybrid wheat 'Sabalan*2' × 'Kal-Blo"s"' (SK) showing 59.67% haploid regeneration ranked as the best female parent followed by 'Falat' × 'MV 17' (FM) (41.33%) and 'MV 17' × 'Falat' (MF) (26.60%). The hybrid wheat 'Sabalan*2' × 'Kal-Blo"s"' (SK) had also the highest amount of DH production with the mean of 9.07% and female genotypes 'Falat' × 'MV 17' (FM) and 'MV 17' × 'Falat' (MF) with the means of 4.56% and 1.63%, respectively, located in later groups.(Tables 3 and 4).

Table 5. Effect of maize and wheat genotypes on the studied traits (two-way analysis of variance).

Source of variation	Degrees of freedom	Mean of squares			
		Seed set	Embryo formation	Haploid efficiency	DH efficiency
Maize	2	283.42 $^{n.s.}$	141.43 $^{n.s.}$	22.97 $^{n.s.}$	2.50 $^{n.s}$
Wheat	2	63.02 $^{n.s}$	115.06 $^{n.s}$	823.29**	42.05**
Wheat × Maize	4	48.62	228.86	6.507	1.64

**; Significant at α=0.01 ns; non significant

Evaluation of DH lines for yellow rust resistance

Of 90 colchicine treated haploid plants, 51 doubled haploid plants were obtained. All DH lines along with two parental cultivars (MV17 and Falat) and Bolani as a control were involved in evaluation against a prevalent race of stripe rust at seedling stage. Analysis of variance showed significant differences among the DH lines for all the resistance parameters (Table 6). Mean separation using Duncan Multiple Range Test (DMRT) revealed that there are significant differences among 51 DH lines in terms of latent period, infection type, and pustule size and pustule density (Table 7). Pooling data recorded on infection type (IT), latent period (LP), pustule size (PS) and pustule density (PD) using cluster analysis revealed three phenotypic resistance classes; resistant (R), moderately resistant (MR) and susceptible (S) against stripe rust pathotype 166E134A+ (Figure 2). Finally, four DH lines were selected as resistant against disease. The most resistant and the most susceptible lines were originated from the crosses 'Sabalan*2' ×'Kal-Blos' and 'FALAT' × 'MV17', respectively.

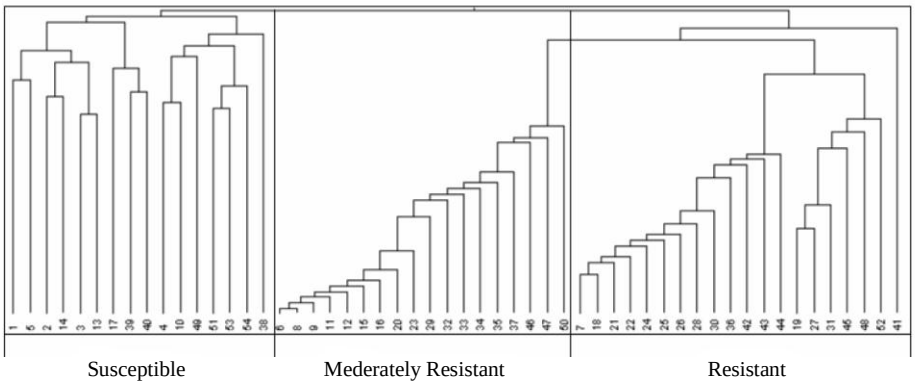

Figure 2. Grouping of 51 DHs, two parental lines (MV17 and Falat) and a check variety (Bolani) in three resistance classes.

Table 6. Analysis of variance for disease resistant traits (at seedling stage) in unbalanced completely randomized design.

S.O.V	df	Mean of squares			
		Latent period	Infection type	Pustule size	Pustule density
Genotypes	53	39.09**	24.75**	47.77**	53.57**
Error	68	0.78	0.53	2.41	3.65

** Significant at α=0.01.

Table 7. Mean separation of studied traits for 51 DHs, two parental lines (MV17 and Falat) and a check variety (Bolani) using Duncan New Multiple Range Test.

Code	line	Latent Period (no. of days)	Infection Type	Pustule Size (mm)	Pustule Density (per cm^2)
1	15-6-fm-1	9^{e}	7^{a}	10.31^{cd}	6.85^{cde}
2	21-8-fm-1	11^{cd}	7^{a}	15.74^{a}	9.51^{abcd}
3	26-8-fm-6	9^{e}	8^{a}	11.78^{bc}	12.03^{a}
4	37-11-fm-4	13^{b}	4^{b}	6^{f}	4.66^{cd}
5	41-11-fm-1	11^{cd}	8^{a}	10.4^{cd}	9.04^{abcde}
6	41-13-fm-3	20^{a}	2^{c}	0^{g}	0.7^{f}
7	45-13-fm-5	20^{a}	0^{d}	0^{g}	0.7^{f}
8	49-14-fm-4	20^{a}	2^{c}	0^{g}	0.7^{f}
9	51-16-fm-1	20^{a}	2^{c}	0^{g}	0.7^{f}
10	57-16-fm-7	13^{b}	4^{b}	6^{f}	6.86^{cde}
11	65-19-fm-3	20^{a}	2^{c}	0^{g}	0.7^{f}
12	73-20-fm-6	20^{a}	2^{c}	0^{g}	0.7^{f}
13	76-21-fm-1	10^{de}	8^{a}	11.2^{c}	10.98^{ab}
14	79-22-fm-1	10^{de}	8^{a}	13.94^{ab}	10.12^{abc}
15	85-24-fm-1	20^{a}	2^{c}	0^{g}	0.7^{f}
16	88-25-fm-1	20^{a}	2^{c}	0^{g}	0.7^{f}
17	93-26-fm-1	12^{bc}	8^{a}	7.5^{ef}	15.8^{a}
18	95-28-fm-1	20^{a}	0^{d}	0^{g}	0.7^{f}
19	100-30-fm-1	20^{a}	1^{cd}	0^{g}	0.7^{f}
20	103-30-fm-4	20^{a}	2^{c}	0^{g}	0.7^{f}
21	106-30-fm-1	20^{a}	0^{d}	0^{g}	0.7^{f}
22	106-31-fm-3	20^{a}	0^{d}	0^{g}	0.7^{f}
23	110-32-fm-2	20^{a}	2^{c}	0^{g}	0.7^{f}
24	112-33-fm-2	20^{a}	0^{d}	0^{g}	0.7^{f}
25	116-33-fm-5	20^{a}	0^{d}	0^{g}	0.7^{f}
26	123-35-fm-3	20^{a}	0^{d}	0^{g}	0.7^{f}
27	126-36-fm-2	20^{a}	1^{cd}	0^{g}	0.7^{f}
28	129-36-fm-2	20^{a}	0^{d}	0^{g}	0.7^{f}
29	132-38-fm-2	20^{a}	2^{c}	0^{g}	0.7^{f}
30	138-38-fm-7	20^{a}	0^{d}	0^{g}	0.7^{f}
31	143-39-mf-3	20^{a}	1^{cd}	0^{g}	0.7^{f}
32	145-39-mf-5	20^{a}	2^{c}	0^{g}	0.7^{f}
33	148-40-mf-1	20^{a}	2^{c}	0^{g}	0.7^{f}
34	149-40-mf-2	20^{a}	2^{c}	0^{g}	0.7^{f}
35	75-22-5-1	20^{a}	2^{c}	0^{g}	0.7^{f}
36	76-22-5-2	20^{a}	0^{c}	0^{g}	0.7^{f}
37	78-22-5-4	20^{a}	2^{c}	0^{g}	0.7^{f}
38	89-28-5-1	20^{a}	7^{a}	6.7^{ef}	7.67^{bcde}
39	90-28-5-2	13^{b}	7^{a}	7.8^{def}	10.25^{abc}

Continue Table 7.

40	90-28-5-3	13^{b}	8^{a}	7.5^{ef}	12.62^{a}
41	93-28-5-5	12^{bc}	1^{cd}	0^{g}	0.7^{f}
42	95-29-5-2	20^{a}	0^{c}	0^{g}	0.7^{f}
43	172-58-5-1	20^{a}	0^{c}	0^{g}	0.7^{f}
44	173-58-5-2	20^{a}	0^{c}	0^{g}	0.7^{f}
45	176-58-5-5	20^{a}	1^{cd}	0^{g}	0.7^{f}
46	190-61-5-2	20^{a}	2^{c}	0^{g}	0.7^{f}
47	192-62-5-1	20^{a}	2^{c}	0^{g}	0.7^{f}
48	194-62-5-3	20^{a}	1^{cd}	0^{g}	0.7^{f}
49	196-63-5-1	13^{b}	4^{b}	5^{f}	0.7^{f}
50	199-64-5-1	20^{a}	2^{c}	0^{g}	0.7^{f}
51	200-64-5-2	13^{b}	7^{a}	5^{f}	5.97^{de}
52	MV17	20^{a}	1^{cd}	0^{g}	0.7^{f}
53	FALAT	12^{bc}	7^{a}	6.51^{ef}	5.78^{de}
54	BOLANI	12^{bc}	7^{a}	5.73^{f}	8.25^{abc}

Means with similar letters do not show significant differences (α= 0.01)

Discussion

Pollination with maize pollens coupled with the post-pollination treatments proved effective in producing haploids and doubled haploids. However, the frequency of both haploid embryo development and DH production varied considerably in different cross combinations. All the wheat F_1 hybrids were crossable with maize as shown by caryopsis and embryo forming in all wheat genotypes. Non of the parental lines of these hybrids are known to carry recessive crossability alleles *kr1* and *kr2*. Thus the wheat × maize system, as demonstrated earlier (Singh et al., 2004; Bakos et al., 2005), is independent of crossability alleles *kr1* and *kr2*. The success in seed set obtained in this study (78.1%) was close to the value reported by Matzk and Mahn (1994) with 12 wheat genotypes. Percent of seed set represents the efficiency of emasculation and pollination procedures. Pollination on the day of anthesis gave the highest frequency of fertilization (data not shown). Cutting the glumes to expose the stigma reduced the success, and if the number of haploid embryos produced per unit of time is considered, the benefit of leaving the glumes intact might be offset by the time saved in emasculating and pollinating florets with cut glumes (Laurie, 1989). Side emasculation resulted in higher seed set.

As reported by Zang et al., (1996) and Brazauskas et al., (2005) application of 2, 4-D significantly improved embryo development. It was observed that placement of coleoptiles upward while transferring to the culture medium improves success in embryo production and plant regeneration. It appears that 2, 4-D concentration and method of it's application as well as timing of pollination are the most important factors determining success (Laurie and O'Donoghue 1994). According to Brazauskas et al., (2005) 2, 4-D induces caryopsis swelling and subsequent haploid embryo development up to 14-17 days after pollination. Most probably 2, 4-D treatment has a critical concentration for the induction of caryopses formation and further increase in concentration has less influence on it.

Several studies have analyzed the influence of wheat and maize genotypes on embryo formation in wheat×maize crosses (Sarrafi et al., 1994; Suenaga, 1994; Li et al., 1996; Zhang et al., 1996; Verma et al., 1999). Almouslem et al., (1998) showed that genotype of the maternal parent does play an important role in haploid wheat production. Verma et al.,

(1999) reported the influence of maize genotypes to be striking in comparison to the milder influence of wheat genotypes. As known from former investigations and also confirmed in this study, the percentage of haploid regeneration and doubled haploid production is dependent on the wheat genotypes, but it is independent of maize genotypes. Although, there was no significant difference among maize genotypes in this study but it could be generally concluded that 'KSC108' is the most suitable male parent for DH production in wheat due to its higher amount of pollen production.

A comparison of various male and female genotypes involved in crosses clearly established that 'Sabalan*2'×'Kal-Blos' hybrid, crossed using the pollens of 'KSC108' maize genotype, gave the highest frequency of embryo formation and resulted in maximum number of haploid plants. Since the frequency of conversion of haploids into doubled haploids after colchicine treatment varied in a narrow range (often around 50%), the 'Sabalan*2'×'Kal-Blos' × 'KSC704' combination is expected to be the most productive cross in terms of DH yields.

Anther culture is an alternative way to produce homozygous fertile plants within one generation. This method uses the microspores (immature pollen) to regenerate plants by androgenesis. With this method, regeneration rate is influenced by the wheat genotype and a lot of albino plants are produced which are unable to survive. Therefore the use of the anther culture in breeding programs is restricted. Although, the expenditure of labor is twofold higher than by using the anther culture (unpublished data), the wheat × maize method is more effective in production of DH plants.

Low genotypic specificity from the wheat side enhances the wheat breeding applicability of the system. Anther culture and the *H.bulbosum* system have been shown to have much stronger genotypic responses of wheat as compared to the wheat × maize system (Inagaki and Tahir, 1990; Kisana et al., 1993). The incompatibility between wheat and rice which is most stronger than between wheat and maize (Bakos et al., 2005), leaves the latter system as the best choice for production of doubled haploids in wheat.

Although a large proportion of the DH lines were identified as susceptible and moderate resistant, transgressive segregation was apparent in the population of lines. Similar results have been reported by other researchers (Zwer and Qualset, 1991; Imtiaz et al., 2003). The mean DH population IT was 2.7, but there were DH lines with ITs ranging from 0 to 8. The numbers of DH lines exhibiting R, MR and S responses were 14, 8 and 19 respectively. This segregation pattern is in accordance with results reported by Imtiaz et al., (2003). From DH lines developed through this research, resistant DH lines were selected to be involved in field evaluation experiments.

The results demonstrated high effectiveness of the method to produce yellow rust resistant homozygous wheat genotypes in short time by doubling of haploid lines generated through pollination of wheat F_1 plants with maize pollens.

Acknowledgment

This work was conducted at Agricultural Biotechnology Research Institute of Iran (ABRII) and authors thank the former head of the institute.

References

Almouslem, A.B., Jauhar, P.P., Peterson, T.S., Bommineni, V.R., Rao, M.B., 1998. Haploid durum wheat production via hybridization with maize. *Crop Sci*., 38: 1080- 1087.

Bakos, F., Jager, K., Barnabas, B., 2005. Regeneration of haploid plants after distant pollination of wheat via zygote rescue. Acta Biologica Cracoviensia 47/1: 167-171.

Barclay, I.R., 1975. High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature 256: 410–411.

Berzonsky W.A., Kleven S.L., Leach G.D., 2003. The effects of parthenogenesis on wheat embryo formation and haploid production with and without maize pollination. Euphytica 113: 285-290.

Brazauskas, G., Paðakinskienë, I., Ruzgas V., 2005. Improved approaches in wheat × maize crossing for wheat doubled haploid production. Biologija 4: 15-18.

Cromey M.G., 1992. Adult plant resistance to strip rust (*Puccinia striformis*) in some New Zealand wheat cultivars. New Zealand J of Crop and Hort. Sci. 20: 413-419.

Eriksen, L., Afshari, F., Christiansen, M.J., McIntosh, R.A., Jahoor, A., Wellings, C.R., 2004. *Yr*32 for resistance to stripe (yellow) rust present in the wheat cultivar Carstens V. Theor. Appl. Genet. 108: 567–575.

Imtiaz, M., Cromey, M.G., Hampton, J., Hill, G.M.J., 2003. Inheritance of seedling resistance to stripe rust (*Puccinia striiformis* f. sp. *tritici*) in 'Otane' and 'Tiritea' wheat (*Triticum aestivum*). New Zealand J of Crop and Hort Scie, 31: 15–22

Inagaki, M., Tahir, M., 1990. Comparison of haploid production frequencies in wheat varieties crossed with *Hordeum bulbosum* L. and maize. Jpn. J. Breed. 40:209–216.

Inagaki, M., 1997. Technical advances in wheat haploid production using ultra-wide crosses. JIRCAS Journal 4: 51-62.

Kasha, K.J., Kao, K.N. 1970. High frequency haploid production in barley (*Hordeum vulgare* L.). Nature 225: 874–876.

Kisana, N.S., Nkongolo, K.K., Quick, J.S., Johnson, D.L., 1993. Production of doubled haploids by anther culture and wheat × maize method in wheat breeding program. Plant Breeding 110: 96-102.

Knott, DR., 1989. The wheat rusts, breeding for resistance. Monographs on theoretical and applied genetics12. Springer-Verlag. Berlin, Heridelberg, pp. 201.

Laurie, D.A., Bennet, M.D., 1986. Wheat × maize hybridization. Can. J. of Genet. Cytol. 28: 313–316.

Laurie D.A., Bennet, M.D., 1987. The effect of crossability loci *Kr1* and *Kr2* on fertilization frequency in hexaploid wheat × maize crosses. Theor. Appl. Genet. 73: 403–409.

Laurie, D.A., 1989. Factors affecting fertilization frequency in crosses of *Triticum aestivum* cv. 'Highbury'× *Zea mayz* cv. 'Seneca 60'. Plant Breeding 103:133-144.

Laurie, D.A., Bennet, M.D., 1989. The timing of chromosome elimination in hexaploid wheat x maize crosses. *Genome* 32: 953-961.

Laurie, D.A., Bennet, M.D., 1990. Early post-pollination events in hexaploid wheat x maize crosses. Sex. Plant Reprod. 3: 70-76.

Laurie, D.A., O'Donoghue, L.S. 1994. Wheat × maize crosses for the production of wheat haploids. In: Bajaj YPS (ed.), Biotechnology in agriculture and forestry, Vol. 25 Maize.

Li, D.W., Qio, J.W., Ouyang, P., Yoa, Q.X., Dawli, L.D., Jiwen, Q., Ping, O., Qingxiao, Y., 1996. High frequency of fertilization and embryo formation in hexaploid wheat × *Tripsacum dactyloides* crosses. Theor. Appl. Genet. 92: 1103-1107.

Matzk, F., Mahn, A., 1994. Improved techniques for haploid production in wheat using chromosome elimination. Plant Breeding 113: 125–129.

McNeal, F.H., Konzak, C.F., Smith, E.P., Tate, W.S., Russel, T.S., 1971. A uniform system for recording and processing cereal research data. U. S. Dept. Res. Seru. ARS. Pp: 34-121.

Mehta, Y.R., Angra, D.C., 2000. Somaclonal variation for disease resistance in wheat and production of dihaploids through wheat x maize hybrids. Genet. Mol. Biol. 3: 617-622.

Mochida, K., Tsujimoto, H., Sasakuma, T., 2004. Confocal analysis of chromosome behaviour in wheat x maize zygotes. Genome 47: 224-228.

Moeini, A., De vallavieille-pope, C., Sarrafi, A., 1997. Potential use of doubled haploid lines for the screening of resistance to yellow rust (*Puccinia striiformis*) in hexaploid wheat. Plant Breeding 116 (6): 595-597.

Mujeeb-kazi, A., Riera-Lizarazu, O., William MDHM, 1995. Production of polyhaploid wheat plants using maize and tripsacum. In: Mujeeb-kazi, A., Hettel. G.P., eds. 1995. Utilizing wild grass biodiversity in wheat improvement: 15 years of wide cross research at CIMMYT-Research report No. 2, Mexico, D.F. CIMMYT.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 15: 473-497.

Sadasivaiah, R.S., Orshinsky, B.R., Perkovic, S.M., Beres, B.L., 2001. Colchicine-induced chromosome doubling in wheat haploids. Wheat Information Service 93: 1-4.

Sarrafi, A., Amrani, N., Alibert, G., 1994. Haploid regeneration from tetraploid wheat using maize pollen. Genome 37:176–178.

Singh, S., Sethi, G.S., Chaudhary, H.K., 2004. Differential responsiveness of winter and spring wheat genotypes to maize- mediated production of haploids. Cereal Reseach Communications 32: 201-207.

Sitch, L.A., Snape, J.W., 1986. Doubled haploid production in winter wheat and triticale genotypes using the Hordeum bulbosum system. Euphytica 35: 1045–1051.

Snape, J.W., 1989. Doubled haploid breeding. Theoretical basis and practical application. In: Mujeeb-Kazi, A. and L.A., Sitch (eds.): Review of Advances in Plant Biotechnology 1985–1988, pp: 19–31. CIMMYT, Mexico.

Suenaga, K., 1994. Doubled haploid system using the intergeneric crosses between wheat(*Triticum aestivum*) and maize (*Zea mays*). Bull. Natl. Inst. Agrobiol. Resour. 9: 83-139.

Torabi, M., Mardokhi, V., Nazari, K., Afshari, F., Forootan, A.R., Ramai, M.A., Golzar, H., Kashani, A.S., 1995. Effectiveness of wheat yellow rust resistance genes in different parts of Iran. Cereal Rust and Powdery Mildew Bulletin 23: 9–12.

Verma, V., Bains, N.S., Mangal, G.S., Nanda, G.S., Gosal S.S., Singh, K., 1999. Maize genotypes show striking differences for induction and regeneration of haploid wheat embryos in wheat × maize system. Crop Sci. 49:1722-1727.

Zhang, J.B., Friebe, W.J., Raupp, S.A., Harrison, B., Gill, S., 1996. Wheat embryogenesis and haploid production in wheat × maize hybrids. Euphytica 90: 315–324.

Zwer, P.K., Qualset, C.O., 1991. Genes for resistance to stripe rust in four spring wheat varieties. 1. Seedling responses. Euphytica 58: 171–181.