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USE OF INTERGENERIC CROSS FOR PRODUCTION OF DOUBLED HAPLOID WHEAT (*TRITICUM AESTIVUM* L.)

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Abstract

The main purpose of conventional breeding or hybridisation is to bring about homozygosity, for which 6 to 7 years may be required. Wheat and maize crosses have proved to be more efficient in DH lines production than anther culture methods, because of its lower genetic specificity. Doubled haploid technique facilitates the development of homozygous plants within one generation. The system is developed through haploid production, followed by chromosome doubling, to produce homozygous plants in a single generation. For doubled haploid production method wheat and maize crossing system is better than anther culture and ovule culture because maize pollens are highly responsive and produce stable progeny population. Wheat is being used as female parent and maize as a male parent for the production of doubled haploid. Moreover, Silver Nitrate ($AgNO_3$) in tiller culture media can improve the frequency of haploid embryo production in this crossing system. Our result showed that DH production through wheat and maize crossing system was proved to be time saving (2 years) as compared to other conventional breeding methods (6 years).

Keywords: Intergeneric cross, Doubled haploid wheat, Conventional breeding methods.

Introduction

In the conventional breeding programmes of field crops, genetic improvement through hybridisation for elite combinations of genes requires homogeneous population and isolation of homozygous lines. Doubled Haploid (DH) breeding ensures complete homozygosity from heterogeneous material in a single generation. It reduces time period for varietal development and improves efficiency of selection by using complete uniformity using homogenous lines. DH population is also useful for the studies of genetic/cytogenetic, evolution, maintenance of genetic stock, study of mutagenesis, QTL mapping and gene location of various plant species (Jauhar et al., 2009). A DH technique added successfully to any breeding programme should fulfill the following parameters: (i) high frequency of DH line/s should be produced from all genotypes, (ii) DH lines should present a random sample of parental genotypes, (iii) DH populations should be genetically stable and, (iv) DH lines should consist of combinations which give superior performance over parents (Snape et al., 1986).

In wheat, haploid plants can be produced by: (a) Anther culture/microspore culture, (b) megaspore culture/egg cell culture and (c) interspecific crosses/intergeneric crosses (Kasha and Swartz, 1983; Gupta, 1999). The efficiency of first two methods is too low. Moreover, production of albino plants discourages these methods and the frequency of haploid embryo production is highly influenced by genotype (de-Buyser et al., 1985). In wheat, there are several ways to induce haploidy through interspecific crosses successfully. By crossing wheat with barley (Barclay, 1975), pearl millet (Suenaga, 1994; Ahmad and Comeau, 1990), tripsacum (Riera-Lizarazu and Kazi, 1993), teosinte (Suenaga et al., 1998), job's tears (Mochida and Tsujimoto, 2001), sorghum

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(Ohkawa et al., 1992) and maize (Campbell et al., 2000), haploid wheat plants are derived. In such crosses, a hybrid embryo is produced. Preferential chromosome elimination removes chromosomes of pollen parent during embryo development and haploid plants are recovered through embryo rescue on nutrient media.

Wheat and maize crossing system is superior for DH production as compared to other methods extensively adopted for DH developed. In 1984, Zenkteler and Nitzsche reported for the first time haploid wheat embryo production through crossing between hexaploid wheat and maize (Zenkteler and Nitzsche, 1984). After a systematic study, Laurie and Bennett in 1986further confirmed these results. In wheat and maize cross, a hybrid zygote is produced. In first few mitotic divisions, maize chromosomes are eliminated from zygote and haploid embryo with 21 wheat chromosomes produced. The production of haploid embryos is effected by many environmental conditions like soil, humidity, temperature and light intensity. To overcome these factors *in vitro*, tiller culture technique is preferred for optimum haploid embryo production. Several plant hormones are used in tiller culture technique to enhance rate of haploid embryo production. The success rate of crossing is limited due to several problems like partial incompatibility, seasonal retention, poor embryo differentiation and premature senescence (Pickering and Morgan, 1983). Seed development in cross combinations can be significantly increased by plant growth regulators.

The application of 2,4-D stimulates seed development in all wheat and maize crosses. In intact plant systems, 2,4-D is applied immediately or one day after pollination via injection in upper most inter node or application to florets by drops or spray method (Laurie and Bennett, 1986). In detached tiller culture, 2,4-D is applied for 3 days after pollination in tiller culture media (Inagaki, 1997). Silver Nitrate ($AgNO_3$) acts as ethylene inhibiter and promotes cellular activity. In wheat and maize crossing, silver nitrate is also applied through injection, spray or drops in intact method and through tiller culture media in detached tiller culture. In intact method, 3 mg/L2, 4-D plus (120-180 mg/L$AgNO_3$) can produce 18.67% haploid embryos (Bidmeshkipour et al., 2007). Almouslem et al., (1998) enhanced haploid embryo in intact method by using a combination of 3mg/L2, 4-D and 180mg/L$AgNO_3$. It also improved embryo differentiation and plant production.

The objective of this experiment was to improve haploid embryo production in detached tiller culture by using combinations of 2,4-D and silver nitrate. Maleic acid was used to evaluate its chances for sulfurous acid replacement.

Material and Method

Twenty F_1Wheat genotypes were grown in the field in the month of November. Standard agronomic practices were followed for irrigation and fertiliser application. Five maize genotypes were grown under controlled conditions in green house. Maize sowing was carried continuously after 15 days interval to ensure a regular and sufficient maize pollen supply for crossing.

By visual observation, healthy wheat spikes at optimum stage for crossing were selected made suitable one day before anthesis, when the spike partially emerged from flag leaf. The immature florets at the top and base of the spikes were removed. All the anthers of a floret were removed without injuring the stigma. After emasculation, the spikes were covered with plastic bag to avoid contamination and moisture loss. Fresh maize pollen was used for pollination. After pollination, tillers were kept in tiller culture for three days containing media and containing following treatment combinations in one liter of water:

1. Control H_2O (Control)
2. 30g sucrose + H_2O
3. 30g sucrose + 100mg 2,4-D
4. 30g sucrose + 100mg 2,4-D + 8ml H_2SO_3
5. 30g sucrose + 100mg 2,4-D + 8ml H_2SO_3 + 100mg $AgNO_3$
6. 30g sucrose + 100mg 2,4-D + 8ml H_2SO_3 + 150mg $AgNO_3$
7. 30gsucrose + 100mg 2,4-D + 8ml maleic acid (200mg/L)

After three days, tillers were shifted to sucrose and sulfurous acid solution. 21^oC temperature, 10000 Lux light intensity and 65% humidity was optimum for seed and embryo development. Fresh tiller culture solution was added regularly.

The optimum stage for embryo rescue was influenced by genotype, temperature and humidity after pollination. Generally, the

embryos were developed 14-16 days after pollination. Hybrid seeds were whitish in colour, smaller in size and endosperm was absent. The seeds were sterilised with washing solution having 10ml\L sodium hypochloride and 1-2 drops of tween twenty. After that, seeds were washed 3 to 4 times with autoclaved water. The sterilised seeds were dissected under 10x magnification stereoscope in sterilised laminar air flow cabinet. The haploid embryos were placed on ½ MS media for regeneration of haploid plants. The embryos were kept in dark for 14 days at 22^0C until cell differentiation. Embryos were then kept under 24^0C and 16 hr. light regime. When plantlets were well developed, they were transferred to soil and peat mixture under controlled conditions. Plant roots were also treated with 1% antifungal solution before transferring to soil.

When plants reached at 3-5 tiller stage, 0.1 to 0.2% colchicines was applied for 3 hrs. The solution was supplied continuously with fresh air by air pump during colchicines application. After treatment, the seedlings were kept at 15-20 ^{0}C with very little or no light. After 2 weeks, the seedlings were moved towards light. When new tillers appeared, seedlings were moved to temperature around 20^{o}C under sufficient light. Seeds were collected 4-5 weeks after colchicines application.

Result and Discussion

The number and percentage of seed setting and haploid embryo formation is described in Table 1. In treatment 1, no caryopsis was observed and tillers were shriveled in 4-5 days. In treatment 2, 16.28% caryopsis occurred with small seed size but within 8-12 days of pollination, fungus covered the florets and destroyed the seed growth. In treatment 3, healthy seeds were produced with 30.52% but fungal attack was also severe at this time. All the seeds were damaged within 12 days after pollination. The addition of sulfurous acid in treatment 4 yielded 48.99% seed setting and 23.71% haploid embryo formation. The fungus attack was absent at this time. The 100mg/L $AgNO_3$ enhanced caryopsis upto 53.52% and haploid seed production to 28.95%. The seeds were vigorous and healthy. In treatment 6, 150mg/L $AgNO_3$ produced maximum seeds (54.50%) but embryo production was at 26.96%. The seeds were a little bit shriveled. The maleic acid treatment yielded 40.10% seed production and 16.05% embryos (Table1). Fungal attack did not occur this time. The treatment 5 yielded maximum number of haploid embryos (Figure 1).

Table 1. Seed setting and embryo formation in different tiller culture media

T	No of crosses	No of seeds produced	% of seeds produced	No of Haploid embryo	% of Haploid embryo
1	115	Zero	zero	Zero	Zero
2	172	28	16.28	Zero	Zero
3	154	47	30.52	Zero	Zero
4	198	97	48.99	23	23.71
5	142	76	53.52	22	28.95
6	211	115	54.50	31	26.96
7	202	81	40.10	13	16.05

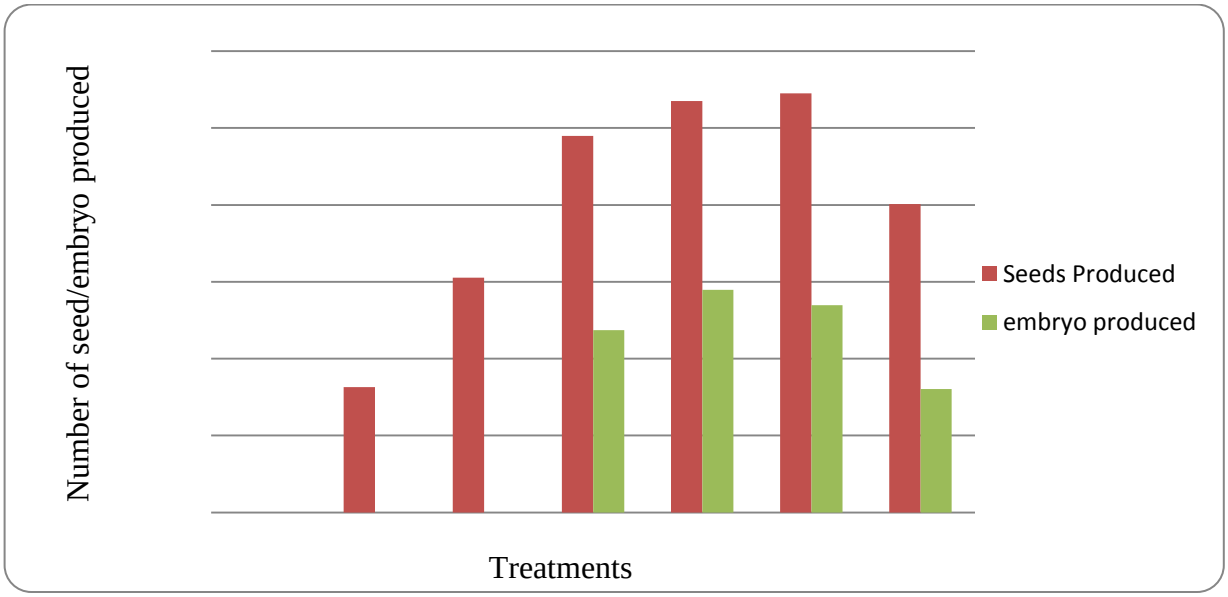

Fig. 1: Frequency of seed and haploid embryo production in various tiller culture media.

Application of $AgNO_3$ provided silver ions and other silver salt solution inhibit the action of ethylene exogenously applied to whole plants and plant parts. In this study, haploid seeds and embryos were produced from a hexaploid wheat genotype but frequency of haploid seeds ranged between 0 to 54.50% and haploid embryos ranged from 0 to 28.95% in hexaploid wheat and maize crosses (Table1). These results are consistent with the report of other studies (Laurie and Bennett, 1986; O'Donoughue and Bennett, 1994; Singh et al., 2001). In present treatments 100mg/L 2,4-D plus 150mg/L $AgNO_3$ gave the highest frequency of haploid embryos (Table 1). These results are consistent with the studies of Almouslem et al., (1998). The phenomenon observed in untreated ovaries of *Triticum* species pollinated with maize seems to involve abscission of the ovary from the mother plant and senescence. It may occur due to ethylene production in the mother plant and the $AgNO_3$ application might counteract this effect. Auxins can enhance ethylene production in plants and the internal level of auxin may promote the rate of ethylene production. Perhaps $AgNO_3$ inhibits the effect of ethylene produced in response to the exogenous application of 2,4-D. Addition of $AgNO_3$ to the detached tiller culture solution increased the cross-ability (expressed as embryo formation frequency) of wheat with maize.

In bread wheat, the effect of 2,4-D on seed setting and embryo formation in wheat and maize crosses was favourable but the effect of 2,4-D was strongly influenced by the durum wheat genotypes used for crosses with maize. In durum wheat with rye crosses, 2,4-D treatment showed no advantage in haploid embryo formation. Nevertheless, 2,4-D treatment is effective for enhancing embryo formation in some durum wheat genotypes. Adding $AgNO_3$ to the 2,4-D treatment improved overall frequencies of embryo production. More caryopses and embryos were observed in the 2,4-D with $AgNO_3$, treatment as compared to the control and 2,4-D alone. Almouslem et al., (1998) have constructed a hypothesis that $AgNO_3$ added to 2,4-D solution inhibits ethylene production which is produced as a side effect by the 2,4-D application. The application of $AgNO_3$ is likely to facilitate embryo development by delaying the abscission process.

In summary, the results of this experiment indicate that successful fertilisation of hexaploid wheat genotypes with maize can be achieved at relatively high frequencies and by using $AgNO_3$ alongwith 2,4-D. Moreover, maleic acid can also be used as antifungal agent in this crossing system.

2,4-D improves cell growth and replication. It increases the frequency of seed setting and

haploid embryo production. 100mg/L concentration for 3 days is optimum (Khan and Ahmad, 2011). Increase in 2,4-D concentration greater than 100mg/L affects haploid production negatively and reduces plantlet regeneration when transferred to nutrient media. Moreover, 2,4-D application method in tiller culture media is superior over injection method. It ensures continuous and sufficient supply of hormones.

No normal endosperm was observed in hybrid seeds with or without haploid embryo. The maize pollen did not fertilise endosperm nuclei and later the nuclei were degenerated. It was a marker for wheat and maize crossed embryo. It can replace cytologic confirmation by chromosome counting of haploid plantlet. The absence of endosperm resulted in nutritional shortage for developing haploid embryo. The immature haploid embryo should be transferred to nutrition media after two weeks of development for plantlet regeneration.

The environmental conditions are crucial for the DH production. 10,000 lux light intensity, 20-24^{0}C temperature and 60-65% humidity level haploid seed formation and haploid embryo development were sufficient.

References

Ahmad, F. and A. Comeau, 1990. Wheat x pearl millet hybridization: Consequence and potential. *Euphytica*, 50: 181-190.

Almouslem, A.B., P.P. Jauhar., T.S. Peterson, V.R. Bommineni and M.B. Rao. 1998. Haploid durum wheat production via hybridization with maize. *Crop Sci*. 38: 1080-1087.

Barclay, I.R. 1975. High frequencies of haploid production in wheat (*Triticumaestivum*) bychromosome elimination. *Nature*, 256: 410-411.

Bidmeshkipour, A., R.J. Thengane, M.D. Bahagvat, S.M. Ghafari, V.S. Rao.2007. Production of haploid wheat via maize pollination. *J. Sci., Islamic Republic of Iran*, 18: 5-11.

Campbell, A.W., W.B. Griffin, D.J. Burritt and A.J. Conner. 2000. The effects of temperature and light intensity on embryo numbers in wheat doubled haploid production through wheat x maize crosses. New Zealand J. Crop and Hort. Sci., 28: 185-194.

de-Buyser, J., Y. Henry and G. Taleb. 1985. Wheat androgenesis: cytogenetical analysis and agronomic performance of doubled haploids. *Z. Pflanzenzuchtung*. 95: 23-34.

Gupta, P.K. 1999. Haploidy in higher plants. pp.116-119. In: Cytogenetics. Rastogi Publication, Shivaji Road Meerut, India.

Inagaki, M. 1997. Technical advances in wheat haploid production using ultra-wide crosses.*JIRCAS Journal*. 4: 51-62.

Jauhar, P.P., S.S. Xu and P.S. Baenziger, 2009. Haploidy in Cultivated Wheat: Induction and Utility in Basic and Applied Research. *Crop Science*, Vol. 49:737-755.

Kasha, K.J. and S. Swartz, 1983.Haploidy in crop improvement. pp. 19-68. In: M.S. Swaminathan, P.K. Gupta and U. Sinha (eds.), Cytogenetics of Crop Plants, Macmillan India Limited, Delhi.

Khan, M.A. and J. Ahmad. 2011. In-vitro wheat haploid embryo production by wheat x maize cross system under different environmental conditions. *Pak. J. Agri. Sci.*, Vol. 48(1) pp.49-53

Laurie, D.A. and M.D. Bennett.(1986). Wheat × maize hybridization. *Can. J. Gen. Cytol*. 28: 313-316.

Mochida, K. and H. Tsujimoto. 2001. Production of wheat doubled haploids by pollination with Job's Tears (Coixlachry-majobiL.). *J. Hered*., 92: 81-83.

O'Donoughue L.S. and Bennett M.D. 1994.Durum-wheat haploid production using maize wide-crossing. *Theoretical and Applied Genetics*, 89: 559-566.

Ohkawa, Y., K. Suenaga and T. Ogawa. 1992. Production of haploid wheat plants through pollination of sorghum pollen. Japan. *J. Breed.*, 42: 891-894.

Pickering, R.A. and P.W. Morgan. 1983. Plant regeneration from cultured embryos derived from *Hordeum vulgar* L. pollinated with *H. bulbosum* L. *Euphytica.* 32: 585-591.

Riera-Lizarazu, O. and A.M. Kazi. 1993. Polyhaploid production in the Triticeae: wheat x Tripsacum crosses. *Crop Sci.*, 33: 973-976.

Singh, N., R.K. Behl and M.S. Punia. 2001. Production of double haploids via maize pollination in wheat. *Cereal Res. Communication*. 29: 289-296.

Snape, J.W., J. de-Buyser, Y. Henry and S. Simpson.1986. A comparison of methods of haploid production in a cross of wheat, Triticumaestivum. Z. Pflanzenzuchtung. 96: 320-330.

Suenaga, K. 1994. Doubled haploid system using the intergeneric crosses between wheat (Triticumaestivum) and maize (Zea mays). Bull. Natl. Inst. Agrobiol. Resour., 9: 83-139.

Suenaga, K., A.R. Moreshedi and N.L. Darvey. 1998. Evaluation of teosinte lines as pollen parents for wheat haploid production. *Cereal Res. Comm.*, 26: 119-125.

Zenkteler, M. and W. Nitzsche. 1984. Wide hybridization experiments in cereals. *Theor. Appl. Genet*. 68: 311-315.