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Comparison of different anti-microtubular compounds in *Triticum aestivum* (AABBDD) and *Aegilops tauschii* (DD) crosses

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Abstract
The efficiency of different antimicrotubular compounds on embryo formation frequency was studied in the present study. Colchicine, amiprophos-methyl (APM) and Trifluralin were used at different concentrations on crosses developed between *Triticum aestivum* and *Aegilops tauschii*. Colchicine at 0.1 and 0.2 percent was found to have significant influence on embryo formation during main season and early main season. It did not show any negative effect on caryopsis formation as well.

Keywords: Chromosome doubling, colchicine, embryo formation, pre regeneration

Introduction
Chromosome doubling either spontaneously or artificially becomes the prerequisite for any wide hybridisation program to be successful. Due to low frequency of spontaneous doubling in nature, several attempts have been reviewed devising artificial doubling protocols Jensen (1974) [2], Rao and Suprasanna (1996) [4] and Kasha (2005) [3]. Among the different anti-microtubule agents used colchicine induced chromosome doubling has received much attention in wide hybridisation programs *viz*., doubled haploid, interspecific or intergeneric hybridisation both *in vitro* and *in vivo*. Due to higher affinity of colchicine for animal tubulin than for plant tubulin (Bartels and Hilton, 1973) [1], higher concentrations are required to compensate for the lower affinity which leads to toxic effect on humans. As a part of methodology for devising a standardized protocol for direct crosses between *Triticum aestivum* and *Aegilops tauschii* alternative antimitotic agents having a higher affinity for plant tubulin were included in the study. While working around pre-regeneration methodology refinement in *Triticum aestivum* and *Aegilops tauschii* crosses, anti-microtubular compounds namely Trifluralin and Amiprophos methyl (APM) were used.

The crosses attempted between different *T. aestivum* cultivars and *Ae. tauschii* accessions were used for pre regeneration chromosome doubling treatment. Antimicrotubular compounds viz., APM, Trifluralin and Colchicine were used at concentrations as given in Table 1 and were administered to ovary on pollinated tillers 24 hours post pollination. A fine syringe was used to administer small drops of the chemical to the ovaries by (Plate 1). Each chemical treatment was administered along with DMSO and wetting agent Tween 20. This was followed by embryo rescue using MS (Murashige and Skoog 1962) basal medium to which, Sucrose (30 g/l) + Gelrite (2 g/l) + BAP (0.25 mg/l) + Kinetin (0.25 mg/l) + IAA (1 mg/l) were added. The pH of the medium was adjusted to 5.8. The medium was poured into test tubes and autoclaved at 15 psi (pounds per square inch) for 20 minutes. Each treatment was applied on 100 florets with 3 replications during main season (2014-15) and early main season (2015) in the field area of department of plant breeding and genetics, Ludhiana.

During main season 2014-15, different concentrations of individual treatments were applied (results given in Table 2). Colchicine (0.2%) was observed to enhance the embryo formation frequency to 39 percent and colchicine (0.1%) with 24 percent embryo formation frequency.

APM (250 µm) showed embryo frequency of 0.6 percent over APM (350 µm) which showed low embryo frequency of 0.1 percent. Trifluralin was not significant in increasing the embryo frequency and showed minimum frequency of 0.1 percent and 0.2 percent at 250 µm and 350 µm respectively. The experiment was repeated in the following early main season of 2015.

The experiment was conducted using same concentration as used in previous season for confirmation of results.

The experiment was conducted using same concentration as used in previous season for confirmation of results. On the similar note, Colchicine (0.2%) enhanced embryo formation frequency to 24 percent as compared to 21 percent in colchicine (0.1%). APM (250 µm) showed lower frequency of 0.3 percent compared to previous season while as APM (350 µm) showed same effect of 0.1 percent on embryo frequency. Trifluralin at both the concentrations (250 and 350 µm) was again insignificant.

The experiment showed colchicine solution to be effective in increasing the embryo formation compared to APM and Trifluralin. Also, no negative impact of colchicine solution at both 0.1 and 0.2 percent was observed on caryopsis shape.

Table 1: Concentrations of antimicrotubular compounds for *T. aestivum* x *Ae. tauschii* crosses

Concentrations of AM Compounds used	Ludhiana Main season 2014-15	Ludhiana Early Main season 2015
Colchicine	0.1%, 0.2%	0.1%, 0.2%
APM	250 µM, 350 µM.	250 µM, 350 µM
Trifluralin	250 µM, 350 µM.	250 µM, 350 µM

Table 2: Embryo formation frequency (%) in *T. aestivum* x *Ae. tauschii* crosses using different AM compounds

Location	Treatments	Parameters			
		No. of Florets	No. of Caryopsis	No. of Embryos	Embryo frequency (%)
Ludhiana 2014-15 Early Main season	APM 250µM	100	31	06	0.06
	APM 350 µM	100	12	01	0.01
	Trif 250µM	100	19	02	0.02
	Trif 350 µM	100	11	01	0.01
	Colchicine 0.1% (Control)	100	56	24	0.24
	Colchicine 0.2% (Control)	100	62	39	0.39
Ludhiana 2014-15 Main season	APM 250µM	100	21	03	0.03
	APM 350 µM	100	09	01	0.01
	Trif 250µM	100	12	01	0.01
	Trif 350 µM	100	25	02	0.02
	Colchicine 0.1% (Control)	100	44	21	0.21
	Colchicine 0.2% (Control)	100	48	24	0.24

Conclusion

The experiment however could only differentiate the efficiency of different compounds on embryo frequency, much was still to be studied on regeneration aspects as well. Though an indication of using colchicine solution over other antimicrotubular compounds for future experiments in this direction was put forward. The crosses developed henceforth used colchicine solution as chromosome doubling agent.

References

1. Bartels PG, Hilton JL. Comparison of triflularin, oryzalin, pronamide, propham, and colchicine treatments on microtubules. Pesticide Biochem Physiol. 1973; 3:462-472
2. Jensen CJ. Chromosome doubling techniques. In: Kasha KJ (ed) Haploids in Higher Plants. Advances and Potential University of Guelph, Canada, 1974, 153-190.
3. Kasha KJ. Chromosome doubling and recovery of doubled haploid plants. In: Palmer CE, Keller WA, Kasha KJ (eds) Biotechnology in agriculture and forestry 56. Haploids in crop improvement II. Springer, Berlin Heidelberg, 2005, 123-152.
4. Rao PS, Suprasana P. Methods to double haploid chromosome numbers. In: Mohan SJ, Sopory SK, Veilleux RE (eds) Current plant science and biotechnology in agriculture. *In vitro* haploid production in higher plants, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1996, 1.