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Production of early maturing heat tolerant wheat varieties for southern warm zone by haploid breeding

Tayebeh Bakhshi[1*], FarajolahShahriary Ahmadi[2], Reza Bozorgipour[3], Saeid Malekzadeh Shafaroudi Shafarodi[4], Behzad Kaviani[5]

[1]*Department of Agriculture – Biotechnology and Eugenic, Ferdowsi University of Mashhad, Mashhad, Iran*

[2]*Department of Crop Biotechnology and Breeding, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran*

[3]*Seed and Plant Improvement Institute, Karaj, Iran*

[4]*Biotechnology and Plant Breeding Dept. college of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran*

[5]*Department of Horticultural Science, Rasht Branch, Islamic Azad University, Rasht, Iran*

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Abstract

This research was conducted to compare haploid production methods in wheat using chromosome elimination by wheat and maize crosses. The three methods used were: a) classic b) detached tiller c) intermediary. Parental materials were 11 F1 wheat genotypes (female parents) and maize genotype BC572 (male parent). The traits studied were: seed set, embryo formation, and haploid plant production. The comparison among these methods revealed that the C method was superior with respect to seed set, embryo formation, and haploid production. Overall performance of the three methods was about 76.84%, 25.22% and 51.89% for seed setting, embryo formation and haploid production respectively. Among wheat genotypes DH-133 with 87.28% seed set DH-132 with 32.27% embryo formation and 65.08% haploid production were the best genotypes studied. Considering human resources and glass house conditions all other lines were produced by B method detachedtiller.As far as evaluation is concerned, overall, 1155 wheat lines were evaluated for earliness and heat tolerance. This included 788 lines from international nurseries and local collection and 367 DH lines. The evaluations were conducted preliminary at Karaj and finally at Ahwaz and other tropical sites from 2005 to 2010. Totally, 160 from international nurseries and local collection were identified as early maturing and heat tolerant. These lines will be used as parental materials for DH production in the second phase of the project. From the evaluated DH lines, 21 lined could reach replicated yield trials and finally 6 lines were found to be superior to the check varieties (Chamran and Vee.Nac) both under normal and late sowing conditions at 3 sites namely Ahwaz, Zabol and Darab stations. Three lines of these have already been assessed under farmer conditions at 2 locations and 1 line overyield the best check variety (Vee.Nac) besides being resistant to all kinds of rusts yellow, brown andblackunderlatesowingconditions.

* **Corresponding Author:** Tayebeh Bakhshi ✉ adelehbakhshi@yahoo.com

Introduction

Triticum aestivum has got an extensive compatibility and comparing with the other arable plants, has got the highest number of cultivation in different weather condition in the world (Knox *et al.,* 2005). Controlling growing circulation in different weather condition is the most important factor in attaining maximum of compatibility and consequently maximum of operation (Singh *et al.,* 2005). Appropriate timing for blooming and grain filling can help us in attaining maximum of compatibility (Mehta *et al.,* 2006). Blooming time genetics or in another words, required time for cultivating wheat is somehow complicated. There are 3 main groups of genes which influence on cluster time and blooming (Vrn, Pdh, Eps) (Koltunow *et al.,* 2007; Imtiaz *et al.,* 2010). Eps genes (Earliness) are independent of environmental stimulus and it is thought that they are controlling cluster timing and juvenile stage (Brazauskas *et al.,* 2011).

It is estimated that more than 7 million hectares of wheat lands in the world expose to constant heat stress and up to 40% of water wheat farming are facing heat tension at the end of the season (after pollination) (Singh *et al.,* 2005). The average crop reduction is estimated 10% - 15% (Sharma *et al.,* 2004). Based on the experiments done in farms and controlled environments, when the temperature increase just 1 C°, there will be 0.85-3 mg or 4.1% - 6% reduction (Bakos *et al.,* 2010). Besides, researches have shown that the quality of dough has been decreased considerably, just because of a few days of calorific shock (Bakos *et al.,* 2010).

Therefore high temperature is one of the determinative environmental factors in wheat production in the world and even a partial solution to this problem can have salient economic effect on increasing wheat production in the world. Producing figures sustaining heat is a solution (Bakos *et al.,* 2007).

In order to attain to these resistant figures, there are many different reformative methods. With regard to 1. Reducing the reformative plan duration 2 (Gurel *et al.,* 2009). Increasing selection efficiency, doubled haploid is one of the methods which can be used to produce resistant figure for yellow rust. Intertype confluence is one the valuable techniques in herbal genetics and practical projects in plants reformation (Bakos *et al.,* 2010). In some of these confluences, chromosome incompatibility results in eliminating base male chromosome at the beginning of foetus growth. After saving the foetus and transferring it to the artificial cultivation environment, haploid seedling will be obtained (Berzonsky *et al.,* 2008). For the first time this system was reported to be used by Kasha and Kao in atmosphere in 1970 and with the confluence of *H.bulbosume* × *H.vulgare* (Kasha and Kao., 1970). Then with using the same technique in wheat, haploid produced in 1986 (Eriksen *et al.,* 2008). And finally in 1986 and 1987, Laurie and Bennet reported the production of wheat haploid using confluence with maize (LauriandBennet, 1986, 1987). Recently, the best method is crossing with maize [5] that it can be obtain 4-6 florets for each germinated ear (Sadasivaiah *et al.,* 2006).

The major methods in producing wheat haploid are 1. Intertype confluence 2. Chromosome elimination 3.Intra glass cultivation (Mochida *et al.,* 2008).

There are preventive factors in using reformative projects on stamen, including genetic association (some of the genes' reaction), outburst of albino plants and genetic fluctuations (Kiepha *et al.,* 2010). Pollination with *H.bulbosume*and eliminating *H.bulbosome* chromosomes have been successful just in the figures of wheat in which there were confluence ability in Krl1, Krl2 locus in 5A, 5B chromosomes with recessive alleles (Sirohi *et al.,* 2008). Right now the best way to produce wheat haploid is with the confluence of corn which has been reported by Laurie and Bennet. The main purpose of this project was to produce wheat figures which are early ripening, productive and sustaining heat for the hot regions in the south of the country and furthermore, examining the relations among arable qualities, cluster time, blooming and the period of grain filling in internal

and external wheat in the hot climate of the country.

Materials and methods

Plant materials

In this research, we have used 11 hybrides F1 genotype as the female parent. These female hybrides have been supplied of Khuzestan because of heat sustaining and early ripening.

Genotype ofmaize BC572 has been used for producing pollen.

Compared methods in this research are as follows:

- Classic or normal method (A)
- Detached tiller method (B)
- Intermediary method (C)

Emasculation and pollination

In this research we have used the common method in producing doubled haploid method (A) and cultivating detached tiller of wheat (B) in order to produce doubled haploid wheat lines. For synchronizing pollination stage of maize and wheat, maize weeks have been planted 45 days sooner than wheat ones. All of implants have been done with 15 days distance with 5 seeds of each kind of figures in a pan. They have been kept in a fitotron in 25°C and with the photoperiod of 16 hours lightness, 8 hours darkness. After bud, the seedling transferred to plastic flower vases with 22 centimetres thickness. The vases contained a mixture of leaf mold, sand and farm dust with a 1:1:2 ratio. The resulted seedlings have been kept in a greenhouse with 25 C° temperature and the photoperiod of 16 hours lightness and 8 hours darkness until male inflorescence production and pollination. For improving maize growth, after 5-6 leaves stage, we have added urea fertilizer to the vases every fortnight. About wheat, every time we have implanted 25 seeds in a pan after disinfecting with 20 days distance and the day after implanting they have been kept in 4°C for 48 hours, in order to pollinate. Then they have been transferred to the fitotron in 20°C with the photoperiod of 16 hours lightness and 8 hours darkness. After bud, wheat seedlings transferred to plastic flower vases with 14 centimetres thickness. These vases included a mixture of leaf mold, sand and farm dust with a 1:1:2 ratio. They have been kept in a greenhouse with the 20°C temperature with the photoperiod of 16 hours lightness and 8 hours darkness until cluster production and completing the other stages of experiment.

Classic or normal method (A)

On the common method (A) after ejecting two thirds of stamen pod cluster, we tried to castrate wheat cluster. For doing this, after eliminating mid florets and cutting the upper two thirds of lema and polymerase, the three existing stamen in each floret was taken out by forceps. The fresh corn pollens, which have been collected using a piece of aluminium foil, transferred to the wheat stigma using a paintbrush after 24 hours. During these 24 hours and after pollinate, 2,4-D hormone with the density of 100 mg per litre was injected to the stem (the last internodes) as well as pollinate florets.

Detached tiller method (B)

In Detached tiller method (B) after ejecting two third of stamen pod cluster, wheat stems are cut near land surface and are put in a bark containing water with environmental temperature and transferred to the lab. In the lab, wheat clusters are put in warm water with the temperature of 43°C. Then the wheat stems moved to a dark containing water and environmental temperature and the clusters have been covered using a polyethylene pocket. The fresh maize pollens, which have been collected using a piece of aluminium foil, transferred to the wheat stigma using a paintbrush after 24 hours. In this method after pollination, they put the wheat cut stems in a liquid cultivation environment containing 2,4-D hormone with the density of 100 mg per litre. Then the stems have been transferred to another liquid cultivation environment but without hormones and are kept in fitotron with 22.5°C of temperature for 14 – 16 days. The photoperiod is as follows: 16 hours lightness and 8 hours darkness with 60 to 65 percent of moisture. The resulted seed lacks potential elements (endosperm), because there weren't any doubled

fertilization in the embryos. Then in order to provide haploid foetus with the alimentary needs and creating good condition for foetus growth and turning to a haploid seedling, 16 days after pollination with the use of saving foetus technique haploid foetus have been transferred to MS cultivation environment and have been kept in a dark fitotron with 20°C of temperature. After 1 or 2 weeks, when the plumule is about 1 to 1.5 centimetre, the seedlings have been transferred to a lightening condition with 16 hours lightness and 8 hours darkness. This was done because they had to absorb light and do photosynthesis, then as a result start to grow. After one month (when the seedlings were at least trifoliate and had a strong filamentary system) they were moved from the glass to the land. After wards in tillering stage, haploid seedlings were affected by 0.05% density in order to double the chromosomes.

Intermediary method (C)

Intermediary method is a combination of the other 2 methods. The incipient stages including castrating, pollination and injecting 2,4-D hormone are exactly the same as classic method. 5 or 6 days after the insemination and seed formation, we cut the stems and transferred them to the liquid cultivation environment without 2,4-D. Furthermore, the stems transferred to the growth chamber in order to control environmental situation. In this method the harvest was done 15 or 16 days after the pollination. In this method various advantages of the previous ones are used very well, because important castrating, pollination and applying hormone on the plant stages, are done in the flower vase. This deed results in a better insemination and more appropriate seed formation. Then we cut the stems and put them in the liquid cultivation environment. Through this, transferring materials to the seed and foetus appropriate growth is done very well. Ultimately we expect more seed production as well as more foetus production.

Statistical Analaysis

For doing some primary assessments in arable year 2013 – 2014, doubled haploid lanes in experimental farm of corn research part, were cultivated. In this stage 10 grams of seeds from 60 doubled haploid lanes which have been produced and there were enough of them, implanted in lines with 1 metre long with 30 centimetres distance under mist system. The characteristics such as the appearance of spikes, plant height, number of seeds per skipes and thousand grain weight. The resulted data from different experiments underwent statistical analysis using X^2 test and random pattern.

Results and discussion

Seed formation percentage

Comparing wheat genotyperegarding seed formation percentage in classic method (A)

With regard to Table 1, genotype DH-133 with 82.82% and genotype DH-125 with 62.53% are the best and the weakestgenotypes respectively, regarding seed formation percentage in classic method (A).

Table 1. Seed formation percentage in classic method (A).

Wheat genotypes	Number of pollinated florets	Number of seed formation	Percentage of seed formation	χ^2
DH-124	295	218	73.90	0.31
DH-125	332	207	62.35	3.61
DH-127	286	214	74.83	0.54
DH-128	340	231	67.94	0.49
DH-129	319	228	71.47	0.00
DH-130	374	276	73.80	0.37
DH-131	327	225	68.81	0.25
DH-132	371	263	70.89	0.00
DH-133	355	294	82.82	6.80
DH-134	331	218	65.86	1.30
DH-135	309	215	69.58	0.11
Total	3639	2589	71.14	13.79n.s

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

Table 2. Seed formation percentage in detached tiller method (B).

Wheat genotypes	Number of pollinated florets	Number of seed formation	Percentage of seed formation	χ^2
DH-124	340	242	71.18	0.41
DH-125	317	211	66.56	2.47
DH-127	304	249	81.91	2.46
DH-128	297	194	65.32	3.13
DH-129	290	202	69.66	0.79
DH-130	334	256	76.65	0.28
DH-131	370	278	75.14	0.05
DH-132	381	310	81.36	2.67
DH-133	337	301	89.32	10.44
DH-134	343	242	70.55	0.60
DH-135	305	198	64.92	3.15
Total	3618	2683	74.15	26.81**

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

Using Kay Score test shows that, with 5% probability, there is not any meaningful difference among wheat genotypesregarding seed formation percentage in this method.

Comparing wheat genotypes regarding seed formation percentage in detached tillermethod (B)

With regard to Table 2, genotypeDH-133 with 89.32% and genotype DH-135 with 64.92% are respectively the best and the weakest genotyperegarding seed formation percentage in detached tillermethod (B). Using Kay score test shows that, with 1% probability, there is a meaningful difference among wheat genotypes regarding seed formation percentage in this method. This difference can exist as a result of female parent effect.

Table 3. Seed formation percentage in intermediary method (B).

Wheat genotypes	Number of pollinated florets	Number of seed formation	Percentage of seed formation	χ^2
DH-124	342	266	77.78	2.09
DH-125	358	286	79.89	1.09
DH-127	341	294	86.22	0.06
DH-128	302	248	82.12	0.29
DH-129	298	243	81.54	0.42
DH-130	329	271	82.37	0.26
DH-131	359	334	93.04	2.74
DH-132	367	329	89.65	0.94
DH-133	346	311	89.88	0.98
DH-134	325	267	82.15	0.31
DH-135	349	309	88.54	0.52
Total	3716	3158	84.98	9.70n.s

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

Table 4. Comparing Kay Score in methods of A, B and C regarding seed formation percentage.

Method	χ^2 Calculated	χ^2 Table	
		5%	1%
A	13.79n.s		
B	26.81**	18.3	23.2
C	9.70n.S		

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

Comparing wheat genotyperegarding seed formation percentage in intermediarymethod (C)

As can be seen in Table 3, wheat genotype DH-131 with 93.04% and genotype DH-124 with 77.78% are respectively the best and the weakest genotyperegarding seed formation percentage in intermediary method.

Table 5. Embryo formation percentage in method (A).

Wheat genotypes	Number of seed set	Number of embryo formation	Percentage of embryo formation	χ^2
DH-124	218	41	18.81	0.72
DH-125	207	36	17.39	1.61
DH-127	214	51	23.83	0.55
DH-128	231	45	19.48	0.43
DH-129	228	53	23.25	0.33
DH-130	276	59	21.31	0.00
DH-131	225	46	20.44	0.11
DH-132	263	75	28.52	6.07
DH-133	294	72	24.49	1.24
DH-134	218	44	20.18	0.17
DH-135	215	34	15.81	3.21
Total	2589	556	21.47	14.45n.s

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

Table 6. Embryo formation percentage in method (B).

Wheat genotypes	Number of seed set	Number of embryo formation	Percentage of embryo formation	χ^2
DH-124	242	48	19.83	1.40
DH-125	211	40	18.96	1.87
DH-127	249	54	21.69	0.36
DH-128	194	32	16.49	4.07
DH-129	202	51	25.25	0.26
DH-130	256	63	24.61	0.13
DH-131	278	75	24.61	1.42
DH-132	310	94	26.98	6.10
DH-133	301	87	30.32	3.71
DH-134	242	56	28.90	0.01
DH-135	198	31	23.14	5.20
Total	2683	631	23.52	24.52**

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

Using Kay Score test shows that, with 5% probability, there is a meaningful difference among wheat genotypes regarding seed formation percentage in this method.

With regard to Fig. 1, intermediary method (C) with 84.98% in seed formation percentage, (the number of germinated florets. the number of formed seeds) × 100 excels classic method (A) and detached tillermethod (B). Besides with regard to Table 13, in interstitial and classic regarding weed formation percentage, with 5% probability, there is not any meaningful difference among wheat genotypes, but in detached tillermethod, with 1% probability, meaningful difference is observed. Among all of methods A, B, and C the best genotype, regarding seed formation percentage, is DH-133 with 87.28% and the weakest one is DH-125 with 69.91%.

Sonaga and colleagues (1991) stated the average seed formation percentage 74.2% in their experiments. John Lee Chen and colleagues (1999), combines 12 F1 wheat genotypes with 1 maizegenotype. The extent of seed formation percentage was variant between 68.94% and 89.76%. The average seed formation percentage was stated 82.87%. John Lee Chen and

colleagues stated that no meaningful difference was observed in seed formation percentage. Javid Ahmad and Mohammad Islam Chodari (2005) combined 8 hexaploid F1 wheat genotypes and 4 tetraploid F1 wheat genotypes with 3 maizegenotypesnamed FSH-399, Akbar and Composite. They have used detached tillermethod and hand operated castrating. Seed formation percentage was reported to be 87.0% to 99.5% in hexaploidgenotypeswith the average of 90.3% and in tetraploidgenotypes 60.3% to 78.1% with an average of 71.4%. They have stated that with 1% probability, there is a meaningful difference in the combination of hexaploid with maize as well as tetraploid with maize. Also just like this research, they concurred that the female parent has an effect on seed formation percentage. Bakhtiar and Bozorgipoor (1385) combined 3 wheat genotypes with 3 maizegenotypes named H7, H3, and H1. They have used common method (A) and detached tiller(B) in their research. Seed formation percentage was variant in method A 55.45% with the combination of G1H1 to 76.50% with G2H7, in method B 59.12% with the combination of G1H7 to 69.77% with G3H1.

Table 7. Embryo formation percentage in method (C).

Wheat genotypes	Number of seed set	Number of embryo formation	Percentage of embryo formation	χ^2
DH-124	266	61	22.93	4.14
DH-125	286	71	24.83	2.32
DH-127	294	83	28.23	0.22
DH-128	248	67	27.02	0.62
DH-129	243	80	32.92	6.83
DH-130	271	89	32.84	0.88
DH-131	334	103	30.84	0.14
DH-132	329	126	38.30	8.11
DH-133	311	83	26.69	0.97
DH-134	267	79	29.59	0.00
DH-135	309	97	31.39	0.29
Total	3158	939	29.73	18.52*

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

Table 8. Comparing Kay Score in methods of A, B and C regarding embryo formation percentage.

Method	χ^2 Calculated	χ^2 Table	
		5%	1%
A	14.45n.s		
B	24.52**	18.3	23.2
C	18.52 *		

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

Embryo formation percentage

Comparing wheat genotypes regarding embryo formation percentage in method (A)

With regard to Table 5, the bestgenotype in method A regarding embryo formation percentage, is DH-132 with 28.52% and the weakest one on this method is DH-135 with 15.81%.

Kay Score test shows that, with 5% probability, there isn't any meaningful difference among wheat genotypes in classic method regarding Embryo formation percentage.

Comparing wheat genotypes regarding embryo formation percentage in method (B)

With regard to Table 6, changes extent in embryo formation percentage is variant in method B: genotype DH-135 with 15.66%, genotype DH-132 with 30.32%. Therefore DH-132 is the best genotype in this method and DH-135 is the weakest one regarding e genotype formation percentage.

Kay Score test has shown that, with 1% probability, there is a meaningful difference among wheat genotypes regarding embryo formation percentage.
Among factors influencing embryo formation are:

- Using fresh and ripe pollen seed
- The influence of female parent (wheat genotype)
- Stigma's preparation for accepting pollen in the period of pollination.

Comparing wheat genotypes regarding embryo formation percentage in method (C)

As can be seen in Table 7, the best genotype in method C, regarding embryo formation percentage is DH-132 with 38.30% and the weakest one is DH-124 with 22.93%.

Kay Score test shows a meaningful difference among wheat genotypes with 5% probability, regarding embryo formation percentage.

Table 9. Haploid seedling formation percentage in method (A).

Wheat genotypes	Embryo formation	Number of haploid seedling	Percentage of haploid seedling	χ^2
DH-124	41	13	31.71	2.70
DH-125	36	11	50.56	2.68
DH-127	51	21	41.18	0.76
DH-128	45	16	35.56	1.84
DH-129	53	26	49.06	0.01
DH-130	59	34	57.63	0.72
DH-131	46	29	63.04	1.62
DH-132	75	44	58.67	1.18
DH-133	72	37	51.39	0.04
DH-134	44	27	61.36	1.18
DH-135	34	19	55.88	0.25
Total	556	227	49.82	12.97n.s

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

Table 10. Haploid seedling formation percentage in method (B).

Wheat genotypes	Embryo formation	Number of haploid seedling	Percentage of haploid seedling	χ^2
DH-124	48	14	29.17	4.04
DH-125	40	13	32.50	2.36
DH-127	54	25	46.30	0.12
DH-128	32	9	28.13	2.98
DH-129	51	30	58.82	0.87
DH-130	63	32	50.79	0.02
DH-131	75	46	61.33	2.08
DH-132	94	59	62.77	3.28
DH-133	87	44	50.57	0.02
DH-134	56	29	51.79	0.05
DH-135	31	12	38.71	0.74
Total	631	313	49.60	16.56n.s

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

With regard to Fig. 2, method C with 29.73% regarding embryo formation (the number of formed seeds, the number of formed embryos) × 100 excel method B and A. Also with regard to Table 8, with 5% probability, there isn't any meaningful difference among wheat genotypes in method A regarding embryo formation percentage. But in B and C methods, with 1% and 5% probability respectively, meaningful difference can be observed among wheat genotypes regarding embryo formation percentage.

Among all of methods A, B and C. The best genotype regarding embryo formation percentage is DH-132 with 32.71% and the weakest one is DH-124 with 20.66% (Table 13). Sonaga and colleagues (1991) reported the embryo formation percentage 20.7% on their researches.

Table 11. Haploid seedling formation percentage in method (C).

Wheat genotypes	Embryo formation	Number of haploid seedling	Percentage of haploid seedling	χ^2
DH-124	61	26	42.62	1.61
DH-125	71	28	39.44	3.00
DH-127	83	43	51.81	0.12
DH-128	67	31	46.27	0.86
DH-129	80	39	48.75	0.51
DH-130	89	58	65.17	1.81
DH-131	103	64	62.14	1.06
DH-132	126	89	70.63	5.91
DH-133	83	41	49.40	0.42
DH-134	79	37	46.84	0.88
DH-135	97	57	58.76	0.30
Total	939	513	54.63	16.47n.s

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

Chen (2007), said that using 2,4-D hormone after pollination can be useful for the number of formulated haploid wheat embryos. Dixon (2009) tested the effects of different ways of using 2.4-D hormone on the number of wheat haploid embryos. They found out that using detached tillermethod is more useful than injecting hormone to the stem or dropping it in the floret. They also reported the number of embryos 12% and 28%, in method A and B respectively. Imtiaz (2006) stated the influence of fresh pollen seeds in formulating haploid wheat embryo. Eriksen (2010) recognize the effect of maizegenotype on the number of haploid embryo, besides they stated that the density of 2,4-D hormone just has effect on the size of the embryo. Dixon (2009) stated the seed formation percentage 20.5% and 19.4% in classic and detached tillermethod respectively. Eriksen (2010) reported the extent of embryo formation percentage 10.62% to 25.23% and the average embryo formation percentage 20.13%. Broers and lopz-Atilano (2008) have done some experiments on 8 hexaploid wheat genotype and 4 tetraploid wheat genotype. They reported the extent of embryo formation percentage in wheat hexaploid genotype, something between 13.1% and 25.0% with an average of 12.2%. Emad(2010), stated embryo formation percentage 64.5% in the common method (A) and 55.7% in detached tillermethod (B).

Table 12. Comparing Kay Score in methods of A, B and C regarding haploid seedling formulation percentage.

Method	χ^2 Calculated	χ^2 Table	
		5%	1%
A	12.97n.s		
B	16.56n.s	18.3	23.2
C	16.47n.S		

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

Haploid seedling formation percentage

Comparing wheat genotypes regarding haploid seedling formation percentage in method (A)

With regard to Table 9, the extent of changes in haploid seedling formulation percentage in method A is from 30.56% in DH-125 genotype to 63.04% in genotype DH-131.

Using Kay Score test, with 5% probability, doesn't show any meaningful difference among wheat genotypes regarding haploid seedling formation percentage in this method.

Comparing wheat genotypes regarding haploid seedling formulation percentage in method (B)

With regard to Table 10, the extent of changes in haploid seedling formulation percentage in method B is from 28.13% in genotype DH-128 to 62.77% in genotype DH-132.

Table 13. Overall Table compares wheat genotype for all traits in all there methods.

Wheat genotype	(A) florets	(B) Seed	(C)Embryo	(D)Seedling	%B.A	%C.B	%D.C
DH - 124	977	726	150	53	74.31	20.66	35.33
DH - 125	1007	704	147	52	69.91	20.88	35.37
DH - 127	931	757	188	89	81.31	24.83	47.34
DH - 128	939	673	144	56	71.67	21.40	38.89
DH - 129	907	673	184	95	74.20	27.34	51.63
DH - 130	1037	803	211	124	77.43	26.28	58.77
DH - 131	1056	837	224	139	79.26	26.76	62.05
DH - 132	1119	902	295	192	80.61	32.71	65.08
DH - 133	1038	906	242	122	87.28	26.71	50.41
DH - 134	999	727	179	93	72.77	24.62	51.96
DH - 135	963	722	162	88	74.97	22.44	54.32
Total	10973	8430	2126	1103	76.84	25.22	51.89

Using Kay Score test, with 5% probability, doesn't show any meaningful difference among wheat genotypes in this method.

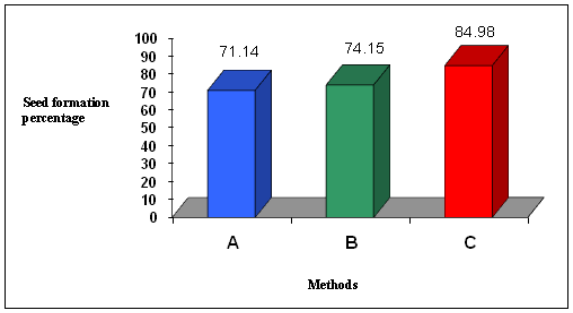

Fig. 1. Comparing methods of A, B and C regarding of seed formation percentage.

Comparing wheat genotypes regarding haploid seedling formulation percentage in method (C)

With regard to Table 11, the extent of changes in haploid seedling formulation percentage in method C is from 39.44% in genotype DH-125 to 70.63% in genotype DH-132.

Using Kay Score test in this method doesn't show any meaningful difference among wheat genotypes regarding haploid seedling formulation percentage.

With regard to Fig. 3, method C with 54.63% in (the number of formulated embryos.formulated haploid seedlings) × 100 is better than method A and B, regarding haploid seedling formulation percentage. Among all of methods A, B and C, the best genotype regarding haploid seedling percentage is DH-132 with 65.08% and the weakest one is DH-124 with 35.33% (Table 13).

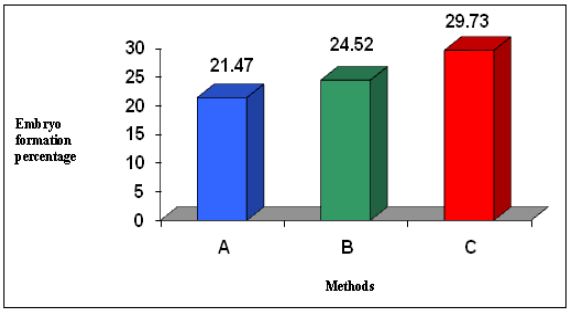

Fig. 2. Comparing methods of A, B and C regarding embryo formation percentage

With regard to Table 12, there is not any meaningful difference in neither method A nor in B and C regarding haploid seedling formulation percentage. Dixon (2009) did not observe any meaningful difference in applying method of hormone 2,4-D and

the abundance of formulated haploid seedling. Emad (2010) reported haploid seedling formulation percentage in common method (A) and detached tillerone (B) 75% in combination with G1H7, G1H3, G1H1, and 62.99% in combination with G2H7, G2H3, G2H1, and 71.15% in combination with G3H7,G3H3,G3H1. These results were drawn form researches done on 3 wheat genotypes (G1, G2, G3) in combination with maizegenotypes (H7, H3, H1). Eriksen (2010)combined 12 F1 wheat genotype with one F1 maizegenotypesand stated the extent changes of haploid seedling formulation percentage form 25.00% to 58.46% with the average of 45.21%. Broers and Lopez-Atilano (2008) used 8 F1 hexaploidgenotypes and 4 F1 tetraploid biotype in combination with maize, in order to compare hexaploid and tetraploid wheat genotype. They used detached tillermethod and hand operated castrating and reported the extent of changes and haploid seedling formulation percentage in hexaploid wheat genotypes as 52.4% to 63.0% with the average of 60.5% and in tetraploidgenotype as 18.1% to 37.6% with the average of 24.6%.

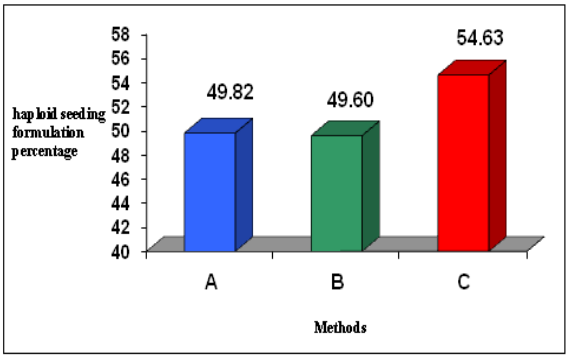

Fig. 3. Comparing methods of A, B and C regarding haploid seedling formulation percentage.

The experiments in this research show that such factors: full ripening of the embryo, Not to hurt the embryo during cultivation stage, Cultivation method of the embryo in culture medium, Temperature situation of cultivated foetuses during incubation stage Can have effect o haploid seedling formulation.

Conclusion

With regard to the following conclusions, among methods A, B and C, intermediarymethod (C) is the best way for keeping cluster: Seed formulation percentage equals 84.98%, Embryo formulation percentage equals 29.73%, Haploid seedling formulation percentage equals 54.63%.

References

Bakos F, DarkoE, Ponya Z, Barnabas B. 2007. Application of wheat (TriticumaestivumL.) microspore culture and ovaries to raise wheat zygotes in vitro. Acta Biologca Cracoviensis Series Botanica. **45,** 107-110.

Berzonsky WA, Kleven SL, Leach GD. 2008. The effects of parthenogenesis on wheat embryo formation andhaploid production with and without maize pollination.Euphytica**113,** 285-290.

Broers L, Lopez-Atilano RM. 2008. Components of adult resistance in bread wheat to stripe rust. Proceding of the 6th International Congress of Plant Pathology, 85 P.

Bakos F, Barnabas B, Castill A, Valles MP, Pulido A. 2010. Comparison of cytological and ultrastructural changes induced in anther and isolated – microsporeculture in barley. Journal of Structural Biology **149,** 170-181. http://dx.doi.org/10.1016/j.jsb.2010.10.009

Bakos F, Jager K, Barnabas B. 2010. Regeneration of haploid plants after distant pollination of wheat via zygote rescue.Acta Biologcal Cracoviensis Series Botanica **47(1),** 167-171.

Brazauskas G, Paðakinskienë I, Ruzgas V. 2011. Improved approaches in wheat × maize crossing for wheatdoubled haploid production. Biologica **4,** 15-18.

ChenXM. 2007. Epidemiology and control of strip rust *(Pucciniastriiformis*f.sp.*tritici*) on wheat. Plant Pathology **27,** 314 – 337. 1094. FN58.

Dixon J, Braun HJ, Kosina P, Crouch J. 2009. Wheat facts and figures. CIMMYT Publications Catalogue, 95 P.

Eriksen L, Afshari F, Christiansen MJ, McIntosh RA, Jahoor A, Wellings CR. 2008.*Yr*32 for resistanceto stripe (yellow) rust present in the wheat cultivar Carstens V. Theoritical Applied Genetic **108,** 567–575.

Eriksen L, Afshari F, Christiansen MJ, McIntosh RA, Jahoor A, Welling CR. 2010. *Yr32* for resistance to stripe rust present in the wheat cultivar carstens V. Theoritical Applied Genetic **108,** 567-575.
http://dx.doi.org/10.1007/s00122-003-1473-9

Emad MAM, Kazal KA, Faris AF, Hassan II, Azhar KH. 2010. Developing of new wheat cultivar resistant to yellow and brown rust diseases, 8th International Wheat Conference, 1–4 June, St. Petersburg, Russia. 221 P.

Gurel S, GurelE, Kaya Z. 2009. Doubled haploid plant product from unpollinated ovules if sugar beet (Beta vulgarisL.). Plant Cell Report **19(12),** 1155-1159.
http://dx.doi.org/10.2225/vol16-issue2-fulltext-3

Imtiaz M, Cromey MG, Hampton J, Hill Gm J. 2006. Inheritance of seedling resistance to stripe rust in 'Otane' and 'Tiritea' wheat. New Zealand of Crop and HorticulturalSciences **31,** 15-22.
http://dx.doi.org/10.1080/01140671.2006.9514231

Imtiaz M, Cromey MG, Hampton J, Hill GMJ. 2010.Inheritance of seedling resistance to stripe rust(*Pucciniastriiformis*f. sp. *tritici*) in 'Otane' and 'Tiritea' wheat (*Triticumaestivum*).New Zealand J of Crop and Horticultural Sciences **31,** 15–22.
http://dx.doi.org/10.1080/01140671.2006.9514231

Kiepha G. 2010. All about haploid production in wheat. Wadsworth, California. 45- 118 .

Koltunow AM, Grossniklaus U. 2007. A developmental perspective. Annual Review of Plant Biology **54,** 547-574.

Knox RE, Clarke JM, Depaum RM. 2005. Dicamba condition effects on doubled haploid production in durum wheat crossed with maize. Plant Breeding **4,** 289.
http://dx.doi.org/10.1046/j.14390523.2005.00498.x

Kasha KJ, Kao KN. 1970. High frequency haploid production in barley (*Hordeumvulgare*L.). Nature **225,** 874-876.
http://dx.doi.org/10.1038/225874a0

Laurie DA, Bennet MD. 1986. The effect of cross ability loci Kr1 and Kr2 onfertilization frequency in hexaploid wheat × maize crosses. Theoritical Applied Genetic**73,** 403-409.
http://dx.doi.org/10.1007/BF00262508

Laurie DA, Bennett MD. 1987. The production of haploid wheat plants from wheat× maize crosses. Theoritical Applied Genetic**76,** 363-397.
http://dx.doi.org/10.1007/BF00265339.

Mehta YR, Angra DC. 2006.Somaclonal variation for disease resistance in wheat and production of diploids through wheat × maize hybrids.Genetical and Molecular Biology **32,** NoSaopaulo Spet 2000.

http://dx.doi.org/10.5455/ijavms.168

Mehta YR, Angra DC. 2006.Somaclonal variation for disease resistance in wheat and production of dihaploids through wheat x maize hybrids.Genetical and Molecular Biology **3,** 617-622.
http://dx.doi.org/10.1590/S1415475720000003000021

Mochida K, Tsujimoto H, Sasakuma T. 2008.Confocal analysis of chromosome behaviour in wheat x maize zygotes. Genome **47,** 224-228.

Mochida K, Tsujimoto H, Sasakuma T. 2008. Confocal analysis of chromosome behavior in wheat × maize zygotes. Genome **47,** 224-228.

Singh S, Behl RK, Punia MS. 2005. Production of

doubled haploids via maize pollination in wheat. Cereal Research Communication **29,** 289-296.

Sadasivaiah RS, Orshinsky BR, Perkovic SM, Beres BL. 2006. Colchicine-induced chromosome doubling in wheat haploids. Wheat Information Service **93,** 1-4.

Sharma H, Yang Y, Ohm H. 2004.An assessment of doubled haploid production in soft red winter with by wheat × corn wide crosses. Cereal Research Communications. **30,** 269-275.

Sirohi M, Khanna VK. 2008.Influence of age of embryo and method of hormone application on haploid embryo formation in wheat × maize crosses. 4thInternational Crop Science Congress.

Singh S, Sethi GS, Chaudhary HK. 2005. Differential responsiveness of winter and spring wheat genotypes to maize- mediated production of haploids. Cereal Research Communications **32,** 201-207.