Indian J. Genet., 77(3): 428-430 (2017)
DOI: 10.5958/0975-6906.2017.00059.1

Short Communication

Efficiency of double haploid production in wheat through wide hybridization and embryo rescue

Hanif Khan, S. C. Bhardwaj*, O. P. Gangwar, Pramod Prasad and Ruchi Rathore

ICAR-Indian Institute of Wheat and Barley Research, Regional Station, Flowerdale, Shimla 171 002, H.P.

(Received: December 2016; Revised: May 2017; Accepted: June 2017)

Abstract

The effect of haploid induction in wheat F_1s by *Zea mays*, *Imperata cylindrica* and growth environments was investigated. Doubled Haploid (DH) plant production via maize and *I. cylindrica* technique from field grown LWH x VL616 F_1 plants was equally efficient. In second environment, four F_1 hybrids viz. LWH x VL616, HPW266 x *Yr15* (CN25087), HS490 x HI1563 and Local Red x WHD938 were grown in greenhouse conditions and pollinated with maize. Efficiency of DH production was considerably high from greenhouse grown F_1s as compared to field grown F_1s. We also report an efficient protocol for DH production in bread wheat.

Key words: Wheat, doubled haploid, embryo rescue, *Zea mays*, *I. cylindrica*

In conventional breeding and genetic analysis isolation of homozygous breeding lines/population is possible, only if breeding materials are permitted to several cycles of inbreeding and/or selection. This phase of breeding is the most tedious, time consuming and expensive to crop breeding programmes, which significantly delays the cultivar development processes. The production of haploid plants from hybrids, followed by chromosome doubling, provides the wheat breeder a tool for accelerating the process of true breeding line development (Henry and De Buyser 1990). Doubled haploids (DH) were earlier produced by anther culture and using wide hybridization with *Hordeum bulbosum* L. (Barclay 1975) and *Zea mays* L. (Laurie and Bennett 1986). Recently, wheat x *Imperata cylindrica* has also been reported to be more efficient in haploid induction in bread wheat (Chaudhary et al. 2005). Although it is considered very innovative technique for faster breeding and genetic analysis, the low efficiency of development of DH populations has limited harnessing of this technique.

The present study was designed to find out relative efficiency of maize and *I. cylindrica* induced haploid production in wheat F_1 crosses. Four F_1 hybrids of wheat crosses; LWH/VL616, HPW266/*Yr15*, HS490/HI1563 and Local Red/WHD938 (durum cross) produced after hybridization during off-season of 2015. The parental material was staggered sown with a 15 day interval. During winter-spring 2015-16, LWH/VL616 was raised in field for doubled haploid production. In the off-season 2016, all four F_1's *viz*., LWH/VL616, HPW266/*Yr15*(CN25087), HS490/HI1563 and Local Red/WHD938 were raised under controlled conditions in greenhouse for DH production. A local composite variety of maize and a perennial winter grass *I. cylindrica* used as the pollen sources. The wide hybridization programme using the two pollen sources *i.e*. maize and *I. cylindrica* was carried out following haploid induction protocol given by Laurie and Bennett (1986) and Chaudhary et al. (2005) during March-May, 2016 and using maize during July-Oct, 2016. During off-season the pollen of *I. cylindrica* was not available due to its photo-period sensitivity. Wheat plants at proper stage were emasculated and three to five days later pollinated with maize and *I. cylindrica pollen*. 2,4-D (125ppm) was applied in each floret (a drop of ~100µl) 24h after pollination. The immature embryos were rescued at 18-20 days after pollination

*Corresponding author's e-mail: scbfdl@hotmail.com
Published by the Indian Society of Genetics & Plant Breeding, F2, First Floor, NASC Complex, PB#11312, IARI, New Delhi 110 012
Online management by indianjournals.com; http://epubs.icar.org.in/journal/index.php/IJGPB

and transferred to MS medium supplemented with 1mg kinetin/L medium. The rescued embryos were kept at 4-6°C in refrigerator for 48h, subsequently incubated in dark at 20±2°C for two days for regeneration. Afterwards, embryos were grown *in-vitro* in the growth room at 20±2°C with 14h light/day regime for 3-5 weeks until they developed roots and shoots. The regenerated plantlets were transferred after 20-25 days to root trainer block with 12 cells of 300cc size, each cell containing sand, coco-peat and vermiculite mixture and were hardened for about two weeks in a growth room. At 2-3 leaf stage the haploid plants were subjected to colchicine treatment (aqueous solution with 0.075% colchicine and 2.0% DMSO) for 4h at room temperature. Colchicine treated plants were transplanted into 10cm pots initially kept in the same growth room for one week and subsequently transferred to greenhouse maintained at temperature range of 15-25°C and 14h day length to complete the growth cycle. The evaluated traits were statistically analyzed making proportion comparisons by the Z means test.

In the first season, a total of 1640 and 1255 florets were used for pollination with maize and *I. cylindrica*, respectively (Table 1). The proportion of caryopsis developed was significantly more in the cross with *I. cylindrica* as compared to maize. However, the proportion of embryo regenerated from the recovered embryos and the doubled haploid plants produced from regenerated embryos did not differ significantly for maize and *I. cylindrica*. The results are in agreement with Chaudhary et al. 2005. However, *I. cylindrica* cannot be used as pollinator for chromosome elimination throughout the year. During off-season 2016 with the F_1 plants raised in greenhouse, the proportions differed significantly for four crosses (Table 2). The percentage of caryopses developed ranged from 68.9 (Local Red/WHD938) to 91.0 (HS490/HI1563). The corresponding percentage of embryos recovered ranged from 5.9 in Local Red/WHD938 to 22.8 in LWH/VL616. The percentage of embryos regenerated ranged from 39.3 (Local Red/WHD938) to 54.9 (LWH/VL616). The doubled haploids from regenerated embryos were highest in LWH/VL616 (69.9%) and lowest in Local Red/WHD938 (54.5%). The same ranking was achieved with the range of 0.86 to 7.64 when the percentage of doubled haploids was calculated from total number of florets pollinated. We obtained 349 haploid and 233 DH plants from all four F_1 cross combinations.

DH production rate was low when the F_1 wheat plants were grown and pollinated in the open field where day and night temperature fluctuation was high. The

Table 1. Relative efficiency of *Zea mays* and *I. cylindrica* with respect to caryopses formation, embryo recovery, regeneration and doubled haploid production during spring season 2016 from F_1 of LWH/VL616 grown in field

Pollinator	No. of florets pollinated	Caryopses developed		Embryos recovered		Embryo regeneration		Doubled haploid plants		
		n	%	n	%	n	%	n	%#	%$
Maize	1640	1334	81.3[a]	59	4.42	31	52.5	28	47.5	1.70
I. cylindrica	1255	1163	92.7[b]	52	4.47	32	61.5	29	55.8	2.49
Total/mean	2895	2497	86.3	111	4.45	63	56.8	57	90.5	1.97

Table 2. Relative efficiency of doubled haploids production by maize pollination in four F_1s grown under greenhouse conditions during off season 2016

Pollinator	No. of florets pollinated	Caryopses developed		Embryos recovered		Embryo regeneration		Doubled haploid plants		
		n	%	n	%	n	%	n	%#	%$
LWH/VL616	1230	1078	87.6	246	22.8[ab]	135	54.9[ab]	94	69.6[a]	7.64[a]
HPW266/Yr15	1432	1285	89.7	196	15.3[a]	89	45.4[a]	57	64.0[b]	3.98[b]
HS490/HI1563	1635	1488	91.0	208	14.0[b]	103	49.5[b]	70	68.0[b]	4.28[c]
LR/WHD938	1370	944	68.9	56	5.9[c]	22	39.3[c]	12	54.5[c]	0.86[d]
Total/mean	5667	4795	88.6	706	14.7	349	49.4	233	66.7	4.11

Percentages bearing different letters differ significantly at P=0.05 (z-test), #: percent calculated from regenerated embryos; $ = percent calculated from no. of florets pollinated; n = number

comparison of data of two environments (Table 1 and Table 2) shows that recovery percentage of haploid embryos was higher under controlled environment of greenhouse. A large number of embryos exhibited very low germination response. This might be attributed partly to poor differentiation of the embryos which led to the development of poorly formed plants. Durum wheat cross Local Red/WHD938 was least responsive for haploid induction. The main criterion of the effectiveness of DH technique is the number of green fertile DH plants produced (Xynias et al. 2014). Further efforts are on to verify the effect of the stress conditions to which haploid embryos are exposed *in vivo* within first two weeks after hybridization with pollinators. A great amount of variation in embryo regeneration between different F_1s in maize pollinated spikes may be due to genotypic interaction present between wheat and *Z. mays*. The results are in agreement to the study conducted by Sood et al. (2003) and Xynias et al. (2014) which revealed that the genotypic interaction present between wheat and *Z. mays* significantly influences the recovery of haploid embryo formation and plant regeneration. DH population of LWH/VL616 will be used for identification of slow rusting genes for leaf and stripe rusts. More DH plants are being generated from the cross HPW266/*Yr15*(CN25087) and HS490/HI1563 for use in wheat breeding programme. With the efficiency achieved in the present investigations, growing of 40 to 60 F_1 seeds would be required to get 200 to 300 fertile doubled haploid plants.

Authors' contribution

Conceptualization of research (HK, SCB); Designing of the experiments (HK, SCB); Contribution of experimental materials (HK, SCB, OPG, PP); Execution of field/lab experiments and data collection (HK, RR, OPG, PP); Analysis of data and interpretation (HK, SCB); Preparation of manuscript (HK, SCB, OPG, PP).

Declaration

The authors declare no conflict of interest.

References

Barclay I. R. 1975. High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature, **256**: 410-411.

Chaudhary H. K., Sethi G. S., Singh S., Pratap A. and Sharma S. 2005. Efficient haploid induction in wheat by using pollen of *Imperata cylindrica*. Plant Breed., **124**: 96-98.

Henry Y. and De Buyser J. 1990. Wheat anther culture: Agronomic performance of doubled haploid lines and the release of a new variety Florin. In: Biotechnology in Agriculture and Forestry: Wheat, (Eds. Y.P.S. Bajaj), vol. 13, 285-352. Springer-Verlag, Heidelberg, Germany.

Laurie D. A. and Bennett M. D. 1986. Wheat × maize hybridization. Can. J. Genet. Cytol., **28**: 313-316.

Sood S., Dhawan R., Singh K. and Bains N.S. 2003. Development of novel chromosome doubling strategies for wheat × maize system of wheat haploid production. Plant Breed., **122**: 493-496, 2003.

Xynias I., Koufalis A., Gouli-Vavdinoudi E. and Roupakia D. 2014. Factors affecting doubled haploid plant production via maize technique in bread wheat. Acta Biologica Cracoviensia series Botanica, **56**(2): 67-73.