SHORT COMMUNICATION

Effect of genotypic background on haploid production through embryo rescue in wheat × maize crosses

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ABSTRACT

Two high yielding wheat (*Triticum aestivum* L.) varieties were pollinated with seven agrarian superior varieties. F_1's thus obtained were pollinated with diverse winter maize lines/composites and treated with a solution containing 2,4-D and GA_3. The haploid embryos were rescued 13 to 16 days post pollination on MS_1 and MS_4 media. Out of 15, only two crosses viz., (UP 2338 × HD 2285) F_1 × maize and (WH 533 × PBW 343) F_1 × maize resulted in callus development and direct germination via embryo rescue.

Keywords: *Triticum aestivum* L.; haploids; wheat × maize hybridisation; genotypic differences

Conventional recombination breeding is not only time consuming but subjected to selection errors. An erroneous selection based on phenotype in the early generation will thus affect the whole breeding effort. Therefore, any method which offers infinite F_2 population in homozygous state would cut short breeding time and help in increasing selection efficiency of breeding programmes. Cytogenetic findings have now presented the double haploid (DH) technique to facilitate the creation of new genetic variability in the form of a multitude of homozygous true breeding lines. Selection during the early generations of pedigree programme if compared with that of double haploids (DH), homozygosity obtained from using a DH system increases efficiency of selection both for qualitative and quantitative characteristics. It is thus easier to identify superior genotypes using a DH system because the frequency of fixation in an F_1 derived DH population is the square root of the probability in an F_2 population (Hu 1997). Other advantages of using DH lines is that additive genetic variance is expressed among the recombinants produced from a cross than among the relative F_2's and F_3's, and dominant variation is absent.

The crossing of wheat and other members of *Triticeae* with maize and the subsequent elimination of maize chromosomes have suggested the possibility of an alternative for haploid production (Laurie et al. 1990). The wheat × maize crosses are not affected by crossability alleles of wheat leading to the recovery of polyhaploids across different genotypes of wheat (Suenaga and Nakajima 1989, Riera-Lizarazu et al. 1992). In the metaphase of zygotes, resulting from wheat × maize crosses, there are 21 wheat chromosomes and 10 smaller maize chromosomes. It indicates that fertilization has taken place, but the karyotype of the hybrid is unstable (Laurie and Bennett 1988). Haploid wheat embryos resulted from the elimination of maize chromosomes in the zygote or later during the first three cell division cycles (Laurie and Bennett 1989).

In the present work we have studied the genotypic influence of wheat crosses on the rate of haploid production through wheat × maize crosses with an aim of large-scale generation of double haploids with the indigenous wheat material.

MATERIAL AND METHODS

Plant material and crossing programme

Fifteen F_1's of wheat (*Triticum aestivum* L.) were developed from pollinating two high yielding cultivars (PBW 343 and HD 2285) with seven agronomically superior varieties viz., WH 533, WH 542, WH 147, HD 2329, PBW 373, Raj 3077 and UP 2338. Parents represent a diverse genetic background and reported to have good crossability and combining ability. These F_1 plants were pollinated with diverse winter maize lines/composites (Prabhat, Vijay, Accessions; 803, 1344, 645 and 1040) having a different maturity period and were maintained in polyhouse/glass house

and field to ensure regular availability of pollen during the entire crossing season.

As soon as the primary spikes of wheat emerged from the boot, the outer two florets were emasculated and the inner one was removed. The upper and 2 to 3 basal spikelets were also removed. The pollination was done after 3 to 5 days after emasculation using randomly collected pollen from maize. After pollination emasculation bags were replaced by brown paper bags.

Pre-treatment and embryo rescue

Immediately after pollination the solution containing 50 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) + 100 mg/l gibberellic acid (GA_3) was injected about a centimeter above the upper most node through leaf sheath as suggested by Pienaar et al. (1997). After 24 hours the spikelets of the pollinated spike were flooded with the same solution using an injection syringe. These spikes were harvested in between the upper node 13 to 16 days of post pollination as suggested by Riera-Lizarazu and Mujeeb-Kazi (1990), Dhaliwal et al. (1995), Kammholz et al. (1996) and Suenaga et al. (1997), and were taken for embryo culture in the above mentioned solution of 2,4-D and GA_3 to laboratory.

The green parthenocarpic caryopsis (GPC's) were removed from the florets by bending them backward with a forceps. White and necrotic caryopsis were removed, as these contain no embryo. The GPC's were surface sterilized and haploid embryos from the disinfected GPC's were excised under aseptic condition. A scalpel was held between the thumb and forefinger and cut along the lateral grooves at the attachment end, the dorsal pericarp and green integuments were folded back and haploid embryo is lifted out. These haploid embryos were plated on callus induction medium MS_1 (Murashige and Skoog medium supplemented with 5 mg/l 2,4-D) to get haploid calli and germination medium MS_4 [MS medium containing 165 mg/l instead of 1650 mg/l NH_4NO_3, 0.40 mg/l thiamine HCl, 20 g sucrose, 1.0 mg/l indole-3-butyric acid (IBA) and 0.5 mg/l 6 benzyl aminopurine (BAP)] for direct haploid plantlet regeneration. Some treated spikes were kept in the field as such up to maturity to confirm the development of swollen ovaries, which would be watery sacks with no embryo.

RESULTS AND DISCUSSION

Fifteen F_1's developed from intervarietal crosses were pollinated with maize to get the embryos with half number of hexaploid wheat chromosomes as the maize chromosomes eliminated preferentially. Moreover, if these embryos were not rescued then no viable embryo is expected at seed maturity. The per cent of enlarged ovaries was highest (87.39) for the cross (HD 2329 × HD 2285) F_1 × maize and lowest (66.04) for (WH 147 × HD 2285) F_1 × maize (Table 1). At seed maturity only watery sacks with aborted embryo were recovered from all the wheat F_1 × maize crosses which failed to germinate when plated on basal Murashige and Skoog (1962) medium.

The embryo excised from caryopsis of wheat F_1's × maize crosses were harvested 13 to 16 days from post pollination and plated on MS_1 medium. The per cent haploid calli obtained was 6.4 for the cross (UP 2338 × HD 2285) F_1 × maize and 5.8 for (WH 533 × PBW 343) F_1 × maize, whereas, rest of the 13 crosses failed to show any callus development (Table 1). Similarly, when rescued embryos were plated on generation medium (MS_4) the per cent of regeneration was 9.50 and 7.65 for the crosses (UP 2338 × HD 2285) F_1 × maize and (WH 533 × PBW 343) F_1 × maize, respectively. The rest of the crosses failed to develop any haploid plantlet as shown in Table 1.

The dates of culturing of the rescued embryo on MS_1 and MS_4 media (Table 1) reveals that the environmental conditions during the rescuing of the embryo were almost similar over the crosses.

Very few investigations on wheat × maize crosses involving Indian wheat varieties and their F_1 have been conducted. Several technical problems affect the efficiency of haploid embryo production. A majority of haploid embryos resulted from wheat × maize hybrids are poorly developed or completely lacking an endosperm (Laurie and Bennett 1986). There is a large variation in the frequencies of haploid embryo recovery in these crosses (Laurie and Bennett 1988, Inagaki and Tahir 1990). Moreover, double fertilization in wheat × maize hybrids is known to occur at a low frequency (Laurie and Bennett 1986, Laurie and Reymondie 1991), therefore, fertilized seed contain either embryo or endosperm nuclei, but not both.

Possibly haploid embryos degenerate prior to rescue, particularly when the endosperm is absent or poorly developed (Zhang et al. 1996). However, endosperm development was not the predominant cause to explain the genotypic differences in our study. Wheat genotypes carrying the dominant genes *Kr*1 and *Kr*2 on chromosome 5B and 5A, respectively, are not crossable with *Hordeum bulbosum* (Snape et al. 1979, Mujeeb Kazi and Asiedu 1990), therefore, crossability of wheat with *Hordeum bulbosum* is restricted to a few genotypes (Falk and Kasha 1983). Crossability in wheat × maize crosses is reported to be independent of the effects of crossability alleles (Laurie et al. 1990). We observed considerably large differences among

Table1. *In vitro* haploid calli development (on MS_1 medium), direct haploid plantlets regeneration (on MS_4 medium) and *in vivo* enlarged ovaries development from wheat F_1's × maize crosses

Variants of wheat F_1 crosses with maize	*In vivo* condition			MS_1 medium (*in vitro*)			MS_4 medium (*in vitro*)			Dates of embrya culturing on MS_1 and MS_4 medium (date/month)
	A	B	C	D	E	F	G	H	I	
HD 2329 × HD 2285	484	423	87.39	321	–	–	92	–	–	26/2, 7/3, 20/3
WH 147 × HD 2285	589	389	66.04	340	–	–	71	–	–	1/3, 6/3, 9/3, 17/3, 26/3
WH 533 × HD 2285	348	259	74.42	500	–	–	70	–	–	2/3, 11/3, 19/3, 27/3
Raj 3077 × HD 2285	458	400	87.34	290	–	–	82	–	–	26/2, 8/3, 18/3, 21/3
UP 2338 × HD 2285	466	391	83.91	500	32	6.4	200	19	9.5	27/2, 10/3, 16/3, 20/3, 26/3
WH 542 × HD 2285	287	221	77.00	278	–	–	64	–	–	27/2, 12/3, 20/3, 27/3
PBW 373 × HD 2285	429	341	79.49	212	–	–	61	–	–	8/3, 9/3, 12/3, 20/3
PBW 343 × HD 2285	349	275	78.80	418	–	–	93	–	–	29/2, 8/3, 21/3, 27/3
HD 2329 × PBW 343	424	370	87.26	183	–	–	69	–	–	14/3, 15/3,17/3, 21/3, 27/3
WH 147 × PBW 343	453	399	88.08	211	–	–	109	–	–	13/3, 15/3, 21/3, 27/3
WH 533 × PBW 343	435	364	83.68	500	29	5.8	170	13	7.65	9/3, 11/3, 14/3, 16/3, 17/3, 26/3
Raj 3077 × PBW 343	400	315	78.75	260	–	–	52	–	–	13/3, 14/3, 23/3, 26/3
UP 2338 × PBW 343	531	450	84.75	470	–	–	90	–	–	11/3, 14/3, 16/3, 21/3, 22/3
WH 542 × PBW 343	274	237	86.50	147	–	–	59	–	–	10/3, 15/3, 22/3, 27/3
PBW 373 × PBW 343	454	377	83.04	290	–	–	78	–	–	15/3, 19/3, 20/3, 22/3

A = total florets pollinated, B = total enlarged ovaries, C = percentage of enlarged ovaries, D = No. of excised ovaries used for culturing, E = No. of calli obtained, F = percentage of calli obtained, G = No. of excised ovaries used for culturing, H = No. of rescued embryos, I = percentage of plant embryo genesis

wheat × maize crosses for getting haploid seedlings directly or through callusogenesis from the rescued embryos. There could be several reasons for this ranging from genetic to environmental.

Genotypic differences in the wheat F_1's × maize crosses may be due to involvement of some other gene(s) independent of *Kr* alleles which might be inhibiting the pre or post embryo development process. Moreover, the possibility of the presence of some modifiers, which affect embryo recovery, cannot be ruled out. A nutrient shortage may also be the cause of embryo abortion at the early stages when the embryos are not rescued (Zhang et al. 1996). It seems that the most potent method for the purpose is yet to be identified. Another possible reason could be the timing of the embryo rescue for these 13 crosses which may be somewhat earlier/later than 13 to 16 days post pollination reported by earlier workers (Inagaki and Tahir 1990, Zhang et al. 1996). Environmental conditions such as temperature, relative humidity and photoperiod greatly effect development rhythms of the embryos post pollination (Kammholz et al. 1996).

It seems that the genetic background may have strong impact on the onset of abortion after pollination. If so, in 13 crosses where we did not succeed to rescue viable embryos, reveals that suitable timing of embryo rescue is earlier than 13 to 16 days as followed in our study.

In the present study the embryo rescue period ranged from last week of February to 3rd week of March. Microenvironment conditions during post anthesis period to embryo rescue were, however, fairly stable with only sporadic rise in temperature of 2 to 3°C coupled with 10 to 15% decrease in relative humidity. However, we were able to rescue embryos 13 to 16 days post pollination only in two crosses out of fifteen. It is therefore suggested that embryo rescue studies for these crosses should be conducted rather early as soon as the embryo becomes rescue able post pollination to standardize the rescue timing vis-à-vis the prevailing ambient temperature and humidity conditions for Indian wheat genotypes adapted to specific environmental niches.

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ABSTRAKT

Vliv genetického pozadí na produkci haploidů získávaných po hybridizaci mezi pšenicí a kukuřicí z exstirpovaných nezralých zygotických embryí

Dvě vysoce výnosné odrůdy pšenice (*Triticum aestivum* L.) byly kříženy se sedmi odrůdami s nejlepšími hospodářskými vlastnostmi. Rostliny z F_1 generace byly sprášeny pylem pocházejícím od různých linií (genotypů) kukuřice a ošetřeny roztoky 2,4-D a GA_3; 13 až 16 dnů po sprášení kukuřičným pylem byla haploidní zygotická embrya pšenice exstirpována a přenesena na kultivační média MS_1 a MS_4. Z 15 variant hybridizačních pokusů došlo k vytvoření kalusu a k přímému klíčení zygotických embryí jen ve dvou typech křížení (tj. mezi UP 2338 × HD 2285 a kukuřicí a mezi WH 533 × PBW 343 a kukuřicí).

Klíčová slova: *Triticum aestivum* L.; haploidy; křížení pšenice × kukuřice; genotypové rozdíly

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