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Effect of different hormones and methods of hormone application on haploid embryo formation in wheat × maize crosses

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Abstract

Research and utilization of poly-haploids in wheat has been limited because of the low frequency of haploid formation. In haploid production through wheat × maize crossing, application of hormones is crucial to produce embryos. Effect of different hormones and application methods were studied in wheat × maize crosses. The wheat genotypes and methods of hormonal treatments *viz.* spray and tiller injection exhibited highly significant differences for embryo formation. Different hormonal treatments *viz.* 100 ppm 2, 4-D, 100 ppm GA_3 and 100 ppm $AgNO_3$ differ significantly for per cent seed set and embryo formation.

Key words: Wheat × maize hybridization, haploid, chromosome elimination

Introduction

The use of doubled haploids enables homozygosity of wheat lines in a single generation, making it a useful technique for both wheat breeding and genetical studies. Of the various methods available, wheat × maize hybridization as a means of producing wheat polyhaploids is the most efficient technique [1]. Maize is insensitive to Kr alleles of wheat, thus embryo recovery frequencies are high across different wheat genotypes [2, 3]. In addition, the variation induced in doubled haploid lines using the maize system was similar to that of doubled haploids obtained from wheat × *Hordeum bulbosum* L. crosses [4]. The significant features of this method include haploid production in high frequency, in all wheat genotypes, without any albino plants and haploids with only wheat chromosomes. After the wheat stigma is pollinated by maize pollen, cytologically unstable hybrid zygotes (with 21 wheat chromosomes and 10 maize chromosomes) are produced [5]. The maize chromosomes are eliminated after a few cell divisions in the hybrid zygote, forming the embryo with only 21 wheat chromosomes. Normally, the embryo soon aborts; however, exogenous treatment with the synthetic auxin 2, 4-D promotes embryo development until the embryo can be excised and plated onto a synthetic medium for continued growth and plant regeneration [6]. Since the role of hormone application is very important in improvement of fertilization frequency and subsequent growth of embryos in wheat × maize crosses, searching the best method of hormonal treatment for optimization of haploid production was undertaken in the present study.

Materials and methods

The experimental material consisted of four wheat F_1s UP 2113 × UP 2338 (W_1), PBW 396 × UP 2338 (W_2), PBW 396 × UP 2425 (W_3) and UP 2113 × UP 2425 (W_4). One genotype of maize 'Surya' with good pollen shedding ability was taken for inter-generic crosses between wheat and maize. Emasculation of wheat spikes was done by hand using cut glume method. Two days later, when the stigma was feathery and receptive, the pistils were hand pollinated by dusting with pollen from the dehiscing anthers of the male parents and spikes were covered with glassine paper bag to avoid any pollination by undesired foreign pollen.

Data collection and statistical analysis: Fifteen to eighteen days after pollination observations were recorded for percent seed set and embryo formation. Data were transformed using $\sqrt{X}$ and $\sqrt{X}$ + 0.5 transformation for percent seed set and embryo formation, respectively. Statistical analysis was done using two factorial completely randomized design (CRD). Mean data from one spike constituted one replication.

Experiment 1: Effect of different hormones on seed set and embryo formation rates: This experiment was conducted with two wheat F_1s × maize crosses with the following three post-pollination hormonal treatments: (i) 2, 4-D @ 100 ppm (T_1), (ii) GA_3 @ 100 ppm (T_2), (iii) $AgNO_3$ @ 100 ppm (T_3). For each treatment, there were 3 replications.

Experiment 2: Comparison of two methods of hormonal treatment for seed set and embryo formation rates: This experiment was conducted with three wheat F_1s × maize crosses with two methods of hormonal treatment *viz.* (T_1) spray and (T_2) tiller injection. In spray method, 100 ppm 2, 4-D solution was sprayed

on the spikes for three consecutive days after pollination with maize in the evening, while in tiller injection method, 5 ml of 100 ppm 2,4-D was injected in the peduncle of wheat plants for three consequent days after pollination.

Results and discussion

Effect of hormones: In the present study, three hormones *viz.*, 2, 4-D, GA_3 and $AgNO_3$ were sprayed on the pollinated florets. In W_1 × Surya, the per cent seed set ranged from 80-90 per cent, 75-80 per cent and 40-50 per cent with 2, 4-D, GA_3 and $AgNO_3$, respectively. In W_4 × Surya the per cent seed set ranged from 80-85 per cent, 75-80 per cent and 40-45 per cent with 2, 4-D, GA_3 and $AgNO_3$, respectively (range represents three replications not shown in the table). The mean value for per cent seed set and embryo formation as influenced by three different hormones is presented in Table 1. Different hormonal treatments differed significantly for per cent seed set but variance due to wheat genotype and interaction between wheat genotype and hormonal treatments were insignificant (Table 2). Maximum seed set was obtained when 2, 4-D was sprayed and this result was consistent with all wheat genotypes. Analysis of variance revealed that hormonal treatments differ significantly for per cent embryo formation. Variance due to wheat genotypes and interaction between genotype and hormonal treatments were non-significant. Highest embryo formation was recorded when 2, 4-D was applied to the pollinated florets. However, low embryo formation was observed when GA_3 and $AgNO_3$ were sprayed. Laurie and Bennett [5] reported that application of 75 mg/l GA_3 spray reduced the mean frequency of embryo formation in both intact and cut florets. O'Donoughue and Bennett [7] obtained haploids in durum wheat and concluded that the recovery of haploids in durum wheat necessitated the addition of $AgNO_3$ to the 2, 4-D treatment. Almouselm *et al.* [8] reported that in durum wheat, post-pollination treatment with 3 mg/l 2, 4-D and 120-180 mg/l $AgNO_3$ gave the best yield of embryos, whereas 3 mg/l 2, 4-D plus 120 mg/l $AgNO_3$ promoted the conversion of embryos into plantlets.

Table 1. Effect of different hormonal treatments on per cent seed set and embryo formation in various wheat x maize crosses

Wheat genotype	Treatment	Number of florets pollinated	No. of seeds obtained	No. of embryos formed
W_1	T_1	60	52 (86.66)	5 (9.61)
	T_2	60	47 (78.33)	3 (6.38)
	T_3	60	26 (43.33)	1 (3.84)
W_4	T_1	60	50 (83.33)	4 (8.00)
	T_2	60	46 (76.66)	2 (4.34)
	T_3	60	25 (41.66)	0 (0.00)

T_1 = 2, 4-D @ 100 ppm; T_2 = GA_3 @ 100 ppm; T_3 = $AgNO_3$ @ 100 ppm. Values in parenthesis are given in percentage.

Table 2. Analysis of variance for seed set and embryo formation among two wheat F_1s × maize crosses in three treatments

Source of variation	df	Mean sum of squares	
		Seed set	Embryo formation
Wheat genotype (W)	1	0.035	1.51
Treatment (T)	2	12.094**	5.41**
W × T	2	0.012	0.12
Error	12	0.068	0.64
Total	17		
C.V.		3.19	37.51

** Significant at 0.01 level of probability

The use of 2, 4-D appears to be critical in promoting seed set and embryo formation in wheat × maize crosses [9, 3]. Techniques using 2, 4-D treatment include floret culture [9], tiller injection [3], spike spraying [10] and floret treatment [11]. Laurie and Bennett [9] also reported increased embryo recovery (from 0.17-26.5%) when 2, 4-D was applied two days after pollination. Thus, production of embryos in wheat-maize crosses will be possible only by the application of hormones to support further growth of the fertilized egg cell and the embryo produced.

Effect of treatment method: Analysis of variance showed that wheat genotypes, treatments and interaction between wheat genotypes, and treatments did not differ significantly for seed set (Table 3). The mean value for per cent seed set and embryo formation as influenced by two methods of treatments is presented in Table 4. For the first treatment i.e. spray (T_1) the per cent seed set ranged from 83.33 to 86.66 and embryo formation ranged from 3.00 to 5.76 among all wheat genotypes. For the second treatment i.e. tiller injection (T_2) the per cent seed set ranged from 85.00 to 85.83 and embryo formation ranged from 8.82 to 10.78 across all wheat genotypes. According to the results obtained from the analysis of variance for embryo formation,

Table 3. Analysis of variance for different methods of hormonal treatment on per cent seed set and embryo formation in wheat × maize crosses

Source of variation	df	Mean sum of squares	
		Seed set	Embryo formation
Wheat genotype (W)	2	0.0125	0.35
Treatment (T)	1	0.0042	4.80"
W × T	2	0.0163	0.04
Error	12	0.0288	0.07
Total	17		
C.V.		1.84	9.82

** Significant at 0.01 level of probability

Table 4. Effect of different methods of hormonal treatment on seed set and embryo formation in wheat × maize crosses

Wheat genotype	Treatment	Number of florets pollinated	No. of seeds obtained	No. of embryos formed
W_1	T_1	120	101 (84.16)	04 (3.96)
	T_2	120	103 (85.83)	10 (9.70)
W_2	T_1	120	100 (83.33)	03 (3.00)
	T_2	120	102 (85.00)	09 (8.82)
W_3	T_1	120	104 (86.66)	06 (5.76)
	T_2	120	102 (85.00)	11 (10.78)

T_1 = Spray; T_2 - Tiller injection; Values in parenthesis are given in percentage.

there was a significant difference among the wheat genotypes. Different methods of hormonal treatments also exhibited highly significant differences for embryo formation. This implies the differential response of both wheat genotypes and methods of hormonal treatments on haploid embryo formation in wheat x maize crossing. In this case the interaction between wheat genotypes and methods of hormone application was not significant (Table 3), indicating genotypic insensitivity.

The results of statistical analysis across three wheat genotypes W_1, W_2 and W_3 showed a non-significant effect of the two methods on seed set. This indicates that both the methods were efficient to induce similar seed formation rates. According to the results obtained from the analysis of variance for embryo formation, there was a significant difference among the wheat genotypes as well as different methods of hormonal treatment for embryo formation. Per cent embryo formation was higher when tiller injection method was used for hormone application. Translocation of hormone is supposed to be better in tiller injection method. The efficiency of injection method has been emphasized by Suenaga [12]. Inspite of this, if easeness of method is considered, spray method is more practical and easy to be performed but more amount of hormone is consumed while injection method is economic.

Per cent haploid formation was also independent from genotypic effect of wheat parent. Once the embryo has germinated, other factors will be effective in its further development into a plantlet. The reason why some cultured embryos failed to develop into plantlet is still unknown; however, in this regard the conditions of media and incubation room, light, temperature etc. could be mentioned. Comeau *et al.* [13] showed that differences between media can result in differences in the recovery efficiency of wheat haploid embryos.

One of the factors which limits the further development of a germinated embryo to a plantlet is deficiency of the embryos in one of their poles for shoot or root induction. In such cases meristems are not properly formed [14]. In the present study some of the embryos were observed which produced only a small shoot and some produce roots but not shoots. Since, for regeneration of the embryos modified B5 medium (B_5 + 0.05 mg/l BAP) was used, it can be concluded that this kind of media only supports the regeneration of complete embryos and those which are deficient in shoot or root cannot be supported well by a media which has a low concentration of growth regulators.

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