Annals of Agri-Bio Research **28** (2): 431-436, 2023

Impact Score : 0.28 (Scopus)

Effect of 2,4-D Dosage on Haploid Embryo Induction in Bread Wheat Following Wide Hybridization with Maize

SANJEET SINGH SANDAL*, PATIL KULBHUSHAN SAVINDRA AND PUNEET WALIA

Department of Genetics and Plant Breeding, Lovely Professional University, Phagwara-144 411(Punjab), India
**(e-mail: sanjeet.23751@lpu.co.in; Mobile: 9417563329)*

(Received: April 10, 2023; Accepted: June 16, 2023)

ABSTRACT

Nine bread wheat genotypes (HD 2851, HD 2967, HD 3086, PBW 502, PBW 550, PBW 677, UNNAT 343, WH 1105 and W H711) were pollinated with pollen of the maize genotype (PRMH 531) followed by 100 ppm 2,4-D application for haploid embryo induction. Data were collected for the number of emasculated florets, number of pollinated florets, number of pseudo seeds formed, pseudo seed formation frequency, pseudo-seed and haploid embryo formation efficiency. Three treatments comprising single dose (after 24 h), 2 doses (after 24 and 48 h) and 3 doses (after 24, 48 and 72 h) were used. The results revealed that the application of 2, 4-D at 100 ppm three times, i.e. 24, 48, and 72 h, resulted in a significantly high frequency of pseudo-seeds and haploid embryos when crossed with PRMH 351.

Key words: Haploid, bread wheat, 2, 4-D, wheat x maize

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most widely cultivated staple food crops in the world, feeding nearly 80% of the population and contributing significantly to the food security mosaic (Chaudhary *et al.*, 2015). Wheat production and productivity must be increased to satisfy the ever-increasing population demand. Development of varieties with high yield potential, resistance to abiotic and biotic stresses and acceptable quality parameters are the most viable and environmentally benign ways to increase agricultural production (Gupta *et al.*, 2016). The wheat breeding strategy underlying the green revolution relied on basic genetic changes, supplemented by better management practices and increased inputs (Srivastava and Bains, (2018). Improving genetic diversity will make crop production more resilient to the biotic and abiotic stresses that pose the greatest hazard in the current and foreseeable future (Chaudhary *et al.*, 2019). The integration of biotechnological techniques with practical plant breeding can accelerate the breeding programmes (Patial *et al.*, 2021). Chromosome elimination, a dynamic process that occurs naturally during wide hybridization in wheat, is utilized globally to produce haploids in both bread wheat and *durum* wheat with great success (Mahato and Chaudhary, 2015). The use of haploid and doubled haploid technology could expedite and significantly reduce the cost of breeding. In a single generation, homozygous lines can be generated from a F_1 hybrid by producing doubled haploids, whereas, it would require 8-9 generations of selfing to achieve over 99.9% homozygosity through conventional breeding (Watts *et al.*, 2018).

Doubled haploid (DH) breeding is a unique biotechnological approach that has allowed for the quick fixation of alien genes in wheat background and the achievement of homozygosity in as little as two years (Badiyal *et al.*, 2016). Doubled haploids (DH) were earlier produced by anther culture and using wide hybridization with *Hordeum bulbosum* L. and Zea *mays* L. Recently, wheat *Imperata cylindrica* has also been reported to be more efficient in haploid induction in bread wheat (Khan *et al.,* 2017). Haploid embryo induction (HEI) is an important biotechnological intervention in crop improvement programs in bread wheat. Double haploid (DH) plants are formed from microspores (male spores) or egg cells (female gametes), both of which are haploid cells along the gametic developmental track. They have numerous applications in the fields of plant breeding, plant genetics and fundamental biology (Wedzony *et al.*, 2015). DH technology fixes rare alleles and may play an important role in evaluation of genetic diversity (Santra *et al.*, 2017). Doubled haploid (DH) plants

derived from such embryos provide instant homozygosity overcoming the need of years of selfing, saving considerable time in making immortal mapping populations and stable cultivars. Wide hybridization with genetically distant pollen parent such as maize followed by Uniparental Genome Elimination (UGE) is a tried and tested method to induce haploid embryo formation in bread wheat. Multiple factors effect haploid embryo induction efficiency which should be at least 20% for practical applications. Pollen parent genotype, concentration of phytohormones, their application methods, ambient conditions and time of pollinations all play a critical role in achieving high efficiency of HEI. Apart from wheat ´ maize system, another protocols that are available for the production of doubled haploids via wide hybridization are bulbosum technique and wheat ´ *Imperata cylindrical.* Wheat x maize system of haploid production is quite successful, but for its practical implementation, maize flowering should coincide with wheat flowering (Mukai *et al.*, 2015).

Various field operations are involved in this process which need optimization to increase the efficiency of HEI. The current research was aimed at studying the per cent of pseudo seeds formed and haploid embryos formed due to the effect of 100 ppm 2, 4-D application at 24, 48 and 72 h after pollination.

MATERIALS AND METHODS

The research was conducted in the experimental fields of Genetics and Plant Breeding, School of Agriculture, Lovely Professional University, Punjab, during the year 2021-22, with nine hexaploid wheat genotypes viz., HD 2851, HD 2967, HD 3086, PBW 502, PBW 550, PBW 677, UNNAT 343, WH 1105 and WH 711 obtained from the from Department of Agriculture, Punjab. The seeds of Hybrid maize, viz PRMH 351, were collected from a regional seed distributor, Jalandhar, Punjab.

A total of four staggered sowings of wheat were done at interval of 10 days during the *rabi* season 2021-22 starting from 1nd week of November to 2nd week of December. Each line was sown in two rows of 2 m length with 30 cm of row to row spacing.

Maize, which was used as the pollen source, was sown twice. The first sowing was done in the first week of October (2nd October 2019) and the second sowing was done in the last week of October (26th October 2019). Maize was sown in 10 rows with 50 cm of row to row spacing and 15 cm of plant to plant spacing.

At ear emergence of 1-2 cm from the leaf sheath, flag leaf was gently removed to expose the ear in wheat. Emasculations were performed in the evening by removing anthers with the help of forceps without cutting lemma and palea (Fig. 1a). The awns were removed prior to cutting using surgical scissors. The central florets, apical and basal spikelets of each spike were removed. Uupper and basal spikelets were removed to maintain ear uniformity. The emasculated spikes were covered with butter paper bags to avoid stray pollen followed by labelling of tags with information viz., date of emasculation and genotype name.

Fresh maize pollen was collected in the Petri plates at the time of anther dehiscence (Fig. 1b). Before collecting the pollen, the Petri plates were sterilized using 70% ethanol. Freshly collected pollen was transferred to wheat stigma preferably in the next day morning of emasculation using fine camel hair brush. Pollen was collected after 15 min interval the prior pollen was discarded. Pollination of wheat spike was performed by individually opening an emasculated floret with a fine tipped forceps and holding it open while a second person shed pollen in it (Fig. 1c). Once this was done for the complete spike it was covered with butter paper bag and secured with a rubber coated paper clip. Information like date of pollination, name of the maize genotype used was also written on the tags.

Hundred ppm of 2, 4-D solution was injected (Fig. 1d) into the uppermost internode of pollinated wheat spikelets. In the first treatment (T_1), only one dose of 2, 4-D @ 100 ppm was injected after 24 h of pollination with maize. In the second treatment (T_2), two doses of 2, 4-D @ 100 ppm were injected at 24 and 48 h after pollination. In the third treatment (T_3), three doses of 2,4-D @ 100 ppm were injected at 24, 48 and 72 h after pollination. In all the cases freshly prepared 100 ppm 2, 4-D solution stored in a black labelled glass bottle kept under cool conditions was used. By using vaseline the injected holes were covered, and leakage was prevented. It has been reported

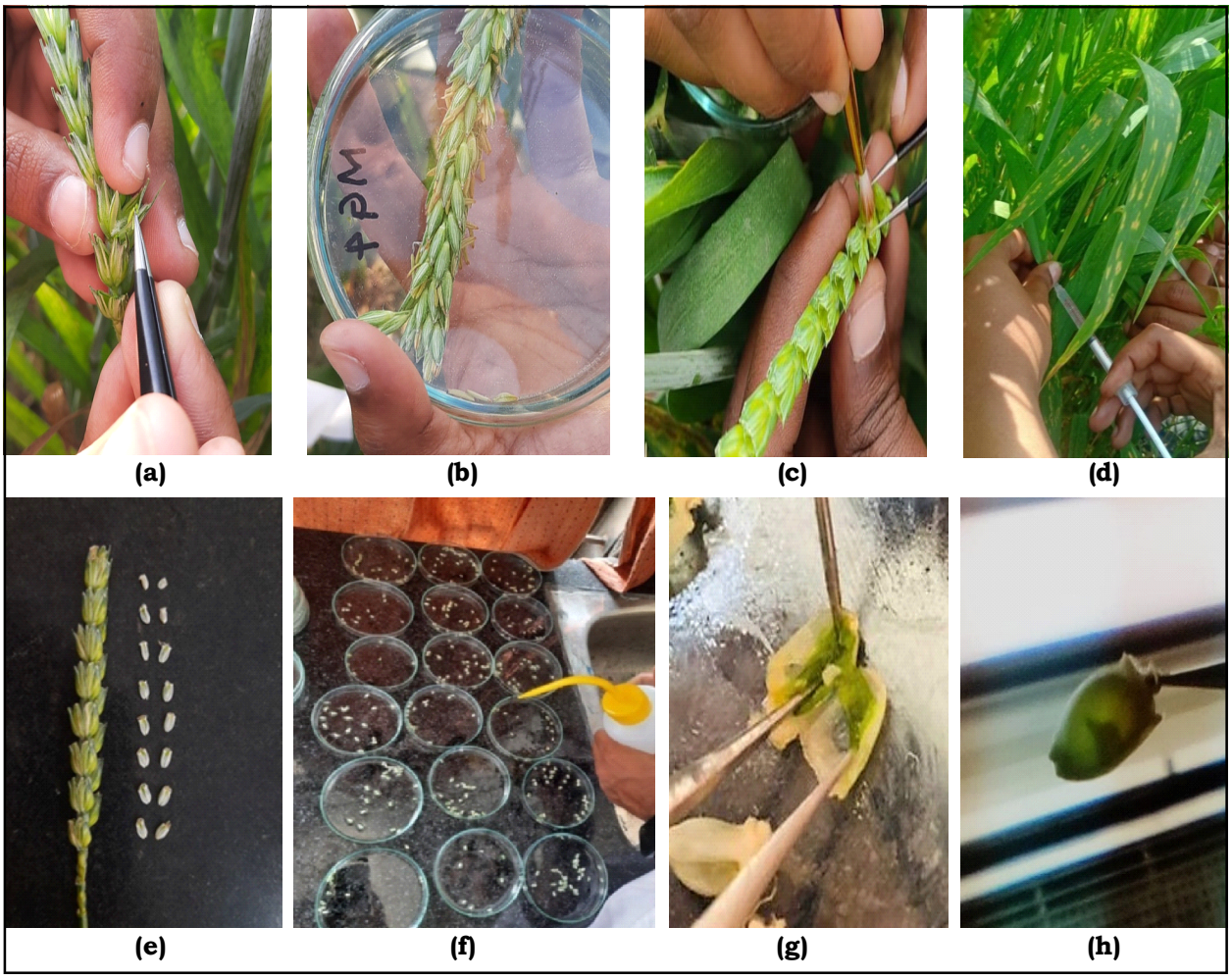

Fig. 1. Haploid embryo production procedure using wheat x maize hybridization technique (a) emasculation in wheat (b) pollen collection from maize, (c) pollination with maize pollen, (d) 2,4-d application, (e) harvested pseudo seeds, (f) pseudo seeds surface sterilization, (g) pseudo seeds are dissected, (h) embryo identification placing pseudo seed against the light source.

that 2, 4-D will aid in the survival of embryos. After 14-18 days of pollination, the spikes were harvested (Fig. 1e) from the base of the plant. The collected pseudo seeds were washed with 70% ethanol and Tween 20 solution under tap water in the lab (Fig. 1f). Under the Laminar air flow cabinet, the pseudo seeds were sterilized with 0.2% mercuric chloride and 0.1% bavistin for 2 min. Finally, autoclaved distilled water was used to rinse the pseudo seeds three times. For haploid embryo identification in the pseudo seed, the surface-sterilized pseudo seeds were dissected (Fig. 1g) using forceps under a laminar airflow chamber. Another method was also followed for haploid embryo identification which is placing the pseudo seeds against the light source and observing from the opposite direction. The dark-colour haploid embryo was observed floating inside the 2, 4-D solution (Fig. 1h). The pseudo seed formation frequency and embryo formation frequency was calculated as:

$$\text{Pseudo Seed Formation Frequency (\%)} = \frac{\text{Total no. of pseudo seeds formed}}{\text{Total no. of florets pollinated}} \times 100$$

$$\text{Embryo Formation Frequency (\%)} = \frac{\text{Total no. of haploid embryos formed}}{\text{Total no. of pseudo seeds formed}} \times 100$$

Temperature and relative humidity during pollination were recorded daily from

information provided by the Department of Meteorology, Lovely Professional University, because the number of haploid wheat embryos produced by the wheat × maize crossing system is known to be influenced by seasonal effects. The average temperature during the experiment was 17.6- 26.43°C.

RESULTS AND DISCUSSION

The pollinated wheat spikelets were subjected to three different treatments with 100 ppm 2,4 D solution. In the first treatment (T_1), 2,4 D was injected into plants 24 h after pollination. The pseudo seed formation frequency ranged from 57.97 to 90.24%, and the embryo formation frequency ranged from 10.47 to 15.41%. The highest pseudoseed and embryo formation frequencies were recorded for genotype WH 1105 (90.24 and 15.41%, respectively).

In the second treatment (T_2), 2,4D was injected into plants at 24 and 48 h after pollination. The pseudo seed formation frequency ranged from 77.78 to 94.79% and the embryo formation frequency ranged from 13.04 to 29.31%. The highest pseudo seed formation frequency was recorded for the genotype HD 2967 (94.79%), and embryo formation frequency was 29.31% for genotype WH 711 (Table 1).

The 2,4D inject in plants 24, 48 and 72 h after pollination in the third treatment (T_3). The pseudo seed formation frequency ranged from 75 to 95.12%, and the embryo formation frequency ranged from 17.54 to 47.27%. The highest pseudo seed formation frequency was recorded for the genotype PBW 677 (95.12%) and embryo formation frequency in genotype WH 1105 (47.27%). Similar results were reported in *durum* wheat x maize by Ltifi *et al.* (2019) and oat x maize by Juzon *et al.* (2022). In the control (T_0) without any application of 2,4D no embryo was formed in any of the nine genotypes (Table 2).

Table 1. Effect of 2, 4-D application on number of florets pollinated (NFP), number of pseudo seed formed (NPF), number of embryo formed (NEF), pseudo seed formation frequency (PFF) and embryo formation frequency (EFF) using treatment (T_1) and treatment 2 (T_2)

Treatment (T_1)						Treatment (T_2)					
Genotypes	NFP	NPF	NEF	PFF (%)	EFF (%)	Genotypes	NFP	NPF	NEF	PFF (%)	EFF (%)
HD 2851	131	106	15	80.91	14.61	HD 2851	127	102	13	80.3	13.21
HD 2967	115	93	13	80.68	14.08	HD 2967	111	105	16	94.79	15.38
HD 3086	132	105	14	79.31	13.04	HD 3086	135	105	15	77.78	14.29
PBW 502	128	108	13	84	11.9	PBW 502	121	109	14	90.2	13.04
PBW 550	121	110	13	90.6	11.87	PBW 550	115	92	13	79.69	13.73
PBW 677	134	115	12	86	10.47	PBW 677	149	135	21	90.83	15.6
UNNAT 343	146	85	13	57.97	15	UNNAT 343	142	112	18	79.17	15.79
WH 1105	144	130	20	90.24	15.41	WH 1105	137	128	31	93.08	24.32
WH 711	130	85	10	65.35	12.05	WH 711	122	108	32	88.78	29.31

Table 2. Effect of 2, 4-D application on number of florets pollinated (NFP), number of pseudo seeds formed (NPF), number of embryo formed (NEF), pseudo seed formation frequency (PFF) and embryo formation frequency (EFF) using treatment 2 (T_3) and Control (T_0)

Treatment 3 (T_3)						Control (T_0)					
Genotypes	NFP	NPF	NEF	PFF (%)	EFF (%)	Genotypes	NFP	NPF	NEF	PFF (%)	EFF (%)
HD 2851	121	107	24	88.54	22.35	HD 2851	129	0	0	0	0
HD 2967	114	105	23	92.16	22.34	HD 2967	101	0	0	0	0
HD 3086	128	116	24	90.7	20.26	HD 3086	124	0	0	0	0
PBW 502	119	110	32	92.41	28.77	PBW 502	120	0	0	0	0
PBW 550	117	106	25	90.34	23.53	PBW 550	111	0	0	0	0
PBW 677	136	129	40	95.12	30.77	PBW 677	141	0	0	0	0
UNNAT 343	150	113	20	75	17.54	UNNAT 343	136	0	0	0	0
WH 1105	128	110	52	85.94	47.27	WH 1105	141	0	0	0	0
WH 711	138	123	31	87.5	25.4	WH 711	128	0	0	0	0

Table 3. Analysis of variance for embryo formation frequency

Source of variation	d. f.	Sum of squares	Mean squares	F-Calculated	Significance
Genotypes	8.00	435.05	54.38	1.87	0.136630
Treatment	2.00	838.80	419.40	14.41	0.000260
Error	16.00	465.57	29.10		
Total	26.00	1739.42			
S. E. (d)	2.54				
C.V.	28.48				
Mean, Standard error and C. D.					
Treatment		Mean		S. E.	
1 dose		13.16		0.57	
2 doses		17.19		1.90	
3 doses		26.47		2.93	
C. D.		5.44			
S. E. (m)		1.80			
S. E. (d)		2.54			
C.V.		28.48			

Analysis of variance showed that significant differences existed among the three treatments for their effects on EFF in bread wheat. However, the genotypes of bread wheat had no effect on the EFF. Among the treatments, the third treatment, that is, application of 2,4-D three times at 24 h intervals was significantly better in terms of EFF, with an average of 25.36% (Table 3). Based on the observations recorded, the highest embryo formation frequency was observed in the third treatment, in which three doses of 2, 4-D were injected 24, 48 and 72 h after pollination with maize. All wheat varieties developed embryos after being treated with 2,4-D, with frequencies ranging from 5.41 to 30.77% among wheat genotypes.

CONCLUSION

The induction of haploid embryos in bread wheat following wide hybridization with maize is a complex process influenced by various factors, including the dosage of the plant growth regulator 2,4-dichlorophenoxyacetic acid (2,4-D). The dosage of 2,4-D application in which three doses of 2, 4-D were injected after 24, 48 and 72 h gave the highest embryo formation frequency in bread wheat cultivars after pollination with maize.

REFERENCES

Badiyal, A., Chaudhary, H. K., Jamwal, N. S., Bhatt, A. K. and Hussain, W. (2016). Comparative assessment of different auxin analogues on haploid induction in triticale× wheat derived backcross generations. *Agric. Res. J.* **53**: 157-168.

Chaudhary, H. K., Badiyala, A. and Jamwal, N. S. (2015). New frontiers in doubled haploidy breeding in wheat. *Agric. Res. J.* **52**: 01-12.

Chaudhary, H. K., Sharma, P., Manoj, N. V. and Singh, K. (2019). New frontiers in chromosome elimination-mediated doubled haploidy breeding: Focus on speed breeding in bread and *durum* wheat. *Ind. J. Gen. Plant Breed.* **79**: 254-263.

Gupta, V., Kumar, S., Singroha, G., Mishra, C. N., Kumar, R. and Tiwari, V. (2016). Induction of haploids in wheat using wheat x maize system of chromosome elimination. *J. Wheat Res.* **8**: 43-48.

Juzon, K., Warchol, M., Dziurka, K., Czyczylo-Mysza, I. M., Marcinska, I. and Skrzypek, E. (2022). The effect of 2, 4-dichlorophenoxyacetic acid on the production of oat (*Avena sativa* L.) doubled haploid lines through wide hybridization. *Peer J.* **10**: e12854.

Khan, H., Bhardwaj, S. C., Gangwar, O. P., Prasad, P. and Rathore, R. (2017). Efficiency of double haploid production in wheat through wide hybridization and embryo rescue. *Ind. J. Gen. Plant Breed.* **7**: 428-430.

Ltifi, A., Sahli, A. and Haouala, F. (2019). Study of some factors influencing the production of durum wheat haploids by cross wheat× maize. *Int. J. Adv. Agric. Sci. Tech.* **6**: 17-23.

Mahato, A. and Chaudhary, H. K. (2015). Relative efficiency of maize and *Imperata cylindrica* for haploid induction in *Triticum durum* following chromosome elimination-

mediated approach of doubled haploid breeding. *Plant Breed*. **134**: 379-383.

Mukai, Y., Okamoto, G., Kiryu, S., Takemoto, S., Sharma, S. K., Suzuki, G. and Yamamoto, M. (2015). The D-genome plays a critical role in the formation of haploid Aegilopstauschii through *Imperata cylindrica* mediated uniparental chromosome elimination. *The Nucleus* **58**: 199-206.

Patial, M., Chaudhary, H. K., Sharma, N., Sundaresha, S., Kapoor, R., Pal, D. and Kumar, J. (2021). Effect of different *in vitro* and *in vivo* variables on the efficiency of doubled haploid production in *Triticum aestivum* L. using *Imperata cylindrica*-mediated chromosome elimination technique. *Cereal Res. Comm*. **49**: 133-140.

Santra, M., Wang, H., Seifert, S. and Haley, S. (2017). Doubled haploid laboratory protocol for wheat using wheat-maize wide hybridization. In: *Wheat Biotechnology. Methods in Molecular Biology,* vol. **1679**, Bhalla, P. and Singh, M. (eds.) Humana Press, New York, NY.

Srivastava, P. and Bains, N. S. (2018). Accelerated wheat breeding: Doubled haploids and rapid generation advance. In: *Biotechnologies of Crop Improvement,* Vol. 1, Gosal, S. and Wani, S. (eds.) Springer, Cham.

Watts, A., Kumar, V., Raipuria, R. K. and Bhattacharya, R. C. (2018). *In vivo* haploid productionin crop plants: Methods and challenges. *Plant Mol. Biol. Rep*. **36**: 685-694.

Wedzony, M., Zur, I., Krzewska, M., Dubas, E., Szechynska-Hebda, M. and Wasek, I. (2015). Doubled haploids in triticale. In: *Triticale,* Eudes, F. (ed.). Springer, Cham.