

## STEPS AND FACTORS IN DOUBLE HAPLOID WHEAT PLANT PRODUCTION THROUGH WHEAT × MAIZE CROSS - A REVIEW

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### ABSTRACT

Double haploid (DH) production is a specific technique to facilitate breeding program for developing pure wheat lines. It involves two major steps i.e. haploid production and doubling of chromosome number. Wheat × maize crosses are used to get earlier homozygosity in bread wheat. Wheat filial generations (F<sub>2</sub> and F<sub>3</sub>) have maximum transgressive segregation and take longer time to get homozygous after seven to eight generations. Intraspecific crosses between wheat × maize gives earlier pure lines within two years. In this content, different factors contribute to the specificity of this process. An efficient work with full lab facilities on DH technique is required for early and high yielding wheat varieties development in shorter time comparative to conventional breeding. The literature was reviewed during the year 2015 after consulting research pursuits of world eminent scientists on double haploid studies.

**KEYWORDS:** *Triticum aestivum*; *Zea mays*; chromosomal doubling elements; intrageneric crosses; photoperiod; rescue media; Pakistan.

### INTRODUCTION

Wheat, a self-pollinated allopolyploid is a key feeding crop for human needs. Wheat cultivars are primarily created through conventional breeding (33), which requires self-pollination for several generations to achieve pure lines (23). In contrast, the breeding of double haploid (DH) permit to get 100% pure line only in one generation (33). This success in wheat × maize allowed DH method to become a well-known and very simple access for breeding in wheat. The production of DHs can speed up cultivar development in crop improvement programs and involves two main steps: haploid production followed by doubling of chromosome. Doubling of haploid chromosomes and effectiveness varies in different species of crops. This review covers

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methodologies available to induce haploids in wheat, focusing on DHs, and assesses the features in wheat x maize crosses during DH formation.

### **Methodologies to develop haploids in higher plants**

This article was written during the year 2015 at Agricultural Biotechnology Research Institute, AARI, Faisalabad, Pakistan after consulting research pursuits of world eminent scientists on double haploid studies.

Anther-culture and ovary culture are two methods for haploid plants production. Anther-culture is a process that has male spore cells with 'n' genome convert into embryo-like structure in culture medium to form haploid plantlets (19). Ovary culture is another way to develop haploid production i.e., megaspore embryo-genesis is a process in which unfertilized eggs into haploid plantlets by parthenogenesis (58). Wu *et al.* (57) found success 20 angiosperm species for haploid production through *in vitro* culture. Furthermore, Kristof and Imre (24) reported that gynogenesis has not been dominantly used for the haploid production in many species due to low number of megaspores and difficulties in separating megaspores from plants. In contrast, microspore culture is successfully utilized in many crops for haploid production (19).

De Buyser *et al.* (11) produced wheat cultivars (Florin), followed by (McKenzie) Graf *et al.* (13) and 'AC Andrew' (48) via anther-culture. There are difficulties in anther-culture of wheat, such as high dwarfism, low response of some lines, and large time scale of induction and as well as regeneration (28). That's why its more specific and acceptable method compared to anther culture. The development of a new wheat variety requires homogeneous lines and is a time wasting and laborious method that needs several years (22). DH enables the pure line development directly from a crop (23). Homozygous wheat lines produced in a single step by different ways such as ovary culture, anther culture and elimination of chromosome through hybridization, haploid inducer genes, variety development in short time though DH (wheat x maize) is major step in wheat comparative to conventional breeding reported by Khan and Javed (22). DH is produced in wheat via intrageneric crosses viz., wheat x barely (3), wheat x pearl millet (1), wheat x sorghum (43), wheat x tripsacum (47), wheat x teosinte (51), wheat x job's tears (38) but changing this method allowed haploid plant production from wheat cultivars (9,19). Laurie and Bennett (31) observed crossing first time between wheat x maize in 1986. Embryo formation between wheat and maize has more efficiency as compared to other crosses

Khan and Javed (22). Khan *et al.* (23) reported that DH production through wheat × maize crossing is superior as compared to ovule and anther-culture because maize pollens are very receptive and produce stable progeny population. Wheat is used as female and maize as male parent for production of doubled haploid. Laurie and Bennett (31) studied that maize pollen grew normally with in embryo sac of wheat and produced hybrid zygote having 21 haploid wheat chromosomes and 10 haploid maize chromosomes. This zygote is destabilized and maize (*Zea mays*) chromosomes unable to arrive the spindle poles during cell divisions and lastly centromere could not adhere to the spindle microtubules as a result centromere loss and finally maize chromosomes removed 3 to 4 mitotic divisions and form wheat embryo with 21 chromosomes described by Khan and Javed (22). This embryo is then rescued and developed on nutrient media for development (60). DH plants in wheat is produced and identified through genetic markers for different improving quality parameters (44).

### **Agents altering the specificity of DH production in wheat into maize intraspecific cross**

Many elements affect the DH efficiency via wheat × maize crosses: the wheat and maize lines, temperature, light intensity, photoperiod and spikelet position during plant growth period (23, 41). (Morshedi *et al.* (37), Pienaar *et al.* (45), Wedzony and Van Lammeren (56) and Campbell *et al.* (9) reported several elements like PGR type and its concentration, rescue media, biochemical elements played important role in successful crossing, colchicine treatments and temperature duration effect on chromosome doubling, when its used.

### **Genotypic effect**

According to various studies it was found that wheat and maize genotypes affect haploid embryo formation but conclusions obtained were still controversial. Suengena and Nakajima (51) crossed four wheat Japanese varieties with five maize cultivars and observed embryo percentage affected only by maize cultivars, not wheat genotypes. Martn-Lopes *et al.* (36) and Niroula and Thapa (39) studied numerous other investigations and proved wheat genotypes distinctly effect the production of embryos per florets. Lefebvre and Devaux (30) observed production of haploids affected both wheat and maize genotypes, result concluded from eighteen F<sub>1</sub> wheat pollinated with five maize varieties. They noticed that bread wheat ploidy level has vital function in double haploid. Niroula *et al.* (39) investigated four maize

(*Zea mays*) cultivars (Arun-1, Arun-2, Khumal yellow and Rampur) and found proficiency of maize at certain scales (pollinated florets number, ovaries established number, number of developed embryos, number of embryos cultured and Haploid “n,, plants) for wheat haploid formation. All spikelets pollinated by hand with fresh maize pollen, and 1 ml of 100 ppm 2, 4-Dichlorophenoxy acetic acid and inoculated on top node. One day after the 2,4-D inoculation, florets were again injected with 2,4-D solution repeatedly two days. After one week pollination, embryos separated and cultured in 1/2MS medium containing 30g/l sucrose with 7g/l agar. The cultured embryos stored at temperature (25C<sup>0</sup>) at 16/18 hours (day/night) period and incubated in dark for one week at 25C<sup>0</sup>. After pollination, application of 2, 4 -Dichlorophenoxy acetic acid necessary to obtain reasonable embryo size. The maize genotypes had significant effect on ovary development percentage, embryo formation and haploid plant. The embryo development percentages and haploid plants ranged from 5.17 to 21.45 and 0.96 to 10.15 respectively, depend used of maize varieties. The number of embryo recovery largest was investigated when wheat (*Triticum aestivum*) was crossed with Arun 2 and Arun 1, respectively. It is recommended that DH production is enhanced by using responsive maize (*Zea mays*) for pollination in wheat.

#### **Location of spikelets in flower and period for pollination**

Wheat inflorescence starts from central florets. It completes its flowering on spike in about three days. The fertilization efficiencies depend upon the floret stage and better results found at feathery stage of stigma. The location of spikelet positively interacts with number of embryo formation and highest ratios were found at middle position, but in other study explained that spikelet location does not affect the fertilization rate in a certain period of pollination. Pollination at the time of close anthesis not only gives better cross ability but also in good quality embryo with maize, that determines rate of germination of rudimentary plant with in seed.

#### **Role of external environmental factors on DH**

Temperature and light strength enhances ratio of haploid formation ratio. Light strength having larger effect can alter the pollen tube formation, important steps for successful fertilization which affects vigour of female plant, egg cells fertilization ability, pollen tube form in female. Environment also affects survival of wheat embryo and pollen tube formation. It was observed when two wheat varieties were pollinated with same maize genotype at 250 to 750  $\mu\text{mol}/\text{m}^2/\text{s}$ , PAR) irradiance levels, light strength

affected in 'Karamu' on pollen tube formation while 'Kotuku' were unaffected. Khan *et al.* (23) reported environmental conditions are vital role for DH production. 10,000 lux light intensity, 20-24C<sup>0</sup> temperature and 60-65% humidity level optimum for haploid seed formation and haploid embryo development. Niu *et al.* (41) kept pollinated plants for 2 weeks at 14 to 16°C for 16/8 day/night in a growth chamber till new seedlings were formed then plants shifted in green house at 20–24°C temperature till maturity.

### **Post pollination treatment**

Double haploid production through maize hybridization poses two problems. Proportion of embryo was less formed and unavailability of endosperm in F<sub>1</sub> (26, 59) that leads to embryo abortion when they were allowed to develop in floret of plant (26). Treatments after pollination as cited by Laure *et al.* (31), Laurie and Reymordie (29) were (i) immediately culturing for three weeks of pollinated spikelets (26), (ii) consistent application of 2,4-D (0.5 mg/l) for a period of 14 to 21 days to pollinated spikes (iii) sprinkling 2, 4-D (100 mg/l) to internode and the spikelets of the pollinated spikes for one to two times (32) (iv) application of solution containing auxin [picloram (4-amino - 3, 5, 6-trichloropicolinic acid), 2, 4-D, or 2, 4, 5-T with 6-benzylaminopurine (BAP) or combination of 2, 4-D and GA3 on florets, 24 to 30 hours post pollination (46, 49) and (v) solution of dicamba (3, 6-dichloro-o-anisic acid) or ZEN (zearalenone) after pollination and result found treatment no. 4 result with sufficient embryos formed as compared to other treatments (4, 14). Wedzony and Lammeren (56) observed 2,4-D application and GA3, pollen tubes formed and sperm cells response well in the pollen tube good and formed haploid embryo in durum wheat (*Triticum turgidum*). Myers *et al.* (35) observed using 2, 4-D (213.05 mg/L) at pH 10.36, maximum number of haploid embryos/100 ovaries formed after pollination. Almouslem *et al.* (2) observed four post pollination on 10 durum wheat genotypes hybridized three maize genotypes and better post pollination mixture of 2,4-D and AgNO<sub>3</sub> and formed 142 haploid plants. Niu *et al.* (2014) noted post pollination and spray that spikes after 24 hours with 2.4-D (213.05 mg/L) removed seeds spike for embryo rescue after two weeks.

### **Embryo rescue**

The rescue medium acts as a major element for embryo development. Gamborg *et al.* (12) reported MS medium (34), ½ MS medium and B5 medium affected germination by which repeatedly confirmed these media by Suenaga and Nakajima (51), Comeau *et al.* (10), Cherkaoui *et al.* (15) and

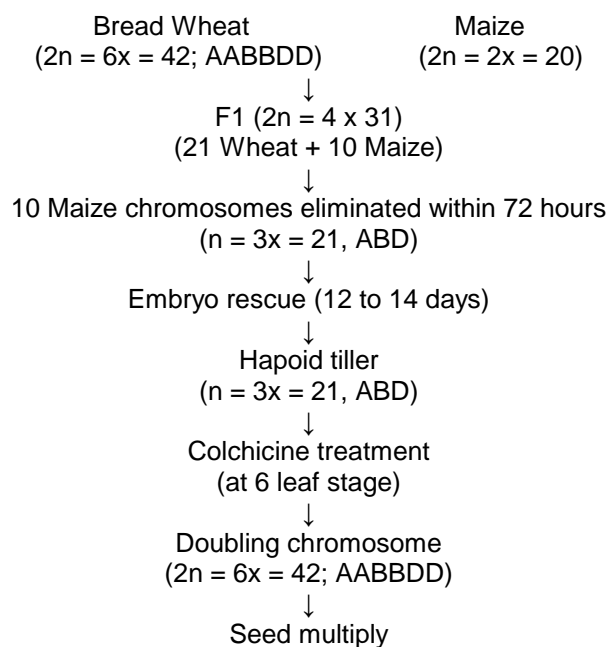
Dogramaci and Jauhar (16). Cherkaoui *et al.* (15) crossed 10 durum wheat genotypes with 8 maize genotypes at three media (B5, MS and ½MS) and found B5 and half strength (½ MS) better as compared to MS. The sucrose concentration in the media is the main element that affects the germination of rescued embryos. Khan *et al.* (23) studied embryos developed 14-16 days after pollination. Colour of hybrid seeds were whitish, small in size and absent of endosperm, seeds sterilised with solution having 10ml/L sodium hypochloride (NaClO) and few drops (1-2) of tween twenty then autoclaved water used to wash seeds 3 to 4 times; seeds were dissected under 10x magnification stereoscope in sterilized laminar air flow cabinet and haploid embryos kept on half strength MS (½ MS) media for haploid plants regeneration and kept in dark for 14 days at 22°C for cell differentiation. After these embryos kept under 24°C for 16 hr. light regime for well plantlets development and, then transferred to soil (1:1 peat and soil) mixture and plant roots treated with 1% antifungal solution before transferring to soil.

### **Colchicine treatment**

Efficient chromosome doubling mechanism is necessary to obtain homozygous plant after gaining haploid plants through wheat x maize. Various doubling chemicals reported caffeine by Thomas *et al.* (55) and N<sub>2</sub>O by Hansen *et al.* (17) but mostly used doubling element is colchicines (53), which restrict mitosis by inhibiting spindle fibers formation, disturb chromosomes movement, leading to chromosome doubling (20). Numerous conditions affect doubling chromosome mechanism like colchicine doses, other synthetic hormones, temperature and length strength, plants growth phase and environment after colchicine treatment. Inagaki (18) and Sood (50) reported colchicine used at several stages but tillering phase found best of haploid plants. Earlier scientists like Jensen (21), Thiebaut *et al.* (54) and Inagaki (18) reported colchicine is normally recommended at 3 to 4 tillers for 5 to 8 hours by putting roots in colchicine (0.1%), Tween 20 (0.3 ml/L), DMS (2%) and GA3 (10mg/L) so that doubling percentage reaches up to 95.6 %. Sood *et al.* (50) reported (1% colchicine solution with 100 ppm 2, 4-D) spray on top first internode fertilized spikes for 48 & 72 hours and reported 33 to 100 percent chromosomes doubling rate. Khan *et al.* (23) studied seven different treatments for DH and embryo rescue and best result found in treatment-5 having (30g sucrose + 100mg 2,4-D + 8ml H<sub>2</sub>SO<sub>3</sub> + 100mg AgNO<sub>3</sub>) and caryopsis formed upto 53.52% and haploid seed production to 28.95%, seeds were vigorous and healthy without fungus attack further 2,4-Dichlorophenoxyacetic acid enhance embryo formation in bread wheat genotypes and AgNO<sub>3</sub> adding with 2,4-D improved overall frequencies of

embryo production and more caryopses and embryos were observed in the 2,4-D with AgNO<sub>3</sub>, as compared to 2,4-D alone. Niu *et al.* (2014) reported at low temperature (14–16°C), colchicine application enhanced survival of colchicines treated plantlets and reached over 90%. Khan *et al.* (23) observed when plants formed 3-5 tiller, 0.1 to 0.2% colchicines applied for 3 hrs. The solution supplied continuously with fresh air by air pump during colchicines application. After treatment, the seedlings kept at 15-20 °C with little or no light. After 2 weeks, the seedlings moved towards light. When new tillers emerged, seedlings were shifted at temperature 20°C under sufficient light and seeds collected after colchicines application from 4-5 weeks.

**EFFICIENT PROCEDURE FOR DH PRODUCTION IN WHEAT**



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