Cereal Research Communications 47(3), pp. 552–565 (2019)
DOI: 10.1556/0806.47.2019.31

Auxin Induced Haploid Induction in Wide Crosses of Durum Wheat

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(Received 31 December 2018; Accepted 30 May 2019;
Communicated by P.S. Baenziger)

Doubled haploidy breeding via wide hybridization has been used in durum wheat haploid production for creating homozygosity in the shortest possible time. Post pollination treatment with hormones is a key factor for inducing haploid embryos in durum wheat wide crosses. An intergeneric hybridization experiment was carried out in seven durum wheat genotypes using *Imperata cylindrica* and two composites of Maize *viz.,* Bajaura Makka and Early Composite, as pollen sources. The pollinated spikes were injected with 2,4-Dichlorophenoxyacetic acid (2,4-D) in five different concentrations i.e., 100, 150, 200, 250 and 300 ppm, for three consecutive days at 24, 48 and 72 hrs after pollination. Analysis of variance revealed that the five concentrations of 2,4-D significantly differed in their ability to induce haploid embryos and 2,4-D at 250 ppm was found to be most effective in durum wheat haploid production through wide hybridization. The highest frequency of embryo carrying seeds was recorded to be 65.75 and 36.73 percent, at 250 ppm with *I. cylindrica* and Bajaura Makka, respectively in first cropping season. During second season, embryo formation frequency was observed to be maximum, 70.69, 32.84 and 27.59 percent, at 250 ppm 2,4-D with all three pollen sources, *viz., I. cylindrica*, Bajaura Makka and Early Composite, respectively.

Keywords: intergeneric hybridization, *Imperata cylindrica*, maize, 2,4-dichlorophenoxyacetic acid

Introduction

The traditional breeding program involves repeated selfing of F_1s for several generations to create homozygous lines. The doubled haploid technology has proven to be an efficient alternative to the conventional procedure that creates 100% homozygosity in a single generation in many crops. Durum wheat (*Triticum durum*), the only commercially cultivated tetraploid species of bread wheat having high demand in pasta and other food industries, has previously been shown to have good response towards different haploid production techniques *e.g.,* microspore culture (Ghaemi et al. 1993; Dogramaci-Altuntepe

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and Jauhar 2001; Cistue et al. 2009), unpollinated ovary culture (Sibi et al. 2001; Slama-Ayed and Slim-Amara 2007) and chromosome elimination mediated wide hybridization with different genera (O'Donoughue and Bennett 1994a and 1994b; Chlyah et al. 1999; Inagaki and Hash 1998; Almouslem et al. 1998; Chaudhary et al. 2013). However, during last decade the chromosome elimination mediated doubled haploid breeding has gained popularity. This technique mainly involves intergeneric hybridization that follows gradual elimination of chromosomes from pollen source during embryonic development producing embryos carrying haploid set of chromosomes from the female genotype (Barclay 1975; Laurie and Bennett 1988; Chaudhary et al. 2005). In durum wheat, wide hybridization to create doubled haploids has been carried out with different genera *viz.,* barley (*Hordeum bulbossum*) (O'Donoughue and Bennett 1994a; Chlyah et al. 1999), pearl millet (*Pennisetum glaucum*) (Inagaki and Hash 1998), maize (*Zea mays*) (O'Donoughue and Bennett 1994b; Almouslem et al. 1998) and *Imperata cylindrica* (Chaudhary et al. 2013; Chaudhary et al. 2015; Mahato and Chaudhary 2015) for producing haploid embryos. But, all these pollen sources may not be equally efficient in inducing haploid formation in durum wheat. Currently, durum doubled haploids have been made with a high level of success when maize and *I. cylindrica* were used as pollinators (Mahato and Chaudhary 2015).

In wide crosses of durum wheat, several factors such as growth condition (O'Donoughue and Bennett 1994b; Knox et al. 2000); male (Mahato and Chaudhary 2015) and female (Amrani et al. 1993; Sarrafi et al. 1994; Inagaki and Tahir 1995; Savaskanet al. 1997; David et al. 1999) genotypes; type, quantity, timing and method of hormone application (Almouslem et al. 1998; Inagaki et al. 1998) etc. have been shown to affect the level of haploid production. Generally, fertilization with alien pollen causes low embryo viability and zygote abortion during the initial stages of embryonic development. Post pollination application of growth hormones can increase the recovery of haploid embryos by inducing ovary enlargement and enhancing the survival and development of haploid embryos to a stage suitable for plant growth on nutrient media. Many growth promoting agents *viz.,* 2,4-D (2,4-dichlorophenoxyacetic acid) (Garcia-llamas et al. 2004), dicamba (3,6-dichloro-2-methoxybenzoic acid) (Knox et al. 2000; Savaskan et al. 1997), gibberellic acid (Almouslem et al. 1998), kinetin (6-Furfurylaminopurine) (O'Donoughue and Bennett 1994b), silver nitrate (O'Donoughue and Bennett 1994b; Almouslem et al. 1998; Inagaki et al. 1998) etc. have been used for increasing the efficacy of haploid production in durum wheat × maize crosses. However, in case of durum wheat × *I. cylindrica,* only 2,4-D has been used in very few previous studies (Chaudhary et al. 2015).

Further, the concentration, time and method of hormone application also influenced the haploid production efficiency of these techniques. Hormonal treatment can be given to the pollinated spike by directly placing the hormone solution inside the floret (Laurie et al. 1990), spraying hormone solution on pollinated spikes (Laurie 1989; Riera-Lizarazu and Mujeeb-Kazi 1990), injecting hormones into the uppermost internode of the wheat stem (Suenaga and Nakajima 1989; Inagaki and Tahir 1995; Riera-Lizarazu and Mujeeb-Kazi 1990) and placing detached spikes into an aqueous solution containing the hormone (Riera-Lizarazu and Mujeeb-Kazi 1990). All these methods are effective in sustaining the

development of haploid embryo (Knox et al. 2000). For determining the best time of hormone application, Suenaga and Nakajima (1989), studied the effect of both pre- and post-pollination applications on embryonic development and recommended post pollination application to be more effective. Later, plant hormones were applied at the time of pollination (Laurie 1989), 1 day (O'Donoughue and Bennett 1994b; Amrani et al. 1993), 1 and 2 days (Inagaki and Tahir 1995) and 2–3 days after pollination (Campbell et al. 1998) to estimate the most appropriate hormone application timing. These studies clearly showed that the application of hormones or growth promoters is an important factor affecting production of doubled haploids through chromosome elimination-mediated technique and till date researchers have not reached to a general consensus in this aspect. Hence, we undertook a study on post-pollination application of different concentrations of 2,4-D, in an attempt to improve the efficiency of haploid production in durum wheat × maize and durum wheat × *I. cylindrica*-mediated approach of doubled haploidy breeding.

Materials and Methods

The present investigation was conducted using seven elite and highly diverse genotypes of durum wheat, in terms of yield, grain quality and parentage, *viz.,* A-9-30-1, HI 8498, PDW 233, PDW 291, PDW 314, WH 896 and WHD 943. Two composite varieties of Himalayan maize, Bajaura Makka and Early Composite, were used as maize pollen sources which had been selected on the basis of their haploid inducing ability in bread wheat *(Triticum aestivum),* triticale (*Triticosecale rimpaui* Wittm.) and their derivatives as reported by Dhiman et al. (2012). The parentage and source of durum wheat and maize genotypes used in the study have been given in (Table 1 and 2). The third pollen source was cogon grass *(Imperata cylindrica),* a wild weedy perennial grass growing abundantly in the fields, bunds etc. of agricultural farms and adjoining areas.

Table 1. Parentage and source of different diverse genotypes of durum wheat

S. No.	Genotype	Parentage	Source
1	A-9-30-1	A206/GAZA	Gujarat Agricultural University, Anand, India
2	HI 8498	CR "S'-GS'S' /A-9-30-1//RAJ911	IARI Regional Research Station, Indore, India
3	PDW 233	YAV'S' / TEZ 'S'	Panjab Agricultural University, Ludhiana, India
4	PDW 291	BOOMER 21/ MOJO2	-do-
5	PDW 314	AJAIA 12/F3 LOCAL (SEL. ETHIO.135.85)//PLATA 13/3/SOMA I- 3/4/SOOTY9/RASCON 37	-do-
6	WH 896	STIL"S"/ YAV"S' //PEN"S	Chaudhary Charan Singh Haryana Agricultural University, Hisar, India
7	WHD 943	GLARE/PLATA-16//AJAIA -3/SILVER16	-do-

Table 2. Parentage and source of different genotypes of Himalayan maize

S. No.	Genotype	Parentage	Source
1	BajauraMakka (Released variety)	Composite of Early Composite, PS-62, FH 3209, FH 3198, FH 3202, 10 half sib progenies of hill early yellow pool and Kullu Local	Highland Agricultural Research and Extension Centre, CSK Himachal Pradesh Agricultural University, Palampur, India
2	Early Composite (Released variety)	Composite of Kullu Local, Abaskajas, Maize No.8, Mex-3CB, Bodhipur Yellow, JML603, VL1, YUZPSC-3, YUZPSC-4, YUZPSC-71C, YUZP-DC-775, YUZPSC-79C, VL2 and VL42	-do-

The experiment was conducted at Experimental Farm, Department of Crop Improvement, CSK Himachal Pradesh Agricultural University, Palampur, India in *rabi* (winter season) 2011–2012 and *rabi* 2012–2013. In first season, scattered sowing of the experimental material *viz.,* seven durum wheat genotypes and one maize composite, Bajaura Makka, was done in crossing block and polyhouse, respectively, to ensure artificial hybridization throughout the season. Five different concentrations of 2,4-D (100 ppm, 150 ppm, 200 ppm, 250 ppm and 300 ppm) were used in the first season. Durum wheat genotypes were manually emasculated and pollinated with both pollen sources. After pollination, a total of 25 spikes per cross were treated with all five treatments, i.e. five spike per cross per treatment. Each treatment was given by injecting in the uppermost internode of pollinated spikes until the fluid comes out through a hole, made with needle, in the upper portion of stem. The same process was repeated for three consecutive days after pollination i.e. at 24, 48 and 72 hrs for a particular treatment.

The pollinated spikes were harvested from the tiller base 18–20 days after pollination and the pseudoseeds were screened for embryo against a light source (Bains et al. 1998). The embryo carrying pseudoseeds were washed with Tween-20 (Merck Limited, India) detergent for 1 to 2 min under tap water followed by surface sterilization with 0.004 M (0.1%) $HgCl_2$ for 3–5 min. The sterilized pseudoseeds were rinsed with autoclaved distilled water for 1 min and subjected to embryo rescue by dissecting the pseudoseeds and culturing the embryos on MS medium (Murashige and Skoog 1962) supplemented with 0.5 mg/l kinetin, 150 mg/l glutamine and 20 mg/l each of L-arginine, L-cystine and L-leucine under aseptic condition. The cultured embryos were immediately subjected to cold treatment at 4 °C for 24 h followed by incubation at 20 ± 2 °C under dark conditions until shoot initiation. The resultant plantlets were then placed under controlled temperature (20 ± 2 °C) and relative humidity (75%) in a 10 h light/14 h dark photoperiod regime. The plantlets at three to four leaflet stage were then transferred to liquid rooting medium consisting of half strength of MS-salts, 1 mg/l naphthalene-3-acetic acid (NAA) and 1 mg/l indole-3-butyric acid (IBA). The haploid plantlets were then subjected to colchicine treatment (0.1% colchicine + 1.5% DMSO) for 4–5 hrs and subsequently transferred to a soil potting mixture after rinsing the colchicine treated plantlets in running tap water

for 1 hr. The root tip cells of resultant plantlets were used to examine their ploidy status following the procedure of Tsuchiya (1971).

During the second season, all the three pollen parents *viz.,* Bajaura Makka, Early Composite and *Imperata cylindrica*, were used to pollinate the female genotypes. The female and male genotypes, except *I. cylindrica,* were sown in a similar manner as that of previous season. Based on the results of previous year, only three concentrations of 2,4-D i.e. 200 ppm, 250 ppm and 300 ppm, which performed better, were used for post pollination hormonal treatment of spikes so as to identify the most appropriate dose for haploid induction. Same procedure as that of *rabi* 2011–2012, was followed for pollination, hormone application, harvesting of pollinated spikes, screening of haploid embryos and regeneration of haploid plantlets.

Observations were recorded with respect to various haploid induction traits viz., pseudoseed formation frequency (number of pseudoseeds formed × 100/total number of florets pollinated), embryo formation frequency (number of pseudoseeds carrying embryo × 100/total number of pseudoseeds formed), haploid embryo regeneration frequency (number of haploid plantlets developed × 100/total number of embryos cultured) and haploid formation efficiency (number of haploid plantlets developed × 100/total number of florets pollinated) at each concentration of 2,4-D for each of the pollen sources, separately. The data thus obtained was subjected to two way analysis of variance after arcsine transformation (Gomez and Gomez 1984) to meet the requirement of normality for the analysed data.

Results

The results obtained from two-way ANOVA indicated that the five concentrations of 2,4-D significantly affected all haploid induction parameters with both pollen sources during first season (Table 3). In durum wheat × *I. cylindrica* crosses, genotypes exhibited significant difference for embryo formation frequency and haploid formation efficiency. Genotype × 2,4-D interactions also differ significantly for all parameters except haploid embryo regeneration frequency. In crosses with Bajaura Makka, significant differences were recorded between genotypes and genotypes × 2,4-D interactions for pseudoseed and embyo formation frequencies.

Most of the earlier reports regarding hormonal influence on durum wheat × maize crosses were based on auxins, mainly 2,4-D either alone or in combination with other growth regulators. In this study 2,4-D alone produced pseudoseeds of varying frequencies at different concentrations, ranging from 22.55 to 55.92% and 19.09 to 37.32% in crosses with *I. cylindrica* and Bajaura Makka, respectively (Table 4 and 5). However, the highest frequencies were recorded at 250 ppm in both cases. When crossed with *I. cylindrica,* the highest percentage of embryo carrying seeds, 15.72, 36.56 and 21.46 per cent, were obtained at 200 ppm, 250 ppm and 300 ppm concentration of 2,4-D, respectively while with Bajaura Makka, the highest mean embryo formation frequency was recorded to be 24.65 per cent at 250 ppm 2,4-D and embryo recovery was nil at 100 ppm and 150 ppm concentration of 2,4-D. The highest frequency of regenerated plantlets and haploid formation

Table 3. Analysis of variance for different haploid induction parameters in durum wheat wide crosses during *rabi* 2011–2012

Sources of variations	Df	*I. cylindrica*				Bajaura Makka			
		PFF	EFF	ERF	HFE	PFF	EFF	ERF	HFE
Genotypes	6	211	1134*	932	204*	472*	402*	227	39
2,4-D concentrations	4	2309*	7575*	10060*	1339*	941*	4920*	7938*	469*
Genotypes × 2,4-D concentrations	24	153*	322*	617	45*	122*	163*	339	17
Error	140	72	88	568	19	45	75	399	12

Df = degrees of freedom; PFF = Pseudoseed formation frequency; EFF = Embryo formation frequency; ERF = Embryo regeneration frequency, HFE = Haploid formation efficiency.
*Mean square values significant at $P \leq 0.05$.

Table 4. Effect of different concentrations of 2,4-D on haploid induction parameters in *T. durum* when pollinated with *I. cylindrica* during *rabi* 2011–2012

Genotypes	2,4-D concentrations																			
	100 ppm				150 ppm				200 ppm				250 ppm				300 ppm			
	PFF	EFF	ERF	HFF	PFF	EFF	ERF	HFF	PFF	EFF	ERF	HFF	PFF	EFF	ERF	HFF	PFF	EFF	ERF	HFF
A-9-30-1	13.16	0.00	0.00	0.00	31.71	17.31	0.00	0.00	38.55	39.06	44.00	6.63	74.40	43.20	42.59	13.69	51.57	26.83	31.82	4.40
HI8498	33.33	0.00	0.00	0.00	31.16	0.00	0.00	0.00	37.01	0.00	0.00	0.00	58.70	21.30	34.78	4.35	42.18	12.90	37.50	2.04
PDW233	34.97	2.00	0.00	0.00	31.88	2.27	100.00	0.72	31.88	13.73	28.57	1.25	50.60	40.48	38.24	7.83	38.03	22.22	33.33	2.82
PDW291	14.38	0.00	0.00	0.00	21.52	0.00	0.00	0.00	25.90	18.60	25.00	1.20	50.00	33.33	34.62	5.77	40.85	8.62	20.00	0.70
PDW314	22.22	0.00	0.00	0.00	24.05	0.00	0.00	0.00	36.99	11.11	16.67	0.68	46.50	65.75	43.75	13.38	64.71	19.32	35.29	4.41
WH896	17.12	0.00	0.00	0.00	40.54	11.67	42.86	2.03	43.18	21.05	41.67	3.79	58.33	39.80	48.72	11.31	34.51	42.86	33.33	4.93
WHD943	22.64	0.00	0.00	0.00	23.78	0.00	0.00	0.00	30.07	6.52	0.00	0.00	52.91	12.09	45.45	2.91	24.84	17.50	14.29	0.62
Mean	22.55	0.29	0.00	0.00	29.23	4.46	20.41	0.39	34.80	15.72	22.27	1.94	55.92	36.56	41.16	8.46	42.38	21.46	29.37	2.85
SE(d)±	3.29	0.29	0.00	0.00	2.49	2.68	14.57	0.29	2.20	4.73	6.76	0.92	3.51	6.48	2.05	1.65	4.82	4.21	3.29	0.68

PFF = Pseudoseed formation frequency; EFF = Embryo formation frequency; ERF = Embryo regeneration frequency; HFE = Haploid formation efficiency.

Table 5. Effect of different concentrations of 2,4-D on haploid induction parameters in *T. durum* when pollinated with Bajaura Makka during *rabi* 2011–2012

Genotypes	2,4-D concentrations																			
	100 ppm				150 ppm				200 ppm				250 ppm				300 ppm			
	PFF	EFF	ERF	HFF	PFF	EFF	ERF	HFF	PFF	EFF	ERF	HFF	PFF	EFF	ERF	HFF	PFF	EFF	ERF	HFF
A-9-30-1	31.34	0.00	0.00	0.00	25.17	0.00	0.00	0.00	30.97	6.25	0.00	0.00	50.33	29.87	47.83	7.19	37.86	20.75	27.27	2.14
HI8498	20.90	0.00	0.00	0.00	19.76	0.00	0.00	0.00	20.28	0.00	0.00	0.00	24.00	19.44	28.57	1.33	12.79	0.00	0.00	0.00
PDW233	26.24	0.00	0.00	0.00	22.22	0.00	0.00	0.00	35.97	0.00	0.00	0.00	43.83	21.13	33.33	3.09	26.00	17.95	28.57	1.33
PDW291	20.42	0.00	0.00	0.00	18.12	0.00	0.00	0.00	16.13	0.00	0.00	0.00	30.14	13.64	33.33	1.37	23.19	9.38	0.00	0.00
PDW314	6.79	0.00	0.00	0.00	11.41	0.00	0.00	0.00	13.58	0.00	0.00	0.00	44.23	24.64	35.29	3.85	35.37	18.97	36.36	2.44
WH896	17.19	0.00	0.00	0.00	13.64	0.00	0.00	0.00	15.58	12.50	0.00	0.00	36.30	36.73	38.89	5.19	10.56	25.00	25.00	0.70
WHD943	10.76	0.00	0.00	0.00	17.19	0.00	0.00	0.00	23.94	0.00	0.00	0.00	32.43	27.08	23.08	2.03	29.08	0.00	0.00	0.00
Mean	19.09	0.00	0.00	0.00	18.22	0.00	0.00	0.00	22.35	2.68	0.00	0.00	37.32	24.65	34.33	3.44	24.98	13.15	16.74	0.94
SE(d)±	3.20	0.00	0.00	0.00	1.79	0.00	0.00	0.00	3.19	1.86	0.00	0.00	3.50	2.84	2.95	0.82	3.94	3.83	6.06	0.39

PFF = Pseudoseed formation frequency; EFF = Embryo formation frequency; ERF = Embryo regeneration frequency; HFE = Haploid formation efficiency.

Table 6. Analysis of variance for different haploid induction parameters in durum wheat wide crosses during *rabi* 2012–2013

Sources of variations	Df	*I. cylindrica*				Bajaura Makka				Early Composite			
		PFF	EFF	ERF	HFE	PFF	EFF	ERF	HFE	PFF	EFF	ERF	HFE
Genotypes	6	119	312	206	70	118	302	369	28	200	293	252	13
2,4-D concentrations	2	2648*	6272*	11553*	1936*	198	372	185	30	3077*	3245	1840	121
Genotypes × 2,4-D concentrations	12	90	369*	221	21	39	54	142	9	86	221	83	5
Error	84	76	166	278	25	38	331	465	28	55	246	306	14

Df = degrees of freedom; PFF = Pseudoseed formation frequency; EFF = Embryo formation frequency; ERF = Embryo regeneration frequency; HFE = Haploid formation efficiency.
*Mean square values significant at $P \leq 0.05$.

Table 7. Effect of different concentrations of 2,4-D on pseudoseed and embryo formation frequencies in *T. durum* when pollinated with *I. cylindrica* and maize during *rabi* 2012–2013

Genotypes	Pseudoseed formation frequency									Embryo formation frequency								
	cylindrica			BajauraMakka			Early Composite			*cylindrica*			BajauraMakka			Early Composite		
	2,4 D Concentrations (ppm)																	
	200	250	300	200	250	300	200	250	300	200	250	300	200	250	300	200	250	300
A-9-30-1	32.39	61.49	44.08	22.44	44.08	31.25	12.94	35.37	22.84	17.54	55.14	38.81	25.71	32.84	22.00	9.09	27.59	18.92
HI8498	16.87	61.31	29.88	18.67	26.88	20.99	6.67	14.61	13.58	0.00	28.16	30.61	16.13	23.26	20.59	10.00	15.38	13.64
PDW233	24.38	49.31	36.99	25.00	32.67	25.97	3.90	23.97	14.67	5.13	45.07	33.33	14.29	18.37	22.50	16.67	14.29	4.55
PDW291	17.83	45.89	43.66	23.85	27.74	19.59	4.88	25.74	10.00	7.14	49.25	29.03	16.13	21.05	17.24	0.00	17.14	60.00
PDW314	22.83	35.15	33.33	26.39	28.38	24.36	5.88	36.36	10.56	11.90	70.69	40.74	23.68	28.57	21.05	0.00	14.29	20.00
WH896	31.11	48.94	33.33	22.02	23.42	16.23	4.49	29.88	12.80	32.14	33.70	30.77	8.11	18.92	16.00	0.00	20.41	19.05
WHD943	18.82	50.68	34.57	25.33	20.63	18.00	6.41	15.12	10.59	9.38	34.67	26.67	5.26	18.18	14.81	0.00	19.23	11.11
Mean	23.46	50.40	36.55	23.39	29.11	22.34	6.45	25.86	13.58	11.89	45.24	32.85	15.62	23.03	19.17	5.11	18.33	21.04
SE(d)±	2.37	3.44	2.05	0.98	2.88	1.97	1.15	3.32	1.68	3.96	5.56	1.95	2.81	2.14	1.17	2.57	1.78	6.82

PFF = Pseudoseed formation frequency; EFF = Embryo formation frequency; ERF = Embryo regeneration frequency; HFE = Haploid formation efficiency.

Table 8. Effect of different concentrations of 2,4-D on haploid embryo regeneration frequency and haploid formation efficiency in *T. durum* when pollinated with *I. cylindrica* and maize during *rabi* 2012–2013

Genotypes	Haploid embryo regeneration frequency									Haploid formation efficiency								
	cylindrica			BajauraMakka			Early Composite			*I. cylindrica*			BajauraMakka			Early Composite		
	2,4 D Concentrations (ppm)																	
	200	250	300	200	250	300	200	250	300	200	250	300	200	250	300	200	250	300
A-9-30-1	30.00	47.46	38.46	22.22	27.27	18.18	0.00	25.00	14.29	1.70	16.09	6.58	1.28	3.95	1.25	0.00	25.00	0.62
HI8498	0.00	31.03	33.33	20.00	30.00	28.57	0.00	25.00	0.00	0.00	5.36	3.05	0.60	1.88	1.23	0.00	25.00	0.00
PDW233	0.00	37.50	38.89	40.00	33.33	22.22	0.00	20.00	0.00	0.00	8.33	4.79	1.43	2.00	1.30	0.00	20.00	0.00
PDW291	50.00	30.30	44.44	20.00	25.00	20.00	0.00	33.33	11.11	0.64	6.85	5.63	0.77	1.46	0.68	0.00	33.33	0.67
PDW314	0.00	34.15	22.73	11.11	8.33	12.50	0.00	25.00	33.33	0.00	8.48	3.09	0.69	0.68	0.64	0.00	25.00	0.70
WH896	11.11	41.94	25.00	0.00	28.57	25.00	0.00	30.00	25.00	1.11	6.91	2.56	0.00	1.27	0.65	0.00	30.00	0.61
WHD943	0.00	38.46	26.67	0.00	33.33	25.00	0.00	20.00	0.00	0.00	6.76	2.47	0.00	1.25	0.67	0.00	20.00	0.00
Mean	13.02	37.26	32.79	16.19	26.55	21.64	0.00	25.48	11.96	0.49	8.40	4.02	0.68	1.78	0.92	0.00	25.48	0.37
SE(d)±	7.46	2.31	3.10	5.31	3.25	2.01	0.00	1.84	5.03	0.26	1.34	0.62	0.21	0.40	0.12	0.00	1.84	0.13

PFF = Pseudoseed formation frequency; EFF = Embryo formation frequency; ERF = Embryo regeneration frequency; HFE = Haploid formation efficiency.

efficiency were recorded at 250 ppm concentration of 2,4-D in both durum wheat × *I. cylindrica* and durum wheat × Bajaura Makka crosses, 41.16 and 34.33 per cent and 8.46 and 3.44 per cent, respectively. These results clearly indicated that only 2,4-D concentrations at higher level affected haploid formation in wide crosses of durum wheat.

During second season, when the same experiment was repeated using 2,4-D concentrations showing maximum effect on various haploid induction parameters based on first season's observations, i.e. 200 ppm, 250 ppm and 300 ppm, significant differences were obtained only in case of durum wheat × *I. cylindrica* crosses (Table 6). These results clearly indicated that higher doses of 2,4-D were at par in inducing haploid production in durum wheat × maize crosses. It is evident from Table 7 and 8, that 250 ppm 2,4-D produced highest mean frequencies for all haploid induction parameters with all three pollen sources. In contrary to the findings of Puja et al. (2011) who proposed the use of different combinations of auxin, these results clearly highlighted that 2,4-D alone was efficient in inducing haploid production in distant crosses of durum wheat with genus *Imperata* and *Zea*.

Discussion

Post-pollination application of 2,4-D is a key step in chromosome elimination mediated approach of doubled haploidy breeding. Earlier, 2,4-D in combination with different growth promoters like, dicamba (Ballesteros et al. 2003; Garcia-llamas et al. 2004), picloram (Puja et al. 2011) and $AgNO_3$ (Almouslem et al. 1998; Inagaki and Hash 1998; Sourour et al. 2011) had been tested for efficient haploid induction in durum wheat × maize crosses. Likewise, Chaudhary et al. (2015) and, Mahato and Chaudhary (2015) reported the significant role of 2,4-D in haploid production via *I. cylindrica*-mediated chromosome elimination approach. It is evident from the findings of present study that higher concentrations of 2,4-D (more than 200 ppm) alone is well efficient in producing greater number of haploid embryos.

O'Donoughue and Bennett (1994b) tested various concentrations and combinations of a synthetic auxin like, 2,4-D and kinetin along with an ethylene inhibitor, silver nitrate ($AgNO_3$) on embryo recovery and suggested *in vitro* treatment of fertilized ovaries using either 2,4-D alone or a combination of 2,4-D and $AgNO_3$ may serve as an effective method for enhanced production of haploids in tetraploid wheat. Jauhar (2003) proposed post pollination application of only $AgNO_3$ by mist spraying is an important factor which delays abscission of young embryos and increases the chances of haploid embryo recovery. Chaudhary et al. (2005) and, Pratap and Chaudhary (2012) reported that 2,4-D at concentrations 100 and 250 ppm played an important role in induction of polyhaploids in crosses of bread wheat and bread wheat-rye derivatives with *I. cylindrica,* respectively. Chaudhary et al. (2015) and, Mahato and Chaudhary (2015) also highlighted that haploid induction in durum wheat × *I. cylindrica* is most responsive at 250 ppm 2,4-D concentration.

Previous studies clearly highlighted that post pollination treatment with hormones is a vital step in haploid production through wide hybridization. The results obtained from this experiment also support the efficiency of 2,4-D in inducing haploid embryos of du-

rum wheat × maize and durum wheat × *I. cylindrica* crosses. But its concentration significantly affects frequency of embryo formation and concentration at 250 ppm is the most appropriate for both haploid production techniques. The findings here brought us to a conclusion that 250 ppm concentration of 2,4-D significantly affected haploid production in durum wheat × *I. cylindrica* crosses however, for maize-mediated approach higher concentrations of 2,4-D i.e. 200 ppm, 250 ppm and 300 ppm are at par in their effect on haploid induction.

Acknowledgement

Authors gratefully acknowledge ICAR-Directorate of Wheat Research, Karnal and Chaudhary Charan Singh Haryana Agricultural University, Hisar for providing durum wheat genotypes used in the study.

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