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Comparison between Conventional and *in-situ* Chromosome Doubling Method in *Triticum Durum x Aegilops tauschii* Crosses

S.R. Cambay[1]*, P. Srivastava[2], S.K. Sandhu[2], N.S. Bains[2] and Maneet Rana[1]

[1]Division of Genetics, IARI, New Delhi, 110012, India
[2]Department of Plant Breeding & Genetics, PAU, Ludhiana, 141012, India

**Corresponding author*

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In this investigation two different methods using aqueous solutions of colchicine were used to treat F_1 tillers from various *Triticum durum x Aegilops tauschii* crosses in order to artificially induce chromosome doubling. Treatment of crown root region (uproot) was found effective compared to *in-situ* (tip) method using treatment of apical meristems of F_1 tillers at 2-3 tiller stage. The aqueous solution of colchicine was administered at two concentrations *viz.*, 0.05 and 0.075% in both the methods. The 0.05% colchicine solution was found more effective as more doubled seed was obtained.

Introduction

The presence of enormous genetic diversity in progenitor species of wheat has always attracted wheat breeders towards wide hybridization. Much attention over decades has been shifted towards it and significant improvement has been brought forth (Mujeeb Kazi *et al.*, 2008). Despite efforts made the difficulties posed by wide hybridization are numerous and demand special interventions at every stage in terms of chromosome doubling, growth hormones, embryo rescue etc. The chromosome doubling becomes the first major concern in wide hybrids for successful gene transfer which in turn largely depends on the stage and method of administering the treatment. To induce polyploidy, chemicals such as colchicine, the mitotic spindle inhibitor has been used in meristemic cells in many plants (Mensah *et al.*, 2007; Saharkhiz, 2007). Different methods to induce polyploidy in plants have been used such as the treatment of seed (Johnson *et al.*, 2004; Quan *et al.*, 2004), germinated seed (Urwin *et al.*, 2007), flower buds (Wu *et al.*, 2007), apical meristems (Lavania and Srivastava, 1991; Hanzelka and Kobza, 2001; Saharkhiz, 2007; Yavari *et al.*, 2009) and roots (Taira *et al.*, 1991), *in-vitro* tissue culture (Adaniya and

Shirai, 2001; Gu *et al.*, 2005; Koutoulis *et al.*, 2005). The most widely used conventional method of chromosome doubling used so far in wide hybrids has been the uproot method. We tried to devise a parallel *in-situ* method of colchicine treatment without uprooting the plant in order to avoid the post treatment transplantation shock. The most effective treatment method and treatment duration, besides colchicine concentration, to induce polyploidy, are species-specific. The main goal of this research was to compare the conventional doubling method with a novel in-situ method of doubling as an alternate one. In the present study F1 plants obtained from crosses conducted between three durum cultivars (PDW 233, PDW 291 and PDW 314) and 11 *Aegilops tauschii* accessions (AT 14, AT 41, AT 51, AT 55, AT 93, AT 95, AT 104, AT 119, AT 304, AT 307 and AT 311) were used. Two methods for doubling the chromosome were used *viz.*, conventional uproot and *In-situ* (tip) method at two concentrations (0.05 and 0.075 per cent) to check the efficiency of one against the other. In case of conventional method of doubling, F_1 plants were uprooted at 3-4 tiller stage. The crown region of F_1 plants to be treated was washed thoroughly before being exposed to the colchicine solution. The roots were trimmed 2-3 cm from tip above for efficient treatment. These plants were divided into two groups one of which was dipped in 0.05% of colchicine solution and other group dipped in 0.075%. The treatment was carried out in containers with a pair of air bubblers inside to ensure proper aeration. The set up for treatment was placed under light and the duration of treatment was for 8 hours. After the completion of treatment the crown region of treated plants was washed under running water thoroughly overnight (12 hours). These treated plants were transplanted back into soil with proper identity maintained (Table 1&2).

Table.1 Seed set in conventional uproot method of colchicine treatment

Pedigree	Col. Conc (%)	No. of tillers treated	Seeds obtained
PDW 233 X AT 55	0.05	09	09
	0.075	09	-
PDW 233 X AT 93	0.05	12	15
	0.075	13	-
PDW 233 X AT 95	0.05	09	09
	0.075	08	-
PDW 233 X AT 104	0.05	10	13
	0.075	11	-
PDW 233 X AT 119	0.05	12	-
	0.075	12	-
PDW 233 X AT 307	0.05	08	08
	0.075	08	-
PDW 291 X AT 14	0.05	06	12
	0.075	05	-
PDW 291 X AT 55	0.05	03	16
	0.075	04	-
PDW 291 X AT 93	0.05	09	-
	0.075	09	-
PDW- 291 x AT- 95	0.05	07	-
	0.075	05	-
PDW- 291 x AT- 304	0.05	06	-
	0.075	07	-
PDW- 291 x AT- 307	0.05	08	-
	0.075	06	-
PDW 314 x AT 311	0.05	08	13
	0.075	07	-

Table.2 Seed set in in-situ (tip) method of colchicine treatment

Pedigree	Col. Conc (%)	No. of tillers treated	Seeds obtained
PDW 233 X AT 55	0.05	03	02
	0.075	01	-
PDW 233 X AT 93	0.05	02	-
	0.075	03	-
PDW 233 X AT 95	0.05	03	03
	0.075	02	-
PDW 233 X AT 104	0.05	01	-
	0.075	01	-
PDW 233 X AT 304	0.05	02	-
	0.075	02	-
PDW 291 X AT 14	0.05	02	-
	0.075	03	-
PDW 291 X AT 55	0.05	01	-
	0.075	02	-
PDW 291 X AT 107	0.05	03	-
	0.075	04	-
PDW 291 X AT 93	0.05	03	-
	0.075	03	-
PDW- 291 x AT- 95	0.05	03	-
	0.075	02	-
PDW- 291 x AT- 304	0.05	02	-
	0.075	01	-
PDW- 314 x AT- 41	0.05	03	02
	0.075	02	-
PDW- 314 x AT- 51	0.05	02	-
	0.075	02	-
PDW 314 x AT 311	0.05	02	-
	0.075	01	-

In an alternate method *viz.*, *In-situ* (tip) method, those tillers of F_1plants which have not reached the boot stage were targeted. Colchicine solution at similar two concentrations as above was used and tips used in the lab were used for this treatment. The F_1 tillers to be treated were given a slant cut at the top and the tip was carefully fixed over it. To check the overflow these tips were filled with water initially. After assuring the fixing of tip on the tiller it was filled with colchicine solution. The tips were covered with aluminum foil to avoid evaporation of colchicine solution. Care was taken to refill the tips timely as per the rate of seepage of colchicine solution inside the culm. This method allowed a single plant to get exposed to two different concentrations at the same time. The treatment was carried out for a period of 6 hours followed by washing. The washing in this method was done differently than usual by injecting water through the tip several times.

The present study used crosses between three durum cultivars (PDW 233, PDW 291 and PDW 314) and 11 *Aegilops tauschii* accessions (AT 14, AT 41, AT 51, AT 55, AT 93, AT 95, AT 104, AT 119, AT 304, AT 307 and AT 311) for the doubling experiment. The results obtained revealed that F_1 tillers subjected to uproot method of chromosome doubling at 0.05% colchicine treatment did not show much symptoms of wilting when compared to plants treated at 0.075% of colchine. The F_1 tillers treated with 0.05% colchicine showed doubled seed set. The number of doubled tillers 11 in number out of

25 treated with an average of 8-10 seeds per ear. On the contrary the tillers treated with 0.075% showed toxic effect due to permanent wilting. The results obtained from *in-situ* tip method showed only two out of 11 treated tillers had seed set at 0.05% colchicine treatment. No seed set was obtained in tillers treated with 0.075% colchicine treatment.

The conventional method of doubling was followed in the similar way as Sehgal (2011) which gave promising results over the alternate one. Nevertheless, the later approach however indicated some feasibility of an alternate method to be successful if attempted at proper stage and over more number of tillers. Since the alternate method reduces the chances of transplantation shock it can be addressed in future with better interventions rather negated completely.

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