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Comparative effect of auxin analogues on induction of polyhaploids in triticale and triticale wheat hybrids through wheat maize system*

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Haploids are the same chromosome complement as the gametes of the species. *In vitro* haploid induction, followed by chromosome doubling by colchicine treatment has played an important role in modern plant breeding programmes as the systematic introduction and rapid fixation of alien chromosomes, chromosome segments or even genes can be achieved in a single step. Doubled haploids, currently in use in a number of crop species including wheat (*Triticum aestivum* L.emend. Fiori & Paol.) and triticale, enable breeders to develop completely homozygous genotypes from heterozygous parents in a single generation, thereby saving 4–6 years. These also enhance the effectiveness of selection of desirable recombinants, especially when quantitative traits are evaluated. Rye (*Secale cereale* L.) is a rich source of genes for improving many important characters in wheat and the useful genes of rye can be incorporated in wheat by using triticale (*x Triticosecale*) as a bridge species. The triticales can also be crossed amongst themselves for the improvement of their hexaploid forms through the recovery of desirable recombinants.

For obtaining haploid wheat and triticale plants, pollination of wheat with maize (*Zea mays* L.), followed by auxin (2,4-D) treatment is being widely used by researchers (Chaudhary *et al.* 2005, Pratap *et al.* 2006, Gill *et al.* 2008). Auxins play a key role in the induction and maintenance of growth of haploid wheat embryos (Wedzony and Van Lammeren 1996) and these are reported to cause the enlargement of ovaries even in the absence of embryos (Wedzony *et al.* 1998). Though the use of 2,4-D in DH breeding has been standardized for *in vivo* application through injections, the information is meager regarding the use of other auxin analogues. Therefore, the aim of the present study was to evaluate two auxin analogues, 2,4-D and picloram (4-amino-3,5,6-trichloropicolinic acid) for their comparative effectiveness in induction of haploids in triticale and triticale × wheat crosses and to standardize their optimum dose.

The study was conducted in Molecular Cytogenetics and Tissue Culture Laboratory, CSKHP Krishi Vishvavidyalaya, Palampur during 2002–06. The plant materials consisted of seven diverse genotypes of triticale (AABBRR), viz DT 126, TL 2900, TL 2908, TL 2919, TL 2920, ITSN 109 and ITSN 105#58 and six of spring wheat (AABBDD), viz PW 565, Raj 3702, RL 14-1, HPW 155, HPW 42 and VL 802. These were hybridized during 2002–03 to generate six triticale × wheat and two triticale × triticale F_1 hybrids. Total 10 genotypes consisting of eight hybrids (TL 2900 × PW 565, TL 2900 × Raj 3702, TL 2908 × RL 14-1, TL 2919 × VL 802, TL 2920 × HPW 155, ITSN 105#58 × HPW 42, ITSN 105#58 × TL 2908 and ITSN 105#58 × ITSN 109) and two triticales (ITSN 109 and DT 126) were raised during 2003– in two replicates in single rows, 1.25 m long and 23 cm apart. Nine main spikes/genotype in each replicate were pollinated with the freshly collected pollen of Madgran Local genotype of maize. All the crossed spikes were given *in vivo* auxin treatment 24 hr after pollination, one replicate subjected to 2,4-D treatment and the other to picloram. For this, two most used and easily available auxin analogues, 2,4-D and picloram (Hi-Media), were filled in three concentrations (100, 250 and 400 mg/l) in the hollow of the uppermost internode of three crossed spikes each by injecting each at their base. The injections were repeated for two more consecutive days (three injections over 72 hr.). The crossed spikes were harvested from the tiller base after 18–20 days of pollination and embryo-carrying seeds were excised in strict aseptic conditions and the embryos rescued on the modified MS medium (Murashige and Skoog 1962), supplemented with 0.5 mg/l kinetin, 150 mg/l glutamine and

*Short note

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20 mg/l each of L- arginine, L- cysteine and L- leucine. The cultured embryos were given cold pre-treatment at 4ºC in dark for 24 hr, followed by incubation at 25±1ºC in 10 hr light/14 hr dark photoperiod regime until they developed to 3–4 leaf stage. At this stage, for profuse rooting, the plantlets were transferred onto liquid medium comprising half the strength of MS salts, 1 mg/l each of naphthalene acetic acid and indole 3-butyric acid, and devoid of sucrose and agar (Pratap *et al.* 2006, Pratap and Chaudhary 2006).

The root tip cells of the regenerated plantlets were used for the determination of somatic chromosome number during 2004–05 following Tsuchia (1971). The true haploids were treated with colchicine as described by Inagaki (1985) and doubled haploids were recovered. Observations were recorded with respect to three haploid induction component traits, viz, seed formation, embryo formation and regeneration frequencies. The significance of the differences between means were estimated using 't' test, χ^2 whereas, test was used for estimating significance of differences between the different concentrations of auxins in pooled data.

All the triticale and triticale × wheat spikes pollinated with maize resulted in seed-set when these were treated *in vivo* with either 2,4-D or picloram, though the frequencies varied at different concentrations of these auxins (Table 1). This indicated that auxin treatment is an important step in the reproduction of haploid embryos through the chromosome elimination technique. The regulatory effect of exogenous auxins on embryo development has also been demonstrated by Matzk (1991). 2,4-D is reported to play a key role in the induction and maintenance of growth of wheat embryos in an earlier study (Wedzony and Van Lammerem 1996). The data pooled over 10 genotypes (Table 1) exhibited that in case of 2,4-D treatment, the three concentrations (100, 250 and 400 mg/l) did not differ significantly with respect to seed formation and embryo formation frequencies as expressed by c^2 test (5.07 and 0.08, respectively) while, significant differences were observed for regeneration frequencies (c^2 = 16.34). On the other hand, among the three concentrations of picloram, significant differences were obtained for all the three parameters of haploid induction (c^2 = 8.50, 6.84 and 14.56, respectively). Further, the varying overall success rate pooled over the 10 genotypes suggested that different concentrations of auxins have varying effects on seed formation, embryo formation and regeneration frequencies and, therefore, optimization of dose is required individually for every auxin analogue for *in vivo* treatment of pollinated spikes for best results.

For the individual concentrations of the two auxins used, the *in vivo* application of of 2,4-D did not exhibit significant differences from picloram at all concentrations with respect to the three component traits of haploid induction ('t' test, $P \leq 0.005$) (Table 2). The non-significant differences between the 2,4-D and picloram treatments with respect to seed formation, embryo formation and regeneration frequencies at 100, 250 and 400 mg/l concentrations indicate that picloram can be a successful alternative of 2,4-D for *in*

Table 1 Relative effectiveness of 2, 4-D and picloram on seed formation, embryo formation and regeneration frequencies in triticale and triticale × wheat F_1s pollinated with maize. Data pooled over 10 genotypes

Auxin analogue	Conc. (mg/l)	Florets pollinated with maize	Seed formation frequency	Embryo formation frequency	Regeneration frequency	Success rate
2,4-D	100	1212	10.6 (128)	14.8 (19)	26.3 (5)	0.4
	250	1398	23.2 (324)	14.2 (46)	50.0 (23)	1.6
	400	1285	21.5 (276)	18.8 (52)	19.23 (10)	0.8
Total		3895	18.7 (728)	16.1 (117)	32.5 (38)	1.0
χ^2			5.1	0.1	16.3	
Picloram	100	1338	10.8 (145)	20.0 (29)	31.0 (9)	0.7
	250	1446	26.6 (385)	12.7 (49)	53.1 (26)	1.8
	400	1326	28.3 (376)	29.5 (111)	22.5 (25)	1.9
Total		4110	22.9 (940)	18.0 (169)	35.5 (60)	1.5
χ^2			8.5	6.8	14.6	

Figures in parentheses represent the numbers obtained

Table 2 Relative efficiency of auxin analogues, 2,4-D and picloram on haploid induction in triticale and triticale × wheat hybrids through chromosome elimination technique

Genotype	2,4-D											
	100 mg/l				250 mg/l				400 mg/l			
	f	sf	ef	r	f	sf	ef	r	f	sf	ef	r
ITSN 109	134	8.9 (12)	25.0 (3)	33.3 (1)	104	49.0 (51)	23.5 (12)	41.7 (5)	153	35.3 (54)	13.0 (7)	14.3 (1)
DT 126	106	3.8 (4)	0	0	116	15.5 (18)	5.6 (1)	100.0 (1)	94	13.8 (13)	15.4 (2)	0
TL 2900 × PW 565	118	17.8 (21)	9.5 (2)	50.0 (1)	161	26.1 (42)	7.1 (3)	66.7 (2)	172	13.4 (23)	17.4 (4)	0
TL 2900 × Raj 3702	96	6.2 (6)	6.7 (4)	25.0 (1)	158	17.1 (27)	40.8 (11)	36.4 (4)	169	13.6 (23)	47.8 (11)	0
TL 2908 × RL 14-1	142	9.1 (13)	7.7 (1)	0	127	11.8 (15)	20.0 (3)	33.3 (1)	103	11.6 (12)	16.7 (2)	0
TL 2919 × VL 802	107	9.3 (10)	50.0 (5)	40.0 (2)	133	16.5 (22)	13.6 (3)	66.7 (2)	142	6.3 (9)	35.4 (4)	25.0 (1)
TL 2920 × HPW 155	89	3.4 (3)	0	0	120	5.0 (6)	50.0 (3)	66.7 (2)	127	12.6 (16)	18.7 (3)	66.7 (2)
ITSN 105#58 × HPW 42	161	14.9 (24)	0	0	158	14.6 (23)	8.70 (2)	50.0 (1)	99	21.2 (21)	28.6 (6)	50.0 (3)
ITSN 105#58 × TL 2908	140	16.4 (23)	17.4 (4)	0	174	32.2 (56)	3.6 (2)	100.0 (2)	104	29.9 (28)	14.3 (4)	25.0 (1)
ITSN 105#58 × ITSN 109	119	10.1 (12)	0	0	147	43.5 (64)	9.4 (6)	50.0 (3)	122	63.1 (77)	11.7 (9)	22.2 (2)
Total	1212	10.6 (128)	14.8 (19)	26.3 (5)	1398	23.2 (324)	14.2 (46)	50.0 (23)	1285	21.5 (276)	18.8 (52)	19.2 (10)
	Picloram											
	100 mg/l				250 mg/l				400 mg/l			
	f	sf	ef	r	f	sf	ef	r	f	sf	ef	r
ITSN 109	166	12.6 (21)	14.3 (3)	0	102	48.0 (49)	16.3 (8)	37.5 (3)	112	45.5 (51)	27.5 (14)	21.4 (3)
DT 126	171	5.3 (9)	11.1 (1)	0	162	13.0 (21)	9.5 (2)	100.0 (2)	159	17.0 (27)	33.3 (9)	22.2 (2)
TL 2900 × PW 565	156	16.7 (26)	11.5 (3)	33.3 (1)	134	24.6 (33)	18.2 (6)	16.7 (1)	87	24.1 (21)	28.6 (6)	16.7 (1)
TL 2900 × Raj 3702	134	5.2 (7)	5.7 (4)	50.0 (2)	142	17.6 (25)	28.0 (7)	42.9 (3)	140	20.7 (29)	55.2 (16)	6.2 (1)
TL 2908 × RL 14-1	98	9.2 (9)	22.2 (2)	0	156	26.3 (41)	12.2 (5)	60.0 (3)	126	30.9 (39)	17.9 (14)	21.4 (3)
TL 2919 × VL 802	147	8.8 (13)	38.5 (5)	40.0 (2)	126	11.9 (15)	13.3 (2)	100.0 (2)	136	9.6 (13)	35.9 (4)	0
TL 2920 × HPW 155	119	5.0 (6)	16.7 (1)	100.0 (1)	134	3.0 (4)	75.0 (3)	66.7 (2)	151	8.6 (13)	69.2 (9)	33.3 (3)
ITSN 105#58 × HPW 42	79	29.1 (23)	0	-	162	27.2 (35)	9.1 (4)	75.0 (3)	118	22.9 (27)	29.6 (8)	62.5 (5)
ITSN 105#58 × TL 2908	128	9.4 (12)	25.0 (3)	33.3 (1)	170	38.3 (65)	9.2 (6)	50.0 (3)	163	32.5 (53)	18.9 (10)	50.0 (5)
ITSN 105#58 × ITSN 109	140	13.6 (19)	36.8 (7)	28.6 (2)	158	55.7 (88)	6.8 (6)	66.7 (4)	134	76.9 (103)	20.4 (21)	9.2 (2)
Total	1338	10.8 (145)	20.0 (29)	31.0 (9)	1446	26.6 (385)	12.7 (49)	53.1 (26)	1326	28.3 (376)	29.5 (111)	22.5 (25)

f, Florets pollinated with maize pollen; sf, seed formation; ef, embryo formation; r, regeneration
Figures in parentheses represent the actual numbers obtained

vivo treatment of pollinated spikes, whereas, earlier, Wedzony *et al.* (1998) had observed significantly better performance of picloram to replace 2,4-D. Dicamba or picloram have been reported to be the successful alternatives of IAA or 2,4-D as previously applied in earlier study (Rogalska and Mikulski 1996). However, in the present context, due to the manifold cost of picloram, it is not economically advisable to replace 2,4-D with it. However, the overall success rate of obtaining haploids over the total number of pollinated florets was found to be the highest (1.6% and 1.9%) at 250 and 400 mg/l concentrations of 2,4-D and picloram respectively.

In case of 2,4-D, the highest average seed formation frequency (23.9%) was recorded at 250 mg/l concentration, followed by 400 mg/l (21.5%) and 100 mg/l (10.6%) (Table 2). However, in case of picloram, the highest seed formation frequency was observed at 400 mg/l (28.3%) followed by 250 mg/l (26.6%) and 100 mg/l (10.8%). Embryos were obtained in all the genotypes at 250 and 400 mg/l concentrations of 2,4-D and picloram, whereas at 100 mg/l concentration of both the auxins, a few genotypes did not respond. The highest embryo formation frequency in case of 2,4-D was recorded at 400 mg/l concentration (18.8%), followed by 100 mgl (14.8%) and 250 mg/l (14.2%). In case of picloram, the highest embryo formation frequency was recorded at 400 mg/l concentration (29.5%), followed by 100 mg/l (29.0%) and 250 mg/l (12.7%).

As far as haploid regeneration frequencies are concerned, all the 10 genotypes responded well with respect to regeneration frequencies at 250 mg/l concentration of 2,4-D, whereas only four and six genotypes showed response for the same parameter at 100 and 400 mg/l concentrations, respectively. At 100 mg/l the embryos after showing initial growth, desiccated and turned black within first fortnight, whereas, at 400 mg/l, the regeneration was substantially reduced by callus formation in the regenerating embryos. In case of picloram, all the 10 genotypes exhibited regeneration of green haploid plantlets at 250 mg/l concentration, whereas nine and six genotypes responded to it at 400 and 100 mg/l, respectively. The highest regeneration frequency was observed at 250 mg/l (53.1%), followed by 100 mg/l (31.0%) and 400 mg/l (22.5%). The cytological examination of regenerated plantlets revealed that in case of triticales, the haploids had a complete set of 21 chromosomes, whereas in case of triticale × wheat derived haploid metaphase cells, the chromosome count was variable barring one cross, TL 2919 × VL 802, where it was complete 21.

The highest seed formation frequency and success rate at 250 mg/l concentration of 2,4-D suggests that this is nearly the optimum concentration of 2,4-D for best results with respect to this trait. On the other hand, the best results for seed formation frequency and success rate at 400 mg/l of picloram suggested that it was the optimum concentration. However, maximum regeneration of haploids at 250 mg/l concentration of both the auxin analogues suggests that 250 mg/l was nearly the optimum concentration of auxins to obtain best regeneration frequencies in triticale and triticale × wheat hybrids. This concentration has also been successfully employed to develop haploid plants in triticale and crosses involving triticale as parents by crossing them with maize, *Imperata cylindrica* and some other Gramineae genera (Pratap *et al.* 2005).

From the above study, it is evident that *in vivo* auxin application is an important step for induction and maintenance of haploid embryos through the wheat × maize system and both the auxins, 2,4-D and picloram, exhibited the best results at a concentration of 250 mg/l. However, owing to huge cost difference between picloram and 2,4-D, use of 2,4-D is more practicable for induction of polyhaploids in wheat and triticale through the wheat × maize system

SUMMARY

The study was designed to evaluate two auxins, 2,4-D and picloram for their effectiveness for induction of polyhaploids in triticale and triticale × wheat crosses and to standardize their optimum dose. Ten F_1 and parental genotypes pollinated with maize were subjected to *in vivo* auxin treatment at three concentrations (100, 250 and 400 mg/l) by injecting at the tiller base of the crossed spikes so as to fill the hollow of their uppermost internode. Both auxins restulted in formation of embryo-carrying seeds, though the performance differed significantly for the three parameters of haploid induction at different concentrations. The highest seed formation, embryo formation, and regeneration frequencies in 2,4-D were recorded at 250, 400 and 250 mg/l concentration, respectively, whereas, 400, 400 and 250 mg/l concentration of picloram, respectively, were best for above traits. All the genotypes led to regeneration of haploid plants at 250 mg/l of both the auxins. On the basis of the three haploid induction parameters and overall success rates, the 250 mg/l concentration of both auxins was optimum.

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