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Overview of Wheat X Maize System of Crosses for Dihaploid Induction in Wheat

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Abstract: The use of gamete based dihaploid technology greatly enhances the production of complete homozygous wheat lines in a single generation and increases the precision and efficacy of selection process in crop breeding. It is a useful tool to reduce the time in attaining homozygosity not only for wheat breeding, but also for various aspects of its genetical studies. Anther culture and wide hybridization are most commonly used method for wheat polyhaploid induction. However, in the recent years it has commonly been experienced that wheat x maize system of crosses following chromosome elimination is an effective and handy tool among available methods for haploid induction in wheat. Its superiority over other techniques includes higher efficacy (2-3 times more efficient for green plant production than the anther culture), simple, less genotype dependent response, less gametoclonal variation and less time consuming. In this paper we try to elucidate several aspects of this system to be used for the production of dihaploid lines in wheat improvement programme.

Key words: Auxin source · Doubled hpaloid · Embryo rescue · Polyhaploid · Wide hybridization

INTRODUCTION

Genetic improvement of major food crops generally comprises three main phases, i. introduction and manipulation of heritable variation, ii. inbreeding couple with selection and iii. extensive evaluation of breeding materials at multilocations to identify the areas of adaptation and commercial worth [1]. Isolation of homozygous and homogeneous breeding lines/population through conventional breeding is possible, only if breeding materials are permitted to several cycles of inbreeding and selection. Thus this phase of breeding is the most tedious, time consuming and expensive to crop breeding programmes, which significantly delays the cultivar development processes. Recent advances in plant tissue culture and its related disciplines open an avenue that greatly facilitated the haplodiplodization (via androgenesis and wide hybridization) breeding scheme, which eliminates the first two phases [2] and permits the extraction of instant homozygous lines/varieties from crop plants with any degree of heterogeneity in a single generation.

Haploids individuals are the sporophytes with gametic chromosome number [3] and by doubling the haploid chromosome complements can instantly be produced dihaploids (DH). In addition, dihaploid breeding methods are the fastest method to create inbred lines and are completely homozygous. Their progenies will be homogeneous and exceptionally uniform. In conventional plant breeding, truly homozygous lines are rare and most selection contains some heterozygous loci [2]. Therefore, DH systems may increase the efficiency of a breeding program for the following reasons: 1) time required to obtain homozygous/homogeneous plants could be shortened by about half or less, 2) more accurate and efficient selection may be possible by using homozygous/homogeneous plants. Additionally, small population needs to be handled to bred the cultivar through DH system, because of the frequency of superior gametes is always higher than the frequency of corresponding superior plants in F_2. At present, DH derived from hybrid progenies can be used as recombinant inbred lines with favorable uniformity in breeding following selection.

Besides that several research papers and theoretical reviews [4-7] indicated that haploid breeding has several advantages over the conventional method and equally important to molecular mapping and genetic studies. Dihaploid breeding also provides a way of combining and fixing the desirable features of diverse wheat genotypes into a common genetic background [8]. At present haploid breeding technique is quite advanced and is being

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routinely employed to generate valuable homozygous breeding materials and creation of new cultivars in varieties of crop plants throughout the world. For example China, which ranks the first to use this technology, has alone released hundreds of rice and wheat varieties within a period of 15 years [9].

Methods Available for Haploid Production: Guha and Maheshwari [10] for the first time produced haploid plants from anthers of *Datura inoxia*, since then this technique has been routinely applied to many plant species [11]. At present a number of DH production techniques such as anther/microspore culture, ovary/ovule culture, chromosome elimination following wide hybridization, haploid inducer gene/s and chemicals have been employed [5, 9]. Regeneration of anther/microspore-derived plants has been successful in most of the cereal crops like rice [12, 13], wheat [14-16], maize [17], barley [18], oat [19] and sorghum [20]. Haploid regeneration from cultured ovaries/ovules has also been reported in barley, maize, rice and wheat [2, 6, 9, 21, 22]. However, the efficiency of haploid regeneration is not as high as that in anther/microspore culture. Presently wide hybridization has been widely applied to wheat and barley. Kasha and Kao [5, 23] for the first time reported haploid barley through barley x *Hordeum bulbosum* crosses (*H. bulbosum* system). Later this system was extended to wheat [24]. This system was also no longer effective due to the presence of Kr1 and Kr2 genes situated on the 5A and 5B wheat chromosomes which markedly reduce the crossability between wheat and *bulbosum* [25]. Despite the limited success, the efficiency of haploid production in wheat through anther/microspores and wheat x *bulbosum* techniques are highly genotype-dependent; which limits the use of these techniques in practical wheat breeding [26-29].

In order to successfully apply the DH systems to a breeding program, any techniques should fulfill the following three criteria: i) DH line/s should be produced efficiently from all genotypes, ii) DH should represent a random sample of the parental gametes and iii) DH should be genetically normal and stable [30]. At present most of the researchers believe that wheat x maize system of crosses fulfill these criteria and is being extensively utilized for haploid induction in wheat [28, 29, 31-35].

Interspecific/intergeneric Hybridization: Several comprehensive efforts have been made to fulfill the criteria proposed by Snape *et al.* [30]. During the course of the investigations, scientists tried to seek alternative way for haploid induction in wheat and to date haploid wheat has been successfully produced by crossing wheat with maize [36-40], wheat x pearlmillet [22, 41], wheat x tripsacum [42], wheat x teosinte [43], wheat x barley [24], wheat x job's tears (*Coislachry-ma jobi* L.) [44] and wheat x sorghum [45]. Such Intergeneric crosses have been found to be effective for the production of dihaploid plants in wheat. Polyhaploids induction from such crosses is possible because of the preferential chromosomes elimination of the pollen parent during embryo development and haploid plants can be recovered following embryo rescue. Several research efforts are concentrated towards the use of intergeneric wheat x maize crosses and suggested that it can be treated as a viable alternative to other method of DH production in wheat [34, 46-48].

Wheat x Maize System of Crosses: It was 1984 for the first time Zenkteler and Nitzsche [49] reported that embryos are frequently formed when hexaploid wheat pollinated with maize. This raised the considerable interest among wheat breeders and Laurie and Bennett [50, 51] at Plant Breeding Institute, Cambridge started systematic study to confirm the previous reports. They were cytologically able to demonstrate that the maize pollen normally germinates and grows into the wheat embryo sac where the wheat egg is fertilized by the maize sperm nuclei. A hybrid zygote with 21 wheat chromosomes and 10 maize chromosomes [51] is produced. The hybrid zygotes are karotypically unstable; therefore, maize chromosomes fail to move to the spindle poles during cell divisions. Possibly, their centromeres fail to attach to the spindle microtubules due to progressive loss of centromere activity, which is seen as reduction in size of and finally the loss of, the primary constriction as reported for *H. vulgare* x *H. bulbosum* hybrid [51]. The maize chromosomes are rapidly eliminated after a few cell divisions and thus forming a haploid embryo with 21 wheat chromosomes [51].

Zhang *et al.* [52] comparatively analyzed the embryogenesis in wheat x maize hybrids and self-pollinated wheat plants using paraffin sectioning. They reported that development of embryo is not accompanied by the formation of an endosperm and the endosperm nuclei remain free in the cytoplasm, fail to advance into the cellular stage and degenerate at a later time. They also obtained wheat polyembryos from spikelet culture resulted from the cleavage of the pro-embryo and the effect of 2, 4-D. Since then, for expediting wheat breeding programmes, the maize mediated haploid

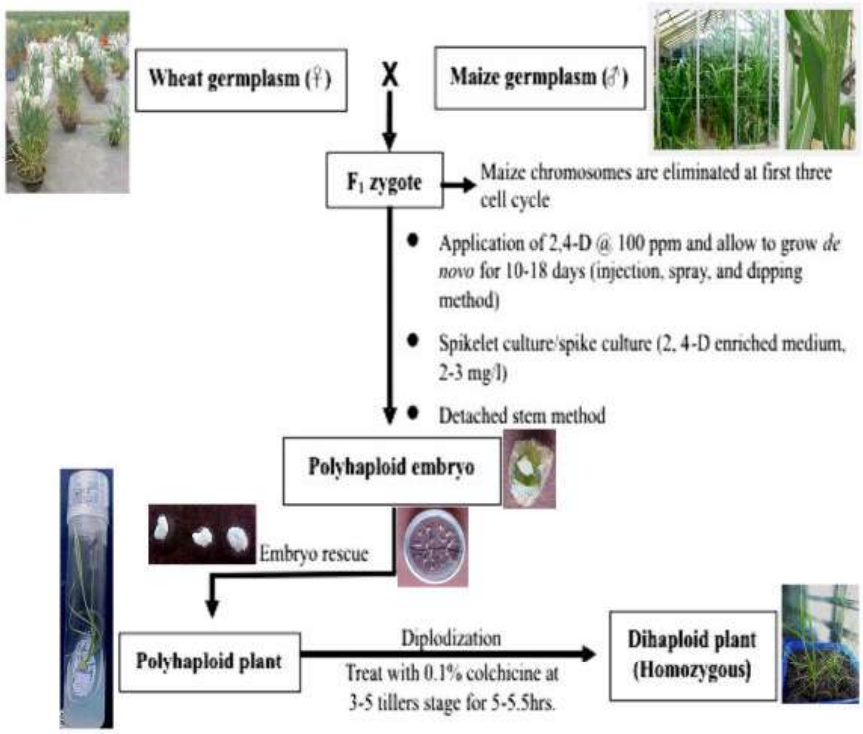

Fig. 1: Simplified protocol of maize pollen mediated dihaploid induction in wheat

breeding technique has been extensively exploited. It has immense practical utility as compared to the androgenesis and *bulbosum* techniques because of haploid production via this technique is very simple and more responsive [27, 28, 37].

Technique of Wheat x Maize System of Haploid Induction: Suenaga [22] and Riera-Lizarazu and Mujeeb-Kazi [33] described the simple and fairly efficient method for crossing and auxin treatment. In wheat x maize system emasculated wheat spikes are pollinated with fresh pollen of maize at the time of anthesis. Cryopreserved (–80°C) maize pollen can also be successfully utilized for pollination of wheat florets. However, the efficiency of haploid formation was quite low as compared with fresh pollen [37]. But this technique is quite advantageous where synchronization is a problem. Once the pollination is complete, the application of auxin is essential (Fig. 1) for the successful recovery of haploid wheat embryos [48, 53].

Suenaga [54] tested different types of hormones including 2,4-D, NAA, IAA, BA Kinetin, Zeatin, GA3, ABA at different concentrations and found that 1 ml of 100 ppm 2,4-D injected at base of the uppermost internode of wheat was very effective. Since then several attempts, such as spray, tiller injection, dipping and spikelets culture, have been made for the effective application of plant hormones, especially, 2, 4-D [35, 35, 54-56]. Kaushik *et al.* [55] found that 2, 4-D supplemented through spikelets culture method was superior for the recovery of polyhaploid embryos formation and plantlets regeneration. In spikelets culture, they used post pollinated spikelets (2 days after pollination) and placed upright on MS medium containing 30g/l sucrose and 2 mg/l 2, 4-D and incubated for three weeks at 20° C with a 16 h day length. But all these procedure are quite tedious and laborious, therefore, non emasculation method and spike culture technique (detached tiller method) has been proposed to eliminate much of the labour required for emasculation and treatment with 2, 4-D from previous methods [35, 37, 55]. Spike culture technique is quite effective to increase the frequency of polyhaploid embryo formation. Recently frequency of polyhaploid embryo formation has greatly improved through the manipulation of Dicamba alone or in combination with 2, 4-D [58]. Garcia-llamas *et al.* [58] obtained 3 haploid plants/spike from those treated with Dicamba. Embryo formation efficiency was also improved in durum wheat following spray with 2, 4-D 3 mg/l plus 120 mg/l $AgNO_3$ [59, 60]. Embryos generally rescue after 14-20 days of post pollination. Embryo rescue is essential to recover haploid plantlets from wheat x maize system [48, 52, 53, 61].

Pre-embryo will abort if it is allowed to develop *in vivo* due to a nutritional shortage in the absence of an endosperm. Excised embryos can be cultured on either full strength of MS [62] or ½ MS or B5 basal medium [63] containing various modifications of organic supplements [34-36, 49, 52] and can be grown *in vitro* for a 3-5 weeks at 20-25°C and 16 h day length. Generally seedlings are ready to transfer up to that period and needs to be hardened for one week in growth chamber at the same environmental regime. Once the seedlings are 3-5 tiller stage, colchicine treatment needs to be practiced (Fig. 1). For the treatment of colchicine, the procedure proposed by Inagaki [37] is very efficient. According to his method, roots of the haploid seedling are pruned leaving a zone of 2-3 cm and submerged in a 0.1% colchicine solution supplemented with 2% dimethyl sulfoxide and ca. 0.05% tween 20 at 20° C for 5 hours. The treated plants need to be hardened under the same conditions for about one month prior to transplant into the pot at external environment.

Stability of DH Lines: Very limited studies have been conducted on this line. Suenaga and Nakajima [64] evaluated 110 wheat DH lines derived from wheat x maize crosses and found that 15 DH_2 lines were variable for traits like extreme dwarfism, low seed fertility, alteration of spike type and strips. Analysis of variance within and between DH lines showed the presence of heterogeneity/heterozygosity in the DH_2 lines/plants. Limited studies have been conducted on this line. They inferred that most of the variations detected in the DH lines were due to the effects of colchicine treatment. Similarly Kammholz *et al.* [65] also found that expected normal segregation pattern for 6 glutenin loci across the 7 crosses indicated that wheat x maize system is stable across the generations and may meet the third criterion proposed by Snape *et al.* [30] for practical wheat breeding programmes. Moreover, Lefebvre and Devaux [66] also reported normal segregation for 1BL-1RS chromosome through wheat x maize system of cross, but deviate from 1:1 in the haploid progenies produced by anther culture.

Influence of Wheat and Maize Genotypes (Genotype Specificity): Although various studied revealed that wheat x maize system is less insensitive or strongly insensitive to wheat genotypes, a substantial report have also been appeared that both wheat and maize genotype showed striking influence on polyhaploid embryo formation to plantlet regeneration. Suenaga *et al.* [22] observed considerable genotypic differences in the ability to produce embryos by evaluating 47 wheat genotypes from diverse geographical regions (Asia, Europe, North America and Australia) and 55 maize varieties and lines. They reported that the overall mean efficiency of haploid embryo formation was 15.2%. However, the efficiency of embryo formation was varied considerably, from 0.9% for Oligo Culm from Israel to 35.8% for Hiyoku-komugi from Japan. Since then effect of parental genotypes on haploid embryo formation to plantlet regeneration have been routinely investigated to assess the utility of this system [27-29, 34, 47, 61]. Most of the studies showed that considerable varietal differences among wheat [22, 39, 46, 67, 68] and maize genotypes [34, 48, 53, 68, 69] were observed. Furthermore, their analysis also revealed that the genotypic interaction between wheat and maize was significantly influenced the recovery of polyhaploid embryo formation and plant regeneration. However, this system is useful method to recover haploid plantlets from recalcitrant wheat genotypes to anther culture [28, 68].

Some researchers found only the influence of maize genotypes and most of the cultivars of wheat used by them were insensitive to crossability barriers. Investigators [34, 52, 53] observed the effect of maize genotypes on the formation of embryos and plantlets regeneration, respectively, regardless of wheat genotypes (Table 1). Verma *et al.* [70] quantified the effect of maize genotypes through line (wheat) x tester (maize) analysis and found that striking differences of maize genotypes on frequency of haploid embryos formation and plantlets regeneration. They also concluded that the genotypic influence of maize genotype was greater than that of wheat. Suenaga *et al.* [69] studied the effect of 55 maize varieties and lines by pollinating Fukuho-komigi as female parent and their study revealed that considerable genotypic differences with embryo formation rate ranging from 16 to 36%. Therefore, haploid induction rate in wheat via maize mediated system can be enhanced by selecting and breeding of either more responsive wheat or maize genotypes [53, 61].

Wheat x Maize vs. Androgenesis and Bulbosum Technique: Very limited studies have been conducted to compare the efficiency of wheat x maize system for haploid plant regeneration with androgenesis and *bulbosum* techniques. Suenaga [54] made comparison of efficiency of wheat embryo formation and reported that average efficiency for embryo formation was comparable to that of most crossable varieties in wheat x *H. bulbosum*. Most of the wheat varieties have very low crossability to

Table 1: Effect of four maize genotypes on ovaries development, embryos formation and subsequent haploid plants regeneration

		F_1 Wheat (Acc #7103/WK 1204)			
Maize genotypes	No. of florets pollinated	No. of developed ovaries	No. of embryos formed	No. of embryos culture	Haploid plants/florets pollinated (%)
Khumal Yellow	116	52 (44.37) [a]	22 (18.91) [a]	15	7 (6.04) [a]
Rampur Composite	91	28 (30.65) [b]	5 (5.17) [b]	4	1 (0.96) [b]
Arun-1	102	36 (35.18) [a]	19 (18.51) [a]	16	7 (6.81) [a]
Arun-2	111	49 (44.32) [a]	24 (21.45) [a]	18	11 (10.15)[a]
Total	420	165 (38.631)	70 (16.01)	53	26 (6.00)
CV	-	8.82	15.31	-	25.22

Figures in parentheses indicate the original mean percentage of well developed ovaries (ovaries/pollinated florets), embryo formation (embryos/pollinated florets) and haploid plant formation (seedlings/pollinated florets). Original means within parenthesis followed by the same letter are not significantly difference at α =0.05 Data adopted from Niroula *et al.* (2007)

2H. bulbosum because of the presence of dominant alleles of the Kr1 and Kr2 genes which restrict application of *H. bulbosum* to haploid wheat breeding. Sadasivaiah *et al.* [28], Fedak *et al.* [71] reported that less genotype dependent response in wheat x maize hybridization system than in anther culture and *bulbosum* method.

The wheat x maize system has many advantages such as higher efficacy [27, 28] less gametoclonal variations [65, 66], less time consuming and less genotype dependent [27, 54]. Based on the previous results, the maize pollen method is about 3-4 times more efficient than anther culture. Sadasivaiah *et al.* [28] obtained 6.29 green haploid plants out of 100 florets pollinated as compared to 1.64 from 100 cultured anthers. The apparent higher efficacy for green haploid plant regeneration through this technique is due to the higher rate of embryo formation, higher germination rate of polyhaploid embryo and no existence of albinism.These are the notorious problems in wheat anther culture. Although few studies have shown that embryo formation rate in anther culture is higher than that of wheat x maize system, most of anther cultured derived embryos either failed to germinate or even if germinated, developed into albino plants whereas all of the wheat x maize derived embryos normally germinated and developed into green plants [29]. The superiority of this system over anther culture is also shown in Fig. 2. Most of the investigators except Oury *et al.* [72] reported that wheat x maize system is superior over androgenesis. Kisana *et al.* [27] and Sadasivaiah *et al.* [28] showed that wheat x maize is quite fast, easiest and 2-4 times superior in terms of overall plants regeneration. The low level of genotype specificity, absence of albinism and ease of application, make this technique more efficient than anther culture for the production of polyhaploids in common wheat. Moreover, wheat x maize-derived dihaploid populations represents unbiased assortment of parental gametes [66].

Future of Wheat X Maize System: From this review, it is known that wheat x maize system has great potentiality to improve the efficiency of future wheat breeding programmes. This technique can be used as viable alternative to anther culture and *bulbosum* technique to fix easily any type of desirable traits into elite wheat background in a single generation. The utility of this system can be improved through integration with convergent crosses and marker aided selection. Additionally, this technique has also great promise to transfer any desirable gene/s from related wild species. However, several technical advancements still need to be addressed so that it can be routinely utilized in wheat breeding programme. The hybrid nature of wheat x maize zygote in future can open new molecular avenue for transferring maize active transposable elements and C_4 cycle into wheat background. Active transposable elements in wheat could be of great value since in theory any gene producing a recognizable phenotype when mutated by transposable element insertion could be cloned for molecular analysis and manipulation [38]. Therefore, investigators should concentrate their efforts to recover wheat x maize hybrids. Once the hybrid will be recovered, it can revolutionize the wheat breeding as well as genomic study of wheat plant.

Concluding Remarks: The present review revealed that the wheat x maize system of crosses is superior over *bulbosum* and anther culture techniques in wheat. The superiority of this method includes are: simple and less time consuming, less genotype dependent, less gametoclonal variation, higher rate of embryo formation and germination, less albinism and higher rate of green plant recovery. Further this technique also fulfills all possible criteria proposedby Snape *et al.* [30]. Although a lot of technical refinements have been made in the past, in order to use this system in practical breeding

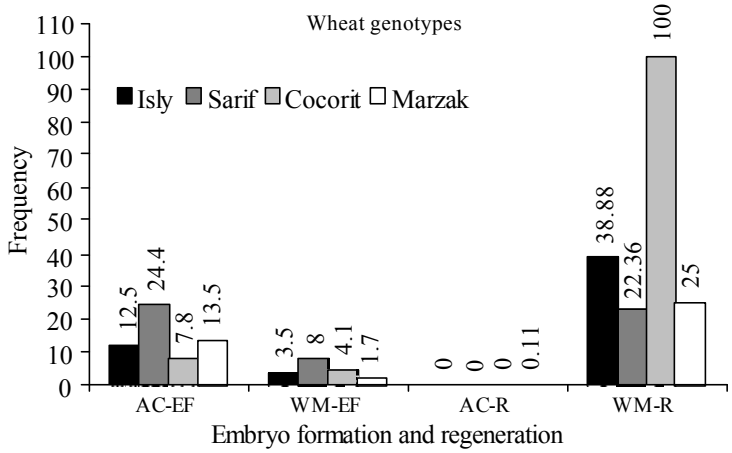

Fig. 2: Comparison between androgenesis and wheat x maize system of cross for embryo and green polyhaploid plant formation. Where AC-EF = embryo formation in anther culture, WM-EF = embryo formation in wheat x maize crosses, AC-R = regeneration from anther culture, WM-R = regeneration from wheat x maize crosses
Data adopted from Saidi *et al.* (1998)

programmes, further strategic research on enhancement of embryo formation, germination and green plant regenerations are urgently needed. These components, in future, can be improved by manipulating suitable auxin sources and various environmental factors such as light and temperature regimes. This technique could thus complement conventional breeding programmes and accelerate the release of new varieties in developed countries, as well as in developing countries where rapid varietal development is critical for sustainable wheat production systems. Therefore, the integration of DH technology via wheat x maize system into wheat genetics and breeding program is extremely useful to reduce the breeding cycle, develop new cultivars and improve our understanding of agronomically important genetic traits.

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