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An Attempt to Produce Oat Haploids Using Oat X Maize Hybridization Technique

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ABSTRACT

The aim of the study was to develop oat haploids through crossing between oat as a female and maize as male parent followed by embryo rescue. Four oats genotypes of diverse origin along with popular fodder maize variety J-1006 were crossed at Forage Research farm, PAU, Ludhiana. Various factors influencing the growth and development of crosses like oat genotype, used as female, method of emasculation and time of pollination, type of growth regulators, their concentration and time of application were investigated. The frequency of embryo formation varied significantly between the genotypes studied. The maximum frequency was observed in genotype IC-372489 (37.50) followed by EC-528919 (14.66), IC-372415 (0.65) and IC-372510 (0.29). Embryo production is strongly influenced by the time interval between emasculation and pollination. The auxins tested have significant influence on the number of enlarged ovaries and embryos. Ovaries treated with Dicamba were generally bigger in size than the ovaries treated with 2,4-D. A higher number of embryos were obtained when oat x maize florets were treated with auxins (both 2,4-D and Dicamba) after 2 days of pollination than after 1 and 3 days.

Key words: *Haploids, Oat x Maize, Pollination, Embryo rescue, Auxins*

INTRODUCTION

Oat (*Avena sativa* L.) is one of the major cereal crops in the world and belongs to the *Poaceae* family. It is ranked sixth after wheat, maize, rice, barley and sorghum[12]. Production of doubled haploid (DH) in cereals is becoming increasingly important in crop breeding programs, but the methods currently applied still remain inefficient. Haploids are plants that contain the gametic number of chromosomes (n). In nature, the spontaneous emergence of haploids in higher plants is very rare (a frequency of 0.001–0.01 %). There are several techniques available by which haploids are produced in vivo and in vitro by androgenesis, gynogenesis, interspecific or intergeneric crosses, followed by chromosome elimination, or pollination with irradiated pollen[3,4,8].

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Haploidization is one of the methods of creating new cultivars that allows decreasing the production time of new cultivars even to one vegetative season[10]. They have many applications in basic plant research, such as cytogenetics, crop evolution, mutagenesis induction, genetic transformation and in breeding programs[6].

Wide crossing with maize is the main and most-effective method for producing oat double haploid (DH) lines[10,18,20]. The wide crossing method (chromosomes elimination) seems to be more effective than androgenesis because there are no albino plants among the regenerants[14]. Currently, doubled haploid (DH) technology for practical breeding is successful in crops like rapeseed, barley and wheat[5]. However, oat still remains recalcitrant to those protocols by which an efficient DH production can be created[10,19]. There are certain barriers that operates at pre and post fertilization phases which hinders the effectiveness of wide crossing hybridization. In wide crossing of oat with maize, endosperm usually fails to develop in seeds, embryos must be rescued transferred to an artificial culture medium to allow them to grow under optimum culture conditions[17]. Selection of regeneration medium is the most important step for the continued growth of the embryo[2]. The efficiency of DH production in wheat is about 20% haploid per florets as compared to oats which is less than one per cent[11,14,15]. So, in our investigation, we were focused on examining critical factors of growth and development which includes pre and post fertilization changes to bring out an efficient system of production of double haploids in oats.

MATERIAL AND METHODS

a) Plant material and growing conditions

Plant material comprising of four oats genotypes namely IC-372510, IC-372415, EC-528919 and IC-372489 used as female parents to cross with popular fodder maize variety J-1006 which has good pollen production capacity. Oat genotype IC-372415 was very early in flowering followed by IC-372415. Similarly, Oat genotype IC-372489 was very late in flowering followed by EC-528919 which allows maximum time period for oat x maize hybridization programme. Oats and Maize genotypes were grown in normal field growing season conditions in field.

b) Method of hybridization

Florets from all the four oat genotypes to be used as female parent were emasculated using fine forceps, needle and scissor before anthesis. Only the florets from middle part of the oat panicle were used for the purpose whereas lower and upper most florets were removed using scissor being too mature and young florets respectively. Secondary and territory florets were also discarded. Only the primary florets of oat spikelets were emasculated as bold seed is formed only from these florets under natural conditions. Fresh pollen grains were collected from male parent (maize variety J 1006) at every 30-45 minutes' intervals on a sheet of paper or petri dish. Old maize pollen was discarded by shaking the tassels prior to pollen collection. Pollination was performed after 1-2 days of emasculation with freshly collected maize pollen grains which were applied to the emasculated oat florets with fine camel brush. Due care was taken to minimize injury to the floral parts, particularly the stigma while applying pollens. After pollination the oats panicles were covered with butter paper bags Fig. (1a – 1c).

c) Application of auxins

After 1, 2 and 3 days of pollination, drops of auxin growth regulators viz; 2,4-D and Dicamba were placed on the floret pistils using a syringe. The concentration of 2,4-D was set at 100 ppm whereas Dicamba was applied at two concentrations i.e. 100 ppm and 150 ppm to check the effects of higher concentration of auxins on frequency of embryos recovered if any.

d) Embryo rescue

i) Procedure of sterilization of oat florets before culturing

Three weeks after pollination with maize pollens the oat florets looking healthy, swollen and bold were excised from panicle and washed in bavistin (fungicide) solution (3% w/v) for 10 minutes and then caryopses were isolated. These caryopses were washed in series of chemicals as followed (Fig. 1d – 1g):

With 0.1% mercuric chloride for 30 seconds followed by 1-minute washing using

95% ethanol, washing in cephotaxime (anti-bacterial) for 2 minutes and then two time washing in sterilized water. Healthy Caryopses were dissected using autoclaved blade and healthy looking embryos were transferred into test tube containing 190-2 medium (Table 1). All these steps of culturing were performed under laminar air flow channel in laboratory.

RESULTS AND DISCUSSION

a) Effect of genotype on embryos production

In total of 4715 florets were pollinated with maize pollen for oat genotype IC-372510, IC-372415, EC-528919 and IC-372489. After 3 to 4 weeks of pollination a total of 1668 healthy caryopses could be recovered culture 83 embryos are obtained from all genotypes. Embryos are varied in size and were poorly developed as compared to the naturally self-pollinated oat embryos. Many of the caryopses did not have endosperm, which is mainly responsible for nourishing the developing embryos. The frequency of embryo formation varied significantly between the genotypes studied. The maximum frequency was observed in genotype IC-372489 (37.50) followed by EC-528919 (14.66), IC-372415 (0.65) and IC-372510 (0.29). In the study of Rines *et al*[16]. (2009), four genotypes tested produced on average 7.2 % of haploid embryos. Sidhu *et al*[17]. (2006) examined 5 oat genotypes for haploid production via pollination by maize and obtained 0.8–6.7 % of haploid embryos and 0.8–1.5 % of haploid plants per emasculated florets. Similar rate of haploid embryo formation (7.8 %) was recorded by Marcin´ska *et al*[10]. The frequency of embryo formation varied strongly between the genotypes studied. Marsincka *et al*[10]., obtained 128 haploid embryos from1576 florets, where it ranges from 23.9% to 2.9% for genotype 82072 and 81350 respectively. According to Bridgen[1], interspecific crosses frequently lead to poor development of the endosperm or lack thereof. Lack of endosperm can cause embryo abortion. This problem may be overcome by aseptic culture of the embryo in nutrient medium which provides nutrients needed for embryo development[9] which was in line with our study.

b) I**nfluence of time between emasculation and pollination on embryo production**

Embryo production is strongly influenced by the time interval between emasculation and pollination. Florets that had pollinated 1 and 2 days after emasculation, produces 1.4% and 4.5% embryo per florets respectively.

c) Influence of auxin on ovaries enlargement and embryo production

Two growth regulators namely 2-4-D and Dicamba were used in the study. There were 290 florets treated with 2-4-D @ 100 ppm, out of which 117 (40.17) enlarged ovaries were obtained. Growth regulator Dicamba was used at different concentrations to check its effect on embryo production and the enlargement of ovaries. Total of 4309 florets were treated @ 100 ppm and 116 florets were treated @ 150 ppm solution out of which 1511(35.06) and 40 (34.48) enlarged ovaries were obtained respectively. Both the growth regulators showed significant effect on the enlargement of ovaries. Ovaries treated with Dicamba were generally bigger in size than the ovaries treated with 2,4-D. From the enlarged ovaries 18 embryos after 2,4-D treatment were obtained. Similarly, 50 and 15 embryos were obtained from the enlarged ovaries treated @ 100 ppm and 150 ppm of Dicamba, respectively.

d) Influence of time between pollination and application of growth regulators and type of regeneration medium for embryo germination

Perusal of the data (not given) exhibited that a higher number of embryos were obtained when oat x maize florets were treated with auxins (both 2,4-D and Dicamba) after 2 days of pollination than after 1 and 3 days.

e) Type of medium for embryo germination

Embryos were cultured on 190-2 medium (Table 1). After three weeks of culturing, 23.7% of all embryos were germinated, from which 10% are developed into green plants. There were three albino plants recovered in this study.

Overall, our results were corroborated by the results obtained by Nowakowska *et al*[13]., Marcin´ska *et al*[10]., Ishii *et al*[7]., and Sidhu *et al*[17]. In conclusion, the oat genotype is the main deciding factor on the frequency of

haploid embryo formation and the efficiency of regenerating plants through pollination with maize. Pollination on the 2nd day after emasculation of the florets was the most effective compared to the 1st day. The increased number of enlarged ovaries following the Dicamba and 2.4-D treatments was similar and there were no statistically significant differences in the production of embryos. It was shown that treatment of florets by auxin on the 2nd day after pollination was more effective than after 1 or 3 days. These investigations have a novel component, because new impacts like time of pollination after emasculation, time between pollination and auxin treatment on haploid embryos and the frequency of plant regeneration have not been tested until now.

We are currently regenerating and hardening the plants with molecular probes to test for the presence of maize DNA. In conclusion, it is clear that genotype, application of growth regulators post-pollination and temperature significantly affect the production of haploid embryos and plants obtained from oat · maize hybridization. However, further technique modifications to improve the efficiency of embryo production will be required before this technique can be applied to breeding programme.

Table 1: Chemical composition of 190-2 medium used for embryo culture

Stock no.	Strength of stock	Constituent salts	Quantity (g)	Use of stock ($ml l^{-1}$)	Actual amount in the culture medium ($mg l^{-1}$)
			2 litre		
	20 X	KNO_3	80g	**50**	2000
		H_3BO_3	0.12g		3.0
		$MnSO_4.H_2O$	0.32g		8.0
		$ZnSO_4.7H_2O$	0.12g		3.0
1		KI	0.02g		0.5
		$CuSO_4.5H_2O$	0.001g		0.025
		$NaMoO_4.H_2O$	0.002g		0.005
		$CoCl_2.6H_2O$	0.001g		0.025
		$NH_4H_2PO_4$	15.2g		380
		K_2SO_4	28.0g		700
2			**½ litre**		
	50 X	$Cacl_2.2H_2O$	3.5g	**20**	140
3			**½ litre**		
	50X	$MgSO_4.7H_2O$	5.0g	**20**	200
			1 litre		
4	**100 X**	$FeSO_4.7H_2O$	2.78g	**10**	27.8
		$NaEDTA.2H_2O$	3.73g		37.3
			I litre		
	100 X	Thiamime HCl	0.2g	**10**	2.0
		Pyridoxine HCl	0.05g		0.5
		Nicotinic acid	0.05g		0.5
5		Glycine	0.2g		2.0
		L-cystine	5.0g		50
		Ethephon	2 ml		0.2ml
		*Myo-inositol			500mg
		*Maltose			105g
		clerigel			3.5g

*Myo-inositol and maltose were added in solid form while preparing the medium.

Table 2: Data on Oat x maize crossing, caryopses recovered and embryo developed

Genotype	Number of oat florets pollinated	Caryopsis formed	Embryos developed
IC-372510	3559	1232	36
IC-372415	854	321	21
EC-528919	186	75	11
IC-372489	116	40	15
Total	**4715**	**1668**	**83**
Percent recovery	**--**	**35.4**	**1.8**

Fig. 1a

Fig. 1b

Fig. 1c

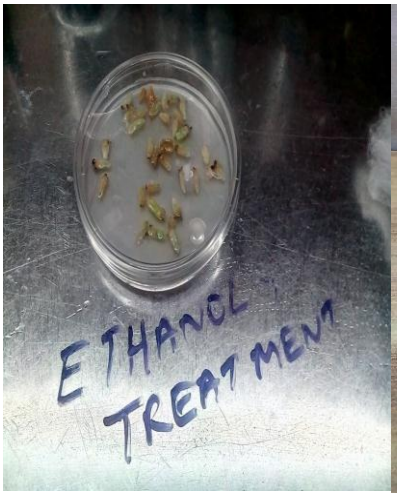

Fig. 1d

Fig. 1e

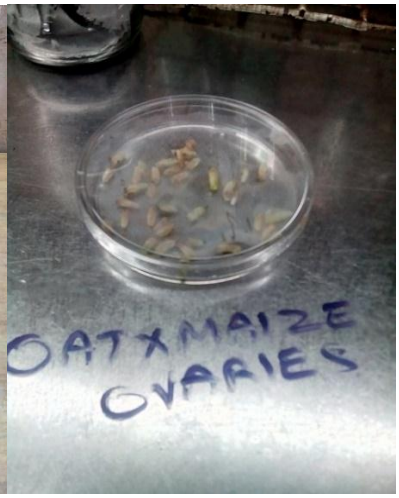

Fig. 1f

Fig. 1g

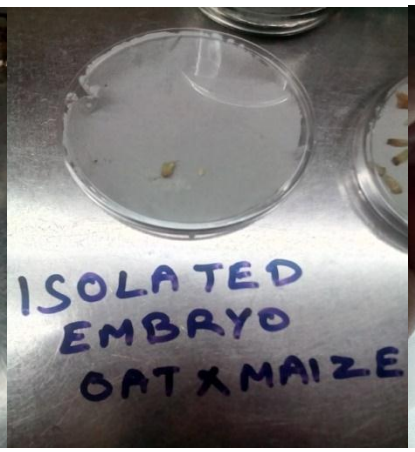

Fig. 1h

Fig. 1i

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