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Haploid production system wheat × maize: A review

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Abstract
By using double haploid technique we can develop complete homozygous line in a short period of time. Anther culture and wheat ×maize wide hybridization are most commonly used techniques to produce haploids. But in wheat × maize system is more advantages than anther culture such as increased embryo efficiency, less time and gametoclonal variations. In these review an introduction of anther culture and wheat × maize system and factors affecting them are stated.

Keywords: Anther culture, auxin treatment, double haploids, wide hybridization and haploid production system

Introduction
Doubled haploids have 100% homozygosity, which can be attained in one generation. In conventional breeding procedures several repeated cycles of selection and evaluation will be required to produce homozygous lines (Mergoum *et al*., 2009) [27]. A number of evaluation and selection cycles, these stages of breeding are the most time-consuming, costly, and cumbersome for crop breeding programmes, slowing the development of cultivars. Therefore by double haploid breeding techniques we can increase the selection efficiency of breeding programs as the time it takes is only one generation as contrast to conventional breeding which takes 6-7 generations to produce homozygous plants could be shortened and also using homozygous plants could allow for more accurate and efficient selection (Snape *et al*., 1990) [24].

Haploids have single set of chromosomes on doubling haploid chromosome complement by using agents like colchicine; we obtain double haploids in wheat (Inagaki, 1997) [13, 14]. Double haploid breeding involves mainly two important steps namely-1) production of haploid plants 2) doubling of haploid plants.

There are different methods for the haploid plants production such as (1) androgenesis (2) intergeneric /interspecific hybridization. An *in vitro* method in which microspore with haploid chromosome complement when placed on culture medium develop embryo-like structure which future develops into a haploid plant is known as anther culture (Jauhar *et al*., 2009) [16]. However more albino plants are produced and also anther culture is genotype dependent (Cistué 2009) [8].

Wide hybridization is the cross between the plants or between the plants and their wild relatives. It is used to transfer desirable genes (Sharma and Gill, 1983) [36]. *Hordeum vulgare* x *Hordeum bulbosum* crosses result in a haploid progeny of *Hordeum vulgare* due to chromosomal elimination of the *H. bulbosum*. (Kasha and Kao, 1970) [19]. When wheat was crossed with H. bulbosum, haploid wheat progeny was obtained (Barclay, 1976). In wheat ×maize wide hybridization crosses haploid embryos were obtained (Laurie and Bennett, 1986) [20]. Wide hybridization can be used on a variety of wheat genotypes as well as maize (Cherkaoui *et al*., 2009). The wheat x maize system of crosses, according to the majority of researchers, meets all criteria and is widely used for haploid induction in wheat (Sadasivaiah *et al*., 1999) [33].

Androgenesis
In Datura spontaneous occurrence of haploids was reported by (Blakeslee *et al* 1922) [5]. Anther culture gained its potential only after it was first reported in the 1970s through *in vitro* methods in *Datura innoxia* by Sipra Guha and S.C. Maheshwari at university in Delhi in India (Guha-Mukherjee, 1999) [12]. Guha and Maheshwari proved that haploids can be obtained by anther culture. From then work has been carried out in some other crops like tobacco.

"FLORIN" is commonly used wheat cultivar which has been developed by anther culture in 1973 (De Buyser *et al*., 1987) [9]. Anther culture in wheat depends on various factors such as high frequency of albino plants generated, genotype dependence (Anderson *et al*., 1987) [3], microspore developmental stage (Ding-Gang and & Jun-Wen, 1984) [10], anther pretreatment (Lazar *et al*., 1990) [28]. Anther culture success has been demonstrated to be highly dependent on the genotype of the parent, as well as its environment during growth and culture conditions (Moien and Sarrafiu, 1995) [28].
Two important issues have been observed in durum wheat androgenesis first is the interaction between genotype and microspore culture technology and second is high proportion of albino plants regenerated (Cistue, 2009) [8]. Problems related to anther culture includes genotype specificity (Orshinsky and Sadasivaiah, 1997) [32], low callus induction rate and albinism (Chaudhary *et al*., 2003) [6].
Any technique used to successfully apply Double haploid production systems to a breeding programme must meet the following three criteria: a) DH line/s should be efficiently produced from all genotypes. b) DH should be genetically normal and stable c) and it should represent a random sampling of the parental gametes (Snape *et al.*, 1986) [37]. The wheat x maize system of crosses, according to the researchers, meets these characteristics and is widely used for haploid induction in wheat (Laurie and Reymondie, 1991) [23] (Saidi *et al.*, 1998) [34].

Wheat × Maize wide hybridization

Frist time report of when Triticum aestivum was pollinated with maize, embryos are usually produced was given by Zenkteler and Nitzsche (Zenkteler and Nitzsche, 1984) [41]. As this haploid production system was a success later several studies were extensively done. Maize was found to be insensitive to crossability genes kr1, kr2 located on chromosomes 5A and 5B when crossed with hexaploid wheat (Laurie and Bennett, 1986) [20]. In durum wheat was crossed with maize it also produced embryos (Amrani *et al*., 1993) [2]. From then wheat x maize crossings in hexaploid and tetraploid wheat were then used in a variety of studies (O'Donoughue and Bennett, 1988; Laurie and Bennett, 1988, 1989; Suenaga and Nakajima, 1989; Laurie and Snape, 1990; Inagaki and Tahir, 1990; O'Donoughue and Bennett, 1994; Almouslem *et al*., 1998) [29, 21, 43, 22, 40, 24, 15, 30, 31, 1]
The wheat x maize system offers several advantages, including increased efficiency, reduced time (Sadasivaiah *et al.*, 1999) [33] and fewer gametoclonal variations (Lefebvre and Devaux, 1996) [26].

Different Strategies of Wheat x Maize System

Simple and effective method for haploid induction include crossing followed by auxin treatment in both hexaploid and tetraploid wheat but during these crosses fresh maize pollen is used (Suenaga, K. 1994; Sarrafi *et al*., 1994) [39, 35]. When maize pollen was cryopreserved for 8 months and then used it reduced the embryo formation frequency (Inagaki *et al.*, 1997) [13, 14]. Application of 2,4-D before pollination did not increase the embryo formation frequency however its frequency was increased to 11.8% when applied after pollination (Suenaga *et al*., 1989) [40]. Other than injection method other method of 2-4 D application include spray, Dipping and spikelet culture method .According to (Kaushik *et al.*, 2004) [18] spikelet culture method of application of 2,4-D proven to produce more haploid embryo when suitable environmental conditions are present when compared to other methods of application of 2,4-D. However, because all of these procedures are tiresome and time-consuming, detached tiller approach is suggested to eliminate much of the labour required. In detach tiller method tillers are cut at the base and then Tillers are cultured for two days after pollination in a solution containing 40 g/1 sucrose, 8 ml/1 sulphurous acid (6 percent S02), and 100 mg/1 2,4-D. They're then grown in a solution containing solely sucrose and sulfuric acid until the embryos are rescued (Inagaki *et al.*, 1997) [13, 14]. In durum wheat tillers were cut 3-5D days post pollination and put in a solution containing 2-4D (100mg/l), sucrose (40mg/l), and ethanol (10mg/l), and incubated in a growth chamber at 20 °C, 16h photoperiod, and 80% relative humidity. Detach tillers gave better results for embryo formation (Cherkaoui *et al*., 2000) [7]. The greatest yield of haploid embryos was obtained after auxin treatment with 3mg/l 2, 4-D and 120-180mg /l AgNO3 in durum wheat (Almouslem *et al*., 1998) [1]. Following treatment with 100 mg/l 2, 4-D, an average of 8.9 caryopses, 2.6 embryos, and 1.3 haploid plants per spike were recovered, while various treatments with Dicamba yielded 15.0 caryopses, 6.0 embryos, and 3.0 haploid plants per spike, the best results for haploid embryos were achieved using Dicamba alone or in combination with 2, 4-D (Garcia-Llamas *et al.*, 2004; Knox *et al.*, 2000) [11, 19].
The majorities of haploid embryos produced by wheat x maize hybridization are underdeveloped or lacks an endosperm (Laurie and Bennett, 1986) [20]. So after crossing and auxin treatment, embryo rescue is the most important step from which future plants haploid plants will be generated (Kaushik *et al.*, 2004) [18]. MS media is used to culture embryos (Kaushik *et al.*, 2004; Inagaki *et al.*, 1997) [18, 13, 14]]. Different researchers has supplemented Ms media with different components one of them is, MS medium is supplemented with other components such as 1-2 mg/l kinetin, 0.1 mg/l of IAA, 60 g/l of sucrose, and 2-3 g/l of phytagel (Zhang *et al.*, 1996) [42]. The optimal number of days after pollination for embryo rescue varies by genotype (Kaushik *et al.*, 2004) [18]. After haploid plant regeneration colchicine treatment is done for chromosome doubling. When 0.005% colchicine is applied at three tiller stage of 29 haploid treated seedlings, 65.5% survived (O'donoughue and Bennett, 1994) [30-31]. The method given by Inagaki, 1997 [13, 14] involves treating haploid plantlets with a 0.1 percent colchicine solution along with 2 percent dimethyl sulfoxide and calcium. 0.05 percent tween 20 for 5 hours at 20° C is found to be effective.

Factors affecting efficiency of haploid production

The efficiency of wheat haploid generation is determined by the genotype of the pollen donor source, whether it is maize, sorghum and pearl millet (Laurie and Bennett, 1988; Chaudhary, 2008). In Durum wheat crosses it was observed the maize genotype was solely important for embryo formation Cherkaoui *et al.*, (2000) [7]. auxin treatment also a factor which influence haploid embryo formation in hexaploid wheat according to Suenaga *et al*., 1989 [40] 1 ml of 100 ppm 2, 4-D injection was very effective .While in durum wheat 250 ppm of 2, 4-D gave good results (Mahato and Chaudhary, 2015) [45].

Conclusion

When compared to other existing haploid production approaches such as anther culture, the wheat ×maize

technology has had more efficiency. It's also more efficient, takes less time, and has less gametoclonal variants. However its practical application is a little difficult. Appropriate environmental conditions and auxin treatment are required for this system to succeed. We can meet the demands of a growing population. By using this technique we can develop new varieties faster as compared to conventional methods.

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