Indian J. Genet., **63**(3): 247-248 (2003)

Short Communication

Effect of pre-regeneration colchicine treatments on the efficiency of wheat × maize system of haploid production in wheat

Rahul Dhawan, Kuldeep Singh[1] and Navtej S. Bains

Department of Plant Breeding, Punjab Agricultural University, Ludhiana 141 004

(Received: April 2002; Revised: June 2003; Accepted: June 2003)

Crossing wheat with maize has emerged as the system of choice for wheat haploid production. Chromosome doubling constitutes an important step in this system. Most of the research on chromosome doubling methodology in wheat has been conducted in the context of interspecific hybrids to generate self-fertile amphiploids. The standard target for these treatments has been the uprooted hybrid seedlings at 2-3-tiller stage. In case of wheat × maize crosses conducted in the field, regenerated haploid plants become available at a time when environmental conditions are not conducive for growing wheat. This led us to investigate some alternative chromosome doubling strategies.

Spring wheat F_1 hybrid (PBW 343 × Inquilab 91) was used in crosses with Pearl Pop Corn, a composite maize cultivar that had been previously identified as an excellent pollinator [1]. The wheat genotype was grown in small plots of 4 rows, 2m each. Each plot was divided into three blocks with colchicine treatments (one per plot) being assigned randomly within blocks. Staggered sowings of the maize genotype were made to ensure synchronization of flowering. The crossing technique, hormonal application, sorting of embryo-carrying caryopses and embryo culture has been described earlier [2]. Three methods of colchicine treatment (*In vivo* tiller injection, treatment via detached tillers and *in vitro* treatment) were followed with varying concentrations and duration/timing of treatment in each case. *In vivo* injection of 100ppm 2,4-D is administered in the uppermost internode, 24, 48, and 72 hours after pollination as a part of our routine methodology to sustain haploid embryo development. Colchicine treatment was combined with the 2,4-D injections. Three concentrations of colchicine, 1.0%, 2.0% and 3.0% were administered 24h and 48h after pollination along with 2,4-D. Control plants received only 2,4-D treatment. In colchicine treatment via detached tillers, tillers pollinated with maize were cultured in liquid medium 15 days after pollination. The tillers were cut just below the second node and placed in jars containing colchicine supplemented liquid medium. The tiller culture medium contained half the strength of MS salts, 40g/l sucrose and 8ml/l sulphurous acid. Colchicine concentration of the medium was adjusted to 0.1%, 0.2% and 0.3%. The tillers were retained in colchicine supplemented medium for 12h, 18h and 24h at 25°C under artificial light. Thereafter, the caryopses were harvested and embryos cultured. The control tillers were cultured in colchicine-free medium. *In vitro* treatment of colchicine involved culturing of 15 day old embryos for 24h, 36h and 48h on the medium supplemented with 0.1%, 0.2% and 0.3% colchicine. After autoclaving, 10ml of MS culture medium (containing MS basal salts + Glutamine 400mg/l + Proline 10mg/l + Arginine 10mg/l + Leucine 10mg/l + Cystine 20 mg/l + Kinetin 0.15mg/l + BAP 0.15mg/l + phytagel 3g/l + sucrose 30g/1) was dispensed into each of the 10cm diameter petridishes. Colchicine was added under aseptic conditions using disposable syringe filters. Subsequently, the embryos were transferred to colchicine-free medium, retaining all other constituents of the previous culture medium. In every treatment, data were recorded on frequency of pollinated florets showing caryopses formation (CFF), frequency of caryopses showing embryo formation (EFF), frequency of embryos showing plant regeneration (PRF). These were used to derive the frequency of florets pollinated that finally resulted in plants, thus giving plant formation efficiency (PFE). Prior to analysis, percentage data with values below 30% was transformed using square root transformation and in experiments where percentage data ranged outside 30 and 70, arc sine transformation was used.

In case of *in vivo* tiller injection, higher concentrations (2% and 3% colchicine levels) were particularly detrimental to caryopses and embryo formation (Table 1). More than 10 fold lowering (from 69.3% to 5.8%) in case of CFF, and about six fold lowering (from 14.4% to 2.2%) in case of EFF was

[1]Department of Genetics and Biotechnology, Punjab Agricultural University, Ludhiana 141 004

Table 1. Treatment means for efficiency parameters CFF, EFF, PRF and PFE in tiller injection experiment using different colchicine concentrations and timings

Treatment	CFF	EFF	PRF	PFE
Control	69.3[a] (679/980)[1]	14.4[a] (141/980)	34.7[a] (49/141)	5.0[a] (49/980)
24h after pollination				
1.0%	52.6[b] (279/530)	8.7[b] (46/530)	56.5[b] (26/46)	4.9[a] (26/530)
2.0%	20.3[c] (93/459)	4.6[c] (21/459)	33.3[a] (7/21)	1.5[b] (7/459)
3.0%	5.8[d] (31/540)	2.2[d] (12/540)	50.0[ab] (6/12)	1.1[bc] (6/540)
48h after pollination				
1.0%	50.8[b] (241/474)	9.5[ab] (45/474)	28.8[a] (13/45)	2.7[a] (13/474)
2.0%	34.3[c] (147/428)	5.1[c] (22/428)	22.7[a] (5/22)	1.1[bc] (5/428)
3.0%	15.5[c] (62/400)	3.5[c] (14/400)	21.4[a] (3/14)	0.8[c] (3/400)

[1]Numbers in parentheses are no. of caryopses/total florets pollinated for CFF, no. of embryos/total florets pollinated for EFF, embryos regenerated/embryos plated for PRF and plants regenerated/total florets pollinated for PFE totaled over three replications. [a]Means followed by same letters are not significantly different at P = 0.01.

observed when 3% colchicine was administered. Interestingly CFF and EFF are depressed to a greater extent in the treatment timed 24 h after pollination whereas PRF is more adversely effected by the '48h-after-pollination' treatment (Table 1). The statistically significant transition to toxicity in terms of overall haploid formation efficiency lies between 1% and 2% in case of both 24h and 48h treatment. Under detached tiller treatment the efficiency parameters CFF and EFF should not be affected because it is a post-embryo formation treatment. Different treatments of cultured tillers with colchicine (0.1%, 0.2%, and 0.3% for 12, 18, and 24h) were statistically all at par in terms of PRF and PFE. No inference except for the possibility of these concentrations being too low or treatment duration being too short could be drawn. In the experiment on *in vitro* colchicine treatment the variation on account of duration and concentration fell into three distinct significant groups (Table 2) representing a three fold lowering of regeneration frequency as compared to control. In accordance with the expectation the effect of concentrations on the PRF and PFE became more acute with increasing duration of treatment. In 48h treatment, the three concentrations stood statistically demarcated from each other. Colchicine concentration of culture medium at 0.1% did not impair regeneration frequency in any of three durations employed, higher concentration of 0.2% and 0.3% led to a significant decrease in plant regeneration frequency (PRF) and consequently plant formation efficiency (PFE) particularly with longer duration of treatment (36h and 48h).

Table 2. Treatment means for efficiency parameters PRF and PFE in detached tiller and rescued embryo treatment experiments using different colchicine concentrations and durations

Treat-	Detached tiller experiment			Rescued embryo treatment		
ment	Dura- tion	PRF	PFE	Dura- tion	PRF	PFE
Control		33.3 (10/30)[1]	3.0 (10/334)		45.6[a] (36/79)	5.8[a] (36/626)
0.1%	12h	38.6 (17/44)	3.1 (17/556)	24h	46.4[a] (26/56)	4.0[a] (26/648)
0.2%		26.3 (10/38)	2.1 (10/490)		25.0[b] (10/40)	3.5[b] (10/288)
0.3%		29.2 (14/48)	2.3 (14/600)		35.4[ab] (17/48)	3.4[b] (17/506)
0.1%	18h	32.0 (16/50)	3.5 (16/460)	36h	34.8[ab] (16/46)	4.8[a] (16/339)
0.2%		28.0 (14/50)	2.5 (14/562)		22.8[b] (8/35)	2.4[b] (8/332)
0.3%		25.0 (10/40)	2.1 (10/480)		23.6[b] (8/34)	2.2[b] (8/363)
0.1%	24h	25.0 (12/48)	2.6 (12/456)	48h	45.6[a] (21/46)	3.6[a] (21/584)
0.2%		31.8 (14/44)	3.4 (14/413)		30.3[b] (10/33)	2.0[b] (10/499)
0.3%		28.6 (12/42)	2.6 (12/455)		15.0[c] (6/40)	1.3[c] (6/458)

[1,a] see Table 1 for legend

The principle for short listing of colchicine treatments for follow-up studies would be to choose the highest treatment that does not significantly impair the haploid formation efficiency. Going by this norm, for tiller injection method, 1% colchicine administered into uppermost internode 24h after pollination seems promising. In this treatment penalties suffered at the level of caryopses and embryo formation are compensated by higher regeneration frequency, thus giving an unimpaired overall efficiency. Twenty four-hour treatments with concentration reaching upto 0.3% and 36h or 48h treatments with concentration not higher then 0.1% represent the useful categories in case of *in vitro* treatment. Thus in case of two methods of colchicine treatments, out of the three investigated, nodal treatment for further experimentation stand identified. A finer array of treatments needs to be devised around these nodal points for actual assessment of their chromosome doubling potential.

References

1. **Verma V., Bains N. S., Mangat G. S., Nanda G. S., Gosal S. S. and Kuldeep Singh.** 1999. Maize genotypes show striking differences for induction and regeneration of haploid wheat embryos in the wheat x maize system. Crop Sci., **49**: 1722-1727.

2. **Bains N. S., Mangat G. S., Kuldeep Singh and Nanda G. S.** 1998. A simple technique for the identification of embryo carrying seeds from wheat × maize crosses prior to dissection. Plant Breeding, **117**: 191-192.