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Evaluating the Production of Doubled Haploid Wheat Lines Using Various Methods of Wheat and Maize Crossing to Develop Heat-Tolerant Wheat Varieties

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Abstract. In this study, chromosome elimination method was used to develop doubled haploid wheat lines via crosses with maize. The plant materials used included 11, F1 wheat genotypes and maize genotype BC572. In these crosses, the maize plant was used as the male parent. Three methods of haploid production in wheat comprising conventional (A), detached-tiller culture (B) and intermediate (C) techniques were used and compared. The traits such as the number of seeds set, the number of obtained embryos and the number of produced haploid seedlings were studied. Comparisons showed that among various methods of storing wheat spikes, method (C) was better than other techniques in terms of the percentage of seed production, embryo formation and haploid seedling production. Also, in all three methods, the percentage of seed production, the percentage of embryo formation and the percentage of haploid seedling production were equal to 76.84, 25.22 and 51.89. Among the wheat genotypes in all three methods, genotype DH-133 with 87.28 percent seed set and genotype DH-132 with 32.71 percent embryo formation and 65.08 percent haploid seedling production were the best genotypes. A total of 92 doubled haploid lines were produced. In the field evaluations of 86 doubled haploid lines, traits such as growing season, plant height, lodging, kernel yield and 1000 kernel weight were examined. Finally, 3 lines were selected for adaptation and stability testing under heat stress conditions.

Keywords: Wheat, Doubled haploid, Chromosome elimination, Detached-tiller culture

Sıcaklık Toleranslı Buğday Çeşitlerini Geliştirmek için Çeşitli Buğday ve Mısır Geçiş Yöntemlerini Kullanan Çift Katlı Haploid Buğday Hatlarının Üretiminin Değerlendirilmesi

Özet. Bu çalışmada, mısır ile çaprazlarla çift katlı haploid buğday hatlarının geliştirilmesi için kromozom eliminasyon yöntemi kullanılmıştır. Kullanılan bitki materyalleri 11, F1 buğday genotipleri ve BC572 mısır genotipini içermektedir. Bu çaprazlarda, mısır bitkisi erkek ebeveyn olarak kullanılmıştır. Geleneksel (A), ayrık-yeke kültürü (B) ve ara (C) tekniklerinden oluşan buğdayda haploid üretiminin üç yöntemi kullanılmış ve karşılaştırılmıştır. Elde edilen tohum sayısı, elde edilen embriyo sayısı ve üretilen haploid fideler sayısı gibi özellikler araştırılmıştır. Karşılaştırmalar, buğday sivrilerini muhafaza etmenin çeşitli yöntemleri arasında tohum üretim yüzdesi, embriyo oluşumu ve haploid fide üretimi açısından (C) metodunun diğer tekniklerden daha iyi olduğunu göstermiştir. Ayrıca, her üç yöntemde, tohum üretim yüzdesi, embriyo oluşum yüzdesi ve haploid fide üretimi yüzdesi sırasıyla 76,84, 25,22 ve 51,89'e eşittir. Her üç yöntemdeki buğday genotipleri arasında,% 87.28 tohumluk seti olan DH-133 genotipi % 32,71 yüzdesiyle embriyo oluşumu ve % 65,08 haploid fide üretimi olan DH-132 genotipi en iyi genotiplerdir. Toplam 92 katlanmış haploid çizgi üretilmiştir. 86 çift haploid hattının değerlendirilmesinde, yetiştirme mevsimi, bitki boyu, barınma, çekirdek verimi ve 1000 çekirdek ağırlığı gibi özellikler incelenmiştir. Son olarak, ısı stres koşulları altında adaptasyon ve stabilite testi için 3 hat seçilmiştir.

Anahtar kelimeler: Buğday, Çift katlı haploid, Kromozom eliminasyonu, Müstakil yeke kültürü

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INTRODUCTION

Bread wheat with the scientific name of *Triticum aestivum* is among the hexaploid wheat group containing 42 chromosomes (2n= 6x= 42), which is known as common or bread wheat (Zenkteler et al., 1984). Common wheat is better than all varieties of cultivated wheat in terms of quality and quantity and its scattering and distributions more than other types of wheat and is larger amount than other types (Imtiaz et al., 2003). Studies have shown that there are considerable differences in the amount of ability to tolerate heat stress among the different genotypes of wheat and also among wild relatives of wheat (Ochida et al., 2004). So, genetic progress to improve heat tolerance in cultivated wheat is possible. Information about the genetic control of this trait would be helpful although most of the published papers indicate that this trait is quantitatively inherited (Sadasivaiah et al., 2006). Under weather conditions of south-west and southern provinces of the Iran including Khuzestan Province, rapid increase in temperature is common at the end of the growing season. Obviously, in these circumstances, developmental stages of wheat, especially grain filling period, are often faced with a critical temperature even in spite of planting the crop in the convenient and recommended date and this creates adverse conditions to produce a good product. Kernel number per spike, spike length, kernel number per unit of area, biomass, hectoliter weight and harvest index are among the important traits affected by heat stress. Also, the kernel number per spike has been suggested as the most sensitive indicator to select resistant genotypes (Ehdaeiand & Nourmohammadi, 2004).Using the genes involved in early maturity from internal and external resources and fixing them in background genotypes and selection in recombinant lines for early maturity along with desirable agronomic characteristics and yield, early maturing and high yielding wheat can be produced for the mentioned areas (Sharma et al., 2004).

By studying the effect of three planting dates (one timely planting date and two late planting dates) on 13 genotypes of spring wheat under the conditions of Mexico, Ayeneh et al. (2002) argued that delay in planting led to a decrease in the number of days to pollination, number of days to maturity, dry matter, kernel yield, kernel number per spike, number of spikes per square meter, number of kernel per square meter and 1000 kernel weight, but harvest index increased. Through the implementation of various tests on planting dates and varieties in the warm regions of Mexico, Sudan and Bangladesh, Badaruddin et al.(1999) declared that delay in planting and increasing average temperature of the growing season reduce the duration of growth, yield and yield components of wheat. While investigating the effect of seven planting dates on spring bread wheat varieties, Ortiz Monasterio et al. (1994) reported that after proper planting date, for each day of delay in planting, the kernel yield of three varieties of PBW34, PBW154, PB226 was respectively reduced by 0.8, 0.7 and 0.7% and with the delay in planting, kernel yield was decreased due to the reduction in the number of kernel per square meter, which is the result of high temperatures before flowering, and kernel weight loss due to high temperatures after flowering. In general, delay in planting and exposure of reproductive growth and grain filling stages to terminal heat stress lead to accelerating the growth, reducing the overall size of the plant, reduction in photosynthesis, increased respiration, reducing the number of spikes per plant, reducing the kernel number per spike, inhibiting the synthesis of starch in the growing kernel, kernel weight loss and finally, accelerating the aging of plant. All of these physiological and morphological changes led to a reduction in yield under heat stress. By studying the effect of three planting dates (timely in the first of November and late in the first days of December and January) in three independent experiments on the average of nine traits of 25 wheat genotypes in Khuzestan, Radmehr et al.(1996) stated that delay in planting date led to the reduction in biological yield, kernel yield, kernel number per spike, 1000 kernel weight, number of days from planting to spike emergence, number of days to physiological maturity, plant height and grain filling period, but most of the genotypes tested were not adapted with the conditions of

the region. Given the previous studies, the present experiment was conducted with the aim of examining the effect of heat stress due to the delay in planting on the varieties existing in the region.

The fastest way to reach pure and stable forms of new recombination is the haploid breeding method (Zlieng et al., 2001). Breeding through haploid plantsis a new chapter in breeding programs. Using this method, the time and cost required to produce a new variety of wheat can be decreased by half. In self-pollinated plants such as wheat, haploid plants and subsequently, doubled haploid lines can be extracted and produced from heterozygous parents (F1, F2, F3) and be used directly to produce new cultivars because each doubled haploid line, due to having specific stabilized recombination, has the potential to become a new cultivar (Bozorgipour, 1990). In order for a system of doubled haploid plant production (DH) to be successfully used in a breeding program, it must have the following conditions to be economical compared with traditional breeding methods (Brazauskas et al., 2005): 1. Easily producing a large number of doubled haploidplants from all genotypes of the breeding program; 2. Doubled haploid lines should be genetically normal and stable; 3. Production of doubled haploid lines should include a random sample of parental gametes.

Main advantages of doubled haploid systems as compared to classical breeding methods are to accelerate the breeding programs and increase the selection efficiency throughout the program (Knox et al., 2005). Haploid wheat plant production has been possible from ancient times through the techniques such as anther culture, pollen kernel and even the culture of ovary and ovule. These methods are still in use but the existence of problems such asproduction of albino plants and great dependence on genotypes caused that other methods are considered and evaluated (Singh et al., 2005). In this respect, chromosome elimination technique which in some distant crosses leads to the production of haploid embryos with mother plant chromosomes has been identified as a useful method (Eriksen et al., 2008). First, by using the cross between common barley (*Hordeum vulgare*) and wild barley (*Hordeum bulbosum*), Kasha and Kao(1970) managed to obtain haploid embryos from common barley and produce haploid plants through cultivating them. Barclay(1975) reported that haploid wheat production by using the cross between *Triticum aestivum* L. And *Hordeum bulbosum*. The cross between hexaploid wheat (*Triticum aestivum*) and maize (*Zea mays*) was first reported by Zenkteler and Nitzsche (1984).

Bozorgipour and Snape (1990) evaluated Haploid production in Iranian wheat cultivars using the cross between wheat and *Hordeum bulbosum*. In this experiment, the rate of cross ability for Iranian wheat cultivarswas reported very low and consequently, using anther culture method or the cross between wheat and maize was proposed for haploid production in Iranian wheat cultivars. But, the biggest disadvantage of this method was its great dependence on genotype so that in some wheat genotypes, the response reaches zero and no embryo is achieved. The reason for this dependence was a controlling cross ability system which is related to *Kr1* and *Kr2* genes. In this system, genotypes having these two genes in dominant state, lose their cross ability with wild barley or may produce a very small percentage of embryos (Koltunow et al., 2007). Finally, Lauria nd Bennet (1986, 1987) could obtain haploid embryo and subsequently wheat haploid plant from crosses between wheat and maize. It seems that this method can fulfill the three conditions mentioned above. In addition, the process of chromosome elimination in this method is much faster than the method using wild barley. In the systems of the cross between wheat, wild barley and maize, if the produced seed and embryo are left on the mother plant, they will discolor after 16 to 18 days and are destroyed. One of the reasons is the absence of normal endosperm in these seeds (Chen, 2007). To prevent the abortion of an embryo, it is necessary to examine the seeds produced after 14 to 16 days from the date of pollination in order to identify the presence of embryos. In the case of embryo formation, the produced embryos should be placed onan artificial nutrient mediumin a germ free environment to grow and produce seedlings (Broersand & Lopez-Atilano, 2006).

Many studies have been so far conducted about wheat × maize crosses, some of which have reported genotypic effects on the percentage of embryo production by wheat plant (Sirohi et al., 2008). Some of these studies have noted the in effectiveness of crossability (*Kr*) genes in wheat × maize crosses (Diaxon et al., 2009). In fact, the method of wheat × maize cross has been used to produce wheat haploid plants and double the number of their chromosomes for other specific purposes including the evaluation of the resistance to yellow rust and baking quality of doubled haploid lines (Kiepha, 2010).Arzani and Darvey (2002), while comparing the doubled haploid lines with their sister lines, demonstrated that doubled haploid lines enjoy more diversity in terms of plant weight, the primary biomass, total dry matter and kernel yield. High kernel yield and high forage in a number of doubled haploid populations as compared with their sister families indicated that modifying the triticale lines to use forage and forage seed through anther culture and selection in early generations not only shortens the period of breeding programs, but also it causes to expand the scope of kernel yield and forage. One of the doubled haploid lines derived from Polony Q × TW cross, besides having superior forage yield, is more resistant to the diseases of leaf rust, stem rust and yellow rust and was introduced as Eleanor cultivar in 2001 in the east of Australia (Arzani & Darvey, 2002). The main objective of this project is to produce early maturing, high yielding and heat-tolerant doubled haploid wheat varieties for the hot southern regions of the country. Moreover, this research intends to investigate the relations between agronomic traits, time of spike emergence, flowering and grain filling period in domestic and foreign wheat in hot conditions of the country.

MATERIALS and METHODS

Plant materials

In this research, 11 hybrids of F1 genotype were used as the female parent. These female hybrids had been provided in Khuzestan region because of heat sustaining and early maturing.Compared methods in this research are as follows: Mean while genotype of maize BC572 was used as pollinator plant for synchronizing of anthesis stage of maize and wheat, maize were planted 45 days sooner than wheat ones (all of implants had done with 15 days distance with 5 seeds of each figures kind in a pan). After anthesis, the seedlings were transferred to the plastic flower vases with 22 centimetres thickness which were contained a mixture of compost, sand and farm soil with a ratio of 1:1:2. The resulted seedlings were kept in a greenhouse with 25 ˚C temperature and the photoperiod of 16/8 hours darkness until male inflorescence production and pollination. For improving maize growth, urea fertilizer was added to the vases every 15 days, after 5-6 leaves stage. Regarding wheat, 25 seeds were sowed in a pan after disinfecting with 20 days distance every time and due to germination, they were first kept in 4°C for 48 hours one day after sowing, and then transferred to the growth chamber in 20°C with photoperiod of 16 hours lightness and 8 hours darkness. After germination, wheat seedlings have transferred to plastic flower vases with 14 centimetres thickness which were included a mixture of compost, 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 spike production and completing the other stages of experiment

Classic or normal method (A)

On the common method (A) after appearance of two thirds of flag leaf stage, we tried to castrate wheat spike. For doing this, after eliminating mid florets and cutting the upper two thirds of lema and palea, the three existing stamen in each floret was taken out by forceps. Following day the fresh maize pollens, which were collected by using a piece of aluminium foil transferred to the wheat stigma using a paintbrush after 24 hours. During this time and after pollination, 2.4-D hormone with concentration of 100 mg/l was injected to the stigma (the last in ternodes) as well as pollinated florets.

Detached tiller method (B)

In Detached tiller method (B) after appearance of two third of flag leaf stage, wheat stems were cut near land surface and were put in a bark containing water with environmental temperature and transferred to the lab. In the lab, wheat spikes are put in warm water with the temperature of 43°C. Then the wheat stems moved to dark containing water and environmental temperature and the spikes have been covered by using a polyethylene pocket. The fresh maize pollens, which have been collected using a piece of aluminium foil, have transferred to the wheat stigma using a paintbrush after 24 hours. In this method after pollination, the wheat cut stems were put in a liquid medium culture containing 2,4-D hormone with the concentration of 100 mg per litre. Then the stems have been transferred to another liquid medium culture but without any hormones and are kept in growth chamber with the temperature of 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% of moisture. The resulted seed lacks potential elements (endosperm), because there weren't any doubled pollination in the embryo sac. 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 by applying foetus technique haploid foetus have been transferred to MS medium culture and kept in a dark growth chamber with 20°C of temperature. After 1 to 2 weeks, when it 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, started to grow. After one month (when the seedlings were at least three leaf stage and had a strong filamentary system), they were moved from the glass to the land. When the seedlings reached the tillering stage, they were removed from the soil and immediately the roots were washed with water to remove the soil from the roots. Root and crown of the above-mentioned plants were placed in a solution containing 0.05 grams of colchicine, 1.5 ml of DMSO (Dimethyl Sulfoxide) and a drop of Tween 20 per 100 ml of distilled water.Seedlings remained at room temperature (20-25) for 5.5 hours in a solution of colchicine (this time is sufficient to complete a cell cycle of wheat).After treating the plants, the roots were exposed to tap running water for 24 hours to completely remove the chemicals from the roots. Then, plants were again transferred to the pots. Seed production in plants treated with colchicine shows the success of chromosomes being doubled since haploid plants are sterile and do not produce any seed.

Intermediary method (C)

Intermediary method is a combination of the 2 other methods. The incipient stages including sterilization, pollination and injecting 2,4-D hormone are exactly the same as classic method. 5 or 6 days after the pollination and seed formation, the stems were cut and transferred to the liquid medium culture without 2,4-D. Furthermore, the stems were 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. This method has various advantages than previous ones because all of the important process such as sterilization, pollination and hormone treatment on the plant are done in the flower vase. This deed results in a better pollination 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.Activities done to assess the lines and cultivars under the natural conditions of the field are as follows: Early maturing spring wheat which was expected to be suitable for the warm climate of the south of the country was selected, after evaluation, from International treasury and collection of native wheat of the country every year in Karaj site and was sent to Ahvaz for main assessment. Also, in Ahvaz site, appropriate varieties and lines were selected and assessed for the project. In order to evaluate the agronomic traits (growing period, plant height, lodging, kernel yield) and for terminal heat stress

tolerance, 50 lines from Cereal Gene Bank collection and 133 lines selected from Karaj international experiments were cultivated in Ahvaz station. Besides, preliminary assessment was performed on 86 doubled haploid lines produced in Karaj.

Note-taking and statistical analysis

During this study, the number of pollinated florets, produced seeds, haploid embryos and haploid seedlings were recorded. Statistical analysis of the collected data was performed using chi-square test. In addition, Excel software was applied to draw the tables and figures.

RESULTS and DISCUSSION

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 [(Number of Seed Formation/ Number of Pollinated Floret) × 100] in classic method (A). Using Chi-square test shows that, with 5% probability, there is not any meaningful difference among wheat genotypes regarding seed formation percentage in this method.

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

WG	NPF	NSF		χ^2
		No	%	
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	13.78^{ns}

ns: Not Significant at 5% Level,WG: Wheat Genotype, NPF: Number of Pollinated Floret, NSP: Number of Seed Formation

Seed formation percentage in detached tiller method (B)

With regard to Table 2, genotype DH-133 with 89.32% and genotype DH-135 with 64.92% are respectively the best and the weakest genotype regarding seed formation percentage in detached tiller method (B). Using Chi-square 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 2. Seed formation percentage in detached tiller method (B).

WG	NPF	NSF		χ^2
		No	%	
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^{**}

**:Significant at 1% Level, WG: Wheat Genotype, NPF: Number of Pollinated Floret, NSF:Number of Seed Formation

Seed formation percentage in intermediary method (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 genotype regarding seed formation percentage in intermediary method. Using Chi-square test shows that, with 5% probability, there is a meaningful difference among wheat genotypes regarding seed formation percentage in this method.

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

WG	NPF	NSF		χ^2
		No	%	
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	$9.70^{n.s}$

ns: Not Significant at 5% Level, WG: Wheat Genotype,NPF: Number of Pollinated Floret, NSF: Number of Seed Formation

Bakoset al. (2005) stated the average seed formation percentage 74% in their experiments. Jain et al. (1996), combines 12 F1 wheat genotypes with 1 maize genotype. The extent of seed formation percentage was variant between 68.94% and 89.76%. The average seed formation percentage was stated 82.87%. Jain et al. stated that no meaningful difference was observed in seed formation percentage. Javid Ahmad (2004) combined 8 hexaploid F1 wheat genotypes and 4 tetraploid F1 wheat genotypes with 3 maize genotypes named FSH-399, Akbar and Composite. They have used detached tiller method and hand operated sterilization. Seed formation percentage was reported to be 87.0% to 99.5% in hexaploid genotypes with the average of 90.3% and in tetraploid genotypes 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. Bakhtiaret al. (2006) combined 3 wheat genotypes with 3 maize genotypes 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.

Embryo formation percentage in method (A)

With regard to Table 4, the best genotype in method A regarding embryo formation percentage [(Number of Embryo Formation/ Number of Seed Formation) × 100], is DH-132 with 28.52% and the weakest one on this method is DH-135 with 15.81%. Chi-square test shows that, with 5% probability, there isn't any meaningful difference among wheat genotypes in classic method regarding Embryo formation percentage.

Table 4. Embryo formation percentage in method (A).

WG	NSF	NEF		χ^2
		No	%	
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	$14.45^{n.s}$

ns: Not Significant at 5% Level, WG: Wheat Genotype,NSF: Number of Seed Formation, NEF: Number of Embryo Formation

Embryo formation percentage in method (B)

With regard to Table 5, changes extent in embryo formation percentage is variant in method B: genotype DH-135 with 15.66%, genotype DH-133 with 30.32%. Therefore DH-133 is the best genotype in this method and DH-135 is the weakest one regarding e genotype formation percentage. Chi-square 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

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

WG	NSF	NEF		χ^2
		No	%	
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	15.66	5.20
Total	2683	631	23.52	24.52**

**: significant at 1% level,WG: Wheat Genotype,NSF: Number of Seed Formation, NEF: Number of Embryo Formation

Embryo formation percentage in method (C)

As can be seen in Table 6, 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%. Chi-square test shows a meaningful difference among wheat genotypes with 5% probability, regarding embryo formation percentage.

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

WG	NSF	NEF		χ^2
		No	%	
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*

*: significant at 5% level,WG: Wheat Genotype,NSF: Number of Seed Formation, NEF: Number of Embryo Formation

Bakoset al. (2007) reported the embryo formation percentage 20.7% on their researches. Mehta and Angra (2000), said that using 2,4-D hormone after pollination can be useful for the number of formulated haploid wheat embryos. Berzonskyet al. (2003) 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 tiller method 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. Inagaki (1997) stated the influence of fresh pollen seeds in formulating haploid wheat embryo. Zhenget al. (2001) recognize the effect of maize genotype 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. Zhenget al. (2001) stated the seed formation percentage 20.5% and 19.4% in classic and detached tiller method respectively. Jain et al.(1996) reported the extent of embryo formation percentage 10.62% to 25.23% and the average embryo formation percentage 20.13%. Javid Ahmad (2004) 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%. Bakhtiaret al. (2006), stated embryo formation percentage 64.5% in the common method (A) and 55.7 percent in detached tiller method (B).

Haploid seedling formation percentage in method (A)

With regard to Table 7, the extent of changes in haploid seedling formulation percentage [(Number of Haploid Seedling/ Number of Embryo Formation) × 100] in method A is from 30.56% in DH-125 genotype to 63.04% in genotype DH-131.Using Chi-square test, with 5% probability, doesn't show any meaningful difference among wheat genotypes regarding haploid seedling formation percentage in this method.

Table 7. Haploid seedling formation percentage in method (A).

WG	NEF	NHS		χ^2
		No	%	
DH-124	41	13	31.71	2.70
DH-125	36	11	30.56	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	$12.97^{n.s}$

ns: Not Significant at 5% Level, WG: Wheat Genotype,NEF: Number of Embryo Formation, NHS: Number of Haploid Seedling

Haploid seedling formulation percentage in method (B)

With regard to Table 8, 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. Using Chi-square test, with 5% probability, doesn't show any meaningful difference among wheat genotypes in this method.

Table 8. Haploid seedling formation percentage in method (B).

WG	NEF	NHS		χ^2
		No	%	
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
	631	313	49.60	$16.56^{n.s}$

ns: Not Significant at 5% Level, WG: Wheat Genotype,NEF: Number of Embryo Formation, NHS: Number of Haploid Seedling

Haploid seedling formulation percentage in method (C)

As we can see to Table 9, 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 Chi-square test in this method doesn't show any meaningful difference among wheat genotypes regarding haploid seedling formulation percentage.

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

WG	NEF	NHS		χ^2
		No	%	
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	$16.47^{n.s}$

ns: Not Significant at 5% Level, WG: Wheat Genotype,NEF: Number of Embryo Formation, NHS: Number of Haploid seedling

Berzonskyet al. (2003) did not observe any meaningful difference in applying method of hormone 2,4-D and the abundance of formulated haploid seedling. Bakhtiaret al. (2006), reported haploid seedling formulation percentage in common method (A) and detached tiller one (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 from researches done on 3 wheat genotypes (G1, G2, G3) in combination with maize genotypes (H7, H3, H1). Jain et al. (1996) combined 12 F1 wheat genotype with one F1 maize genotypes and stated the extent changes of haploid seedling formulation percentage form 25.00% to 58.46% with the average of 45.21%. Javid Ahmad (2004) used 8 F1 hexaploid genotypes and 4 F1 tetraploid biotype in combination with maize, in order to compare

hexaploid and tetraploid wheat genotype. They used detached tiller method and hand operated sterilization 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 tetraploid genotype as 18.1% to 37.6% with the average of 24.6%.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 of haploid seedling formulation. Besides with regard to Table 10, in interstitial and classic regarding seed formation percentage, with 5% probability, there is not any meaningful difference among wheat genotypes, but in detached tiller method, 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%.

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% and the best genotype regarding haploid seedling percentage is DH-132 with 65.08% and the weakest one is DH-124 with 35.33% (Table 10).

Table 10. Overall table compares wheat genotype for all traits in all their methods.

Wheat genotype	Florets (A)	Seed (B)	Embryo (C)	Seedling (D)	%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

Field assessments under natural conditions

In order to evaluate the agronomic traits (growing period, plant height, lodging and kernel yield), 30 lines from Cerea lGene Bank collection and 114 lines selected from Karaj international experiments were cultivated in Ahvaz station. Out of 30 lines received from Cereal Gene Bank, 12 lines were selected with regard to lines appearance, diseases and early maturity. Out of 114 lines from international experiments of cereal section, 54 lines were selected with regard to early maturity and would be cultured for further study in the next year in crossing block test. Furthermore, 92 doubled haploid lines which were related to the first generation of colchicine were cultured for proliferation and preliminary study in Ahvaz site, which 86 of them had become green. However, given that these lines were the first generation of colchicine and were planted with delay (the second half of December), the results obtained are not reliable. Evaluation and judgment about these lines will be reliable after a generation of proliferation and self-pollination.

CONCLUSION

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

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