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RESEARCH ARTICLE

Effect of hormonal treatments on haploid formation and *in vitro* haploid regeneration in wheat x maize system

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SUMMARY

Haploids are useful for basic studies on inter-genomic relationship, molecular studies and in practical breeding. In the present investigation post pollination treatment with different growth regulators and their combinations were analyzed. Twenty nine wheat F_1's and three maize parents were utilized to obtained haploid embryos. Three different hormonal treatments were designed to observe their effect on different parameters in wheat-maize haploid system. Out of three treatments, combination of GA3 and 2,4-D showed highly significant effect on caryopsis formation frequency (64.40), embryo formation frequency (18.84), embryo germination frequency (55.82) and haploid regeneration frequency (36.51). The growth pattern of haploid embryos on nutrient medium was quite different. The differential response of haploid embryos on same nutrient medium might be because of residual effect of different growth regulators.

Key Words : Wheat x maize, Haploid embryo, Hormonal treatment, Residual effect

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Development of pure breeding line/population is the basis of plant breeding programmes. Isolation of homozygous and homogeneous line/ population is possible only if the breeding material is permitted to several cycle of inbreeding and selection. It's a time consuming tedious and expensive method. Dihaploid breeding is an excellent method to create inbred line in shortest possible time. Wheat - maize crossing system was found to be the most promising technique for poly-haploid production in spite of the presence of other technique like pollen culture, microspore culture, ovary and ovule culture. Double haploid (DH) system is a biological tool, which has been widely applied crop improvement of most of the cereal crops (Moieni *et al.*, 1997 and Suenaga, 1991).

The system is developed through haploid production, followed by chromosomal doubling, to produce homozygous plant in a single generation. Haploid production is important for genetic manipulation and crop improvement because it offers a means to rapidly

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advance selected lines to complete homozygosity and increase efficiency of selection in turn reducing the time for cultivar development. There are a lot many factors that affect the success of the technique (Khan and Ahmad, 2011). The role of pre and post fertilization barrier was well documented by different researchers (Kour *et al.*, 2009 and Bhatt and Khanna, 2010). The wheat genotypes as maternal parent and maize genotypes as paternal parent showed significant effect on the embryo formation and other related parameters (Kour *et al.*, 2008). Application of different growth hormones is a key to the success of this method. The role of growth hormones and the method of its application significantly affect the recovery of haploid embryos and its subsequent growth on nutrition medium (Kaushik *et al.*, 2004). The effect of different growth regulators was quite different on developing embryos. The role of 2, 4-D and $AgNO_3$ was reported by many scientists. Almouslem *et al.* (1998) tested effect of different types of hormones at different concentration on the embryo formation. Since these growth regulators have direct effect on embryo development, growth so residual effect of hormones on the growth of embryos on nutrition medium should taken seriously.

In the study the effect of different hormones on haploid formation and residual effects of growth regulators on the growth of embryos was undertaken for which very less reports (Fernanda *et al.*, 2010) were available. Such studies helped the scientists to observe growth and development pattern of haploid embryos that ultimately help in designing suitable growth medium for optimum growth of haploid embryos.

MATERIAL AND METHODS

Plant materials:

Drought tolerant (VL 804, VL 802, UP 2572, PBW 65, PBW 175, WH 730, JOB 666, NI 5439, NP 846, NIAW 34, HI 385, PBN 51, Halna) and drought susceptible (UP 2338, PBW 337, UP 2425) varieties of wheat were crossed to produced F_1's (Table A). These F_1's were used for inter-generic crossing to produce haploid embryos. Maize varieties Pragati, Pearl Pop Corn, New composite were used as pollen parents. Following treatments were given to observe the effect of hormones on the efficiency of inter-generic crosses:

T_1= 100 ppm 2, 4-D + 0.75% colchicine + 2% DMSO injected in the uppermost internode after 24 hr of pollination followed by three sprays of 2, 4-D.

T_2= 300 ppm GA_3 spray first at 24 hr and then at 48 hr after pollination, then apply two more sprays of 2, 4-D at 72 and 96 hr after pollination.

T_3= 100 ppm 2, 4-D spray for 24, 48 and 72 hr after pollination.

The data were transformed by the Arc Sin √x

Table A : wheat crosses used for intergeneric crossing

Sr. No.	Name of cross	Sr. No.	Name of cross
1.	W_1=VL 804 × UP 2425	16.	W_{16}=JOB 666 × UP 2338
2.	W_2=VL 804 × PBW 373	17.	W_{17}=JOB 666 × UP 2425
3.	W_3=VL 802 × UP 2338	18.	W_{18}=NI 5439 × UP 2425
4.	W_4=VL 802 × PBW 373	19.	W_{19}=NI 5439 × UP 2338
5.	W_5=VL 802 × UP 2425	20.	W_{20}=NP 846 × UP 2338
6.	W_6 =UP 2572 × PBW 373	21.	W_{21}= NP 846 × UP 2425
7.	W_7=UP 2572 × UP 2425	22.	W_{22}=NIAW 34×PBW 373
8.	W_8=UP 2572 × UP 2338	23.	W_{23}=NIAW 34 × UP 2425
9.	W_9=PBW 65 × PBW 373	24.	W_{24}=HI 385 × PBW 373
10.	W_{10}=PBW 65 × UP 2425	25.	W_{25}=HI 385 × PBW 373
11.	W_{11}=PBW 65 × UP 2338	26.	W_{26}=PBN 51× UP 2425
12.	W_{12}=PBW 175 × UP 2425	27.	W_{27}=PBN 51× UP 2338
13.	W_{13}=PBW 175×PBW 373	28.	W_{28}=Halna × UP 2425
14.	W_{14}=WH 370 × UP 2425	29.	W_{29}=Halna × PBW 373
15.	W_{15}=WH 370 × UP 2338		

Table B : Analysis of variance for Randomized Block Design

Source of variation	d.f.	S.S.	M.S.	F
Replication	(r - 1)	SS_r	M_r	M_r / M_e
Treatment	(t - 1)	SS_t	M_t	M_t / M_e
Error	(r - 1) (t - 1)	SS_e	M_e	

where,

G= Grand mean; CF= Correction factor; Ti= Total of i^{th} genotype

T= Total number of treatment; R= Total number of replications of i^{th} treatment; N= Total number of observations

r = Number of replications; g = Number of treatment; M_r = Mean square for replications

M_t = Mean square for treatment; M_e = Mean square for error; $CF= G^2 / n$

$CV= \sqrt{\text{Error MS}} / \text{Grand Mean} \times 100$

For comparison of means:

$LSD_{5\%} = t_{5\%} \times sd$; $Sd = \sqrt{2}\, MS_e / r$

The significance among the genotype mean were tested by 'F' value and wherever the 'F' test was found to be significant, LSD had to be calculated to test the significance between the treatment means. LSD helped in making homogenous groups of treatments according to their significant effect.

transformation. Analysis of variance was done in randomized block design (CD) according to appropriate statistical method (Gomez and Gomez, 1984). It has been presented in Table B.

RESULTS AND DISCUSSION

Analysis of variance table showed that the hormonal treatments were significantly different for their effect on different parameters (Table 1). Values given in the mean separation table showed that all the treatments were significantly different from one another and they show maximum heterogenity in their response to affect all four characters (Table 2).

Treatment 2 had the highest effect on cryopsis formation frequency (64.40), embryo formation frequency (18.84), embryo germination frequency (55.82) and haploid regenration frequency (36.51), followed by treatment 1 with moderate cryopsis formation frequency (63.24), embryo formation frequency (16.64), embryo germination frequency (49.43) and haploid regenration frequency (27.39) and treatment 3 had least values for cryopsis formation frequency (60.98), embryo formation frequency (11.37), embryo germination frequency (36.67) and haploid regenration frequency (14.54). These results indicated that effects of T_1, T_2 and T_3 were significantly different for all the parameters (Fig. 1).

The caryopsis formed after treatment 1 were very small in size as compared to caryopsis in treatment 2 and 3 (Fig. 2 b and c). This may be due to higher dose of 2, 4-D. haploid embryo isolated from such crosses are

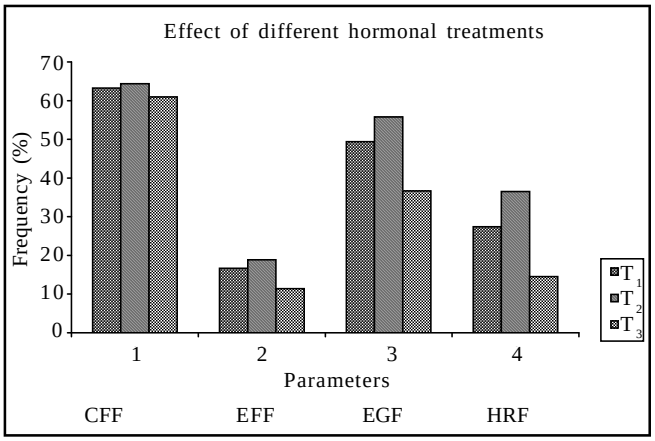

Fig. 1 : Effect of different hormonal treatment on different parameters of wheat x maize crosses

of small in size and have some aberrant callus like structure along with the haploid embryos (Fig. 2 d).

After 14 days of pollination embryo rescue was done on BAP supplemented media to have haploids. In treatment 1 profuse callus growth was observed in the media. As the media was not supplemented with the auxin the profuse growth might be because of residual effect of 2, 4-D. From such callus only shoots were emerged. Multiple shoots were also observed (Fig. 2 i, j) from callus, no roots were observed. The 2,4-D promotes the conversion of embryo into plantlet had also been established (Almouslem *et al.*, 1998). One of the factor that limit the further development of a germinated embryos to a plantlet is deficiency of the embryos in one of their poles for shoot or root induction. In such cases

Table 1 : ANOVA for the effect of hormonal treatments

Source of variation	df	Mean squares			
		CFF	EFF	EGF	HRF
Replication	2.0	0.008	0.026	0.021	0.027
Treatment	2.0	9.091**	44.00**	285.081**	365.659**
Error	4.0	0.023	0.022	0.065	0.008
Total	8.0				
C.D. (P=0.05)		0.341	0.338	0.285	0.066

**indicates significance of value at P=0.01

Table 2 : Mean separation table for effect of hormonal treatments

Treatments	CFF	EFF	EGF	HRF
T_1	63.244 (b)	16.639 (b)	49.429 (b)	27.392 (b)
T_2	64.402 (c)	18.844 (c)	55.817 (c)	36.511 (c)
T_3	60.980 (a)	11.369 (a)	36.671 (a)	14.536 (a)
LSD	0.567	0.563	0.475	0.109
CV	0.240	0.959	0.265	0.112

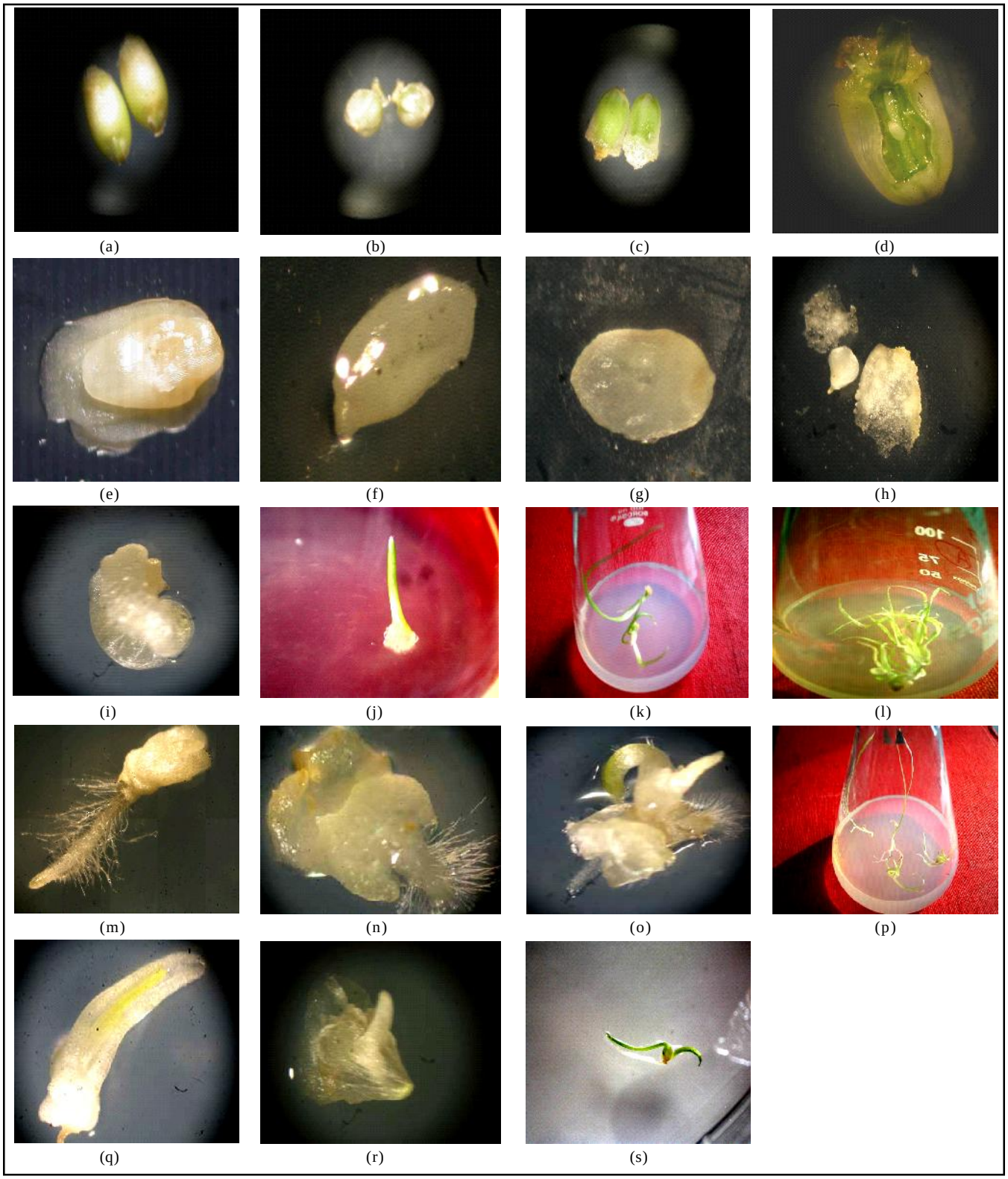

Fig. 2 : Effect of different hormonal treatment on haploid formation by wheat – maize system. Formation of caryopsis in Self (a), treatment 1(b) treatment 2 and 3 (c). Dissected caryopsis having haploid embryos (d). Embryos from Self seed (e), Haploid embryos from wheat –maize system after different hormonal treatment (f, g). Formation of aberrant structure along with the haploid embryo in treatment 1 (h). Figure showing differential growth pattern after treatment 1(i-l), treatment 2 (m-p), treatment 3 (q-s)

meristem is not properly formed (Kaushik *et al.*, 2004; Kammholz *et al.*, 1996, Bains *et al.*, 1995 and Moieni *et al.*, 1997). Plants regenerated from callus were vigorous in their growth and development (Fig. 2 k, l).

Big size heart shaped fully developed embryos were observed in treatment 2. Probably GA_3 and 2,4-D in combination provide better nourishment to developing embryos. GA3 also helped in enlargement and growth of embryos. Such embryo developed into complete plant with well developed root system. There are evidences from several reports that GA_3 only influence cell extension in the presence of auxin which helped in swelling and growth of embryos resulting into a complete plant (Fig.2 m-p). The cumulative effect of hormones *i.e*. 2, 4-D and GA_3 would promote the growth embryos on nutrition medium.

Treatment 3 was the least effective treatment. Only 2, 4-D was provided. Small size, poorly developed embryos with very low frequency were obtained as compared to earlier treatments. Few haploid embryos that germinated could regenerate into a complete plant. The regenerated plants were very weak and could not survive much longer (Fig.2 q-s). In this treatment as well only as single hormone was given in low dose that provide nourishment to the developing embryos but that is not sufficient for proper development of poles for regeneration of embryos in plantlet.

The callus formed from embryos showed shoot proliferation because the media contained low levels of BAP. BAP has been shown to enhance the speed of shoot differentiation in wheat (Kato *et al.*, 1991). For rooting they had to be transferred to a rooting media. Effect of hormonal treatment was also reported by (Almouslem *et al.*, 1998; Sood *et al.*, 2003; Kaushik *et al.*, 2004; Ushiyama *et al.*, 2007 and Fernanda *et al.*, 2010). These studies indicated that hormones plays significant role in deciding the success of the technique. Not only the type of hormone but also the method of application is also matter of concern. The combination of hormones was found better as compare to applying only single hormone. The residual effect of applied hormones had significant effect on the growth of haploid embryos on nutrition medium. These studies help in formulating better combination of hormones to enhance frequency of haploid embryos. By utilizing the information generated by such studies, highly compatible nutrition medium can be prepared for optimum growth of haploid embryo.

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