Dynamics of wheat x *Imperata cylindrica* – a new chromosome elimination mediated system for efficient haploid induction in wheat

Chaudhary HK

Molecular Cytogenetics & Tissue Culture Lab, Department of Plant Breeding & Genetics, CSK Himachal Pradesh Agricultural University, Palampur – 176 062 (HP) INDIA

INTRODUCTION

Doubled haploidy breeding serves as a useful tool in wheat improvement by providing instant homozygosity, which leads to fixation of desirable characters of recombinants as well as reduction in the number of generations required to achieve success. Of the various techniques for haploid production in wheat, chromosome elimination has been studied and investigated by researchers to a great extent and nowadays is being utilized for large-scale practical applications. The technique was initiated in wheat with the investigations of Barclay (1975)[1], who recovered wheat haploids in crosses between the wheat variety Chinese Spring and *Hordeum bulbosum.* The technique was, however, genotype-specific due to the presence of the dominant crossability inhibitor genes *Krl* and *Kr2* in wheat, which are expressed in many wheat varieties. The wheat x maize for haploid production (Laurie and Bennett, 1987[2]) was genotype non-specific because of the insensitivity of maize pollen to the action of *Kr1* and *Kr2* genes thereby rendering the chromosome elimination technique more efficient and of practical value. Although the wheat x maize system of haploid production is quite successful yet the maize crop needs to be grown in greenhouse so as to coincide flowering with wheat thereby increasing the cost of haploid production. Hence there was a dire need to search for another species which may serve, as an efficient pollen source for haploid production in wheat and so dispense with the need to grow maize under greenhouse conditions during the winter season.

Keeping in view the necessity to search for winter season growing plant species as a pollen parent having equal or higher potential of haploid induction in wheat than that of maize, the present investigation was planned.

MATERIALS AND METHODS

Four diverse groups of wheat and triticale encompassing winter x spring wheat F_1s (5 crosses), triticales (5 lines), triticale x wheat F_2s (5 crosses) and triticale x wheat BC_1F_2s (5 crosses) were hybridized with maize (*Zea mays*) and cogon grass (*Imperata cylindrica*) as pollen sources at the Experimental Farm of the Deptt of Plant Breeding & Genetics, CSK HP Agricultural University, Palampur (India) and the relative performance of both the pollen parents was compared in respect of various haploid induction parameters on the basis of overall results. The protocol for developing the haploids was used as per Laurie & Bennett (1987)[2] and Chaudhary *et al.* (2002)[3] with certain modifications for hormonal applications especially in case of triticale and triticale x wheat derivatives. The roots of the haploid regenerants obtained after embryo rescue in wheat were randomly screened for cytological investigations. Hybrid origin of the haploids through *Imperata cylindrica* – mediated approach was confirmed following genomic *in situ* hybridization (GISH) as per the protocol of Mukai & Gill (1991)[4].

RESULTS AND DISCUSSION

The results obtained after crossing five spikes of each of the cross or pure line from each group of wheat and triticale with maize (2n = 20) and *I. cylindrica* (2n = 20) were compiled and average frequency of the haploid induction parameters *viz*., pseudo seed formation, haploid embryo formation and embryo regeneration was worked out and compared (Fig 1). The *I. cylindrica* is performing better than the maize in all the groups of wheat and triticale in respect of all the haploid induction parameters and hence identified as an efficient pollen source. Amongst different groups, the performance of winter x spring wheat crosses was observed significantly better than the triticale and the crosses of the triticale with wheat. It is expected that the D genome might also be playing significant role in determining the success of the haploid production. Genomic *in situ* hybridization analysis of the zygotes of wheat x *I. cylindrica* was exercised and 21 chromosomes of wheat and 10 of *I. cylindrica* were seen in the same prometaphase cell (Fig 2). It was also observed that the chromosomes of *I. cylindrica* are eliminated faster than that of maize and there is no endosperm formation in the former case. Metaphase preparations from the roots of haploid regenerants obtained in wheat after hybridization with *I. cylindrica* were investigated and haploid status (n = 21) of the plants was confirmed (Fig 3). Confirmation for such status of the wheat plants obtained through maize-mediated system has already been exercised by Laurie & Bennett (1988)[5].

Overall, wheat x *I. cylindrica* system of doubled haploidy (DH) breeding in wheat following chromosome elimination approach has following advantages over the existing wheat x maize system:

- **Coincidence of flowering**

  In wheat x maize system, maize has to be raised under artificial conditions in the green houses/polyhouses so as to coincide the flowering with that of wheat or triticale whereas such exercise is not required in case of wheat x *I. cylindrica* system because *I. cylindrica* is a winter season plant and coincides well for flowering with that of wheat and triticale under natural conditions.

- **Raising of pollen source**

  Maize has to be raised every season whereas *I. cylindrica* being a wild weedy perennial grass doesn't require its repeated sowings (Fig 4 & 5). *I. cylindrica* is available under natural conditions in almost all parts of the world wherever wheat is cultivated.

- **Genotype specificity**

  Both the plant species viz. maize and *I. cylindrica* are genotype non-specific for hybridization with any variety of wheat, triticale or their derivatives.

- **Availability of pollen**

  Likewise maize, pollen of *I. cylindrica* is readily available in abundance during the wheat hybridization period.

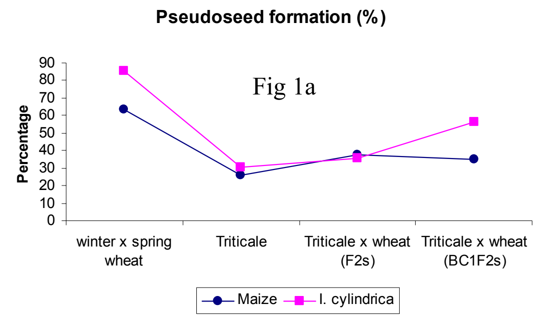
- **Haploid induction performance**

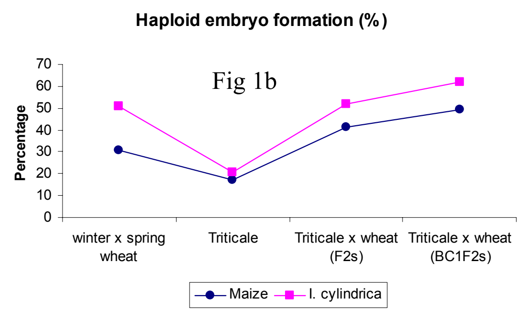
  I. cylindrica performs significantly better than maize for all the haploid induction parameters in wheat and triticale and their derivatives and can produce more DH populations in comparison to the maize-mediated system in the same time period.

Hence, the *I. cylindrica* mediated system of DH breeding has been proved a very efficient and economically viable approach for the acceleration of wheat breeding endeavours with enhanced precision and efficiency. This innovation has globally opened new vistas for the commercial utilization of the new system for large scale production of doubled haploids of wheat to be used directly as improved varieties or gene mapping populations.

REFERENCES

1. Barclay, I.R. 1975. High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature 256: 410-411.
2. Laurie, D.A. and Bennett, M.D. 1987. Wide crosses involving maize (*Zea mays*). Annual Report of the Plant Breeding Institute, 1986-87, pp.66.
3. Chaudhary, H. K., Singh, S. and Sethi, G.S. 2002. Interactive influence of wheat and maize genotypes on haploid induction in winter x spring wheat hybrids. Journal of Genetics & Breeding 56: 259-266.
4. Mukai, Y. and Gill, B. S. 1991. Detection of barley chromatin added to wheat by genomic *in situ* hybridization. Genome 34: 448-452.
5. Laurie, D. A. and Bennett, M. D. 1988. Cytological evidence for fertilization in hexaploid wheat x sorghum crosses. Plant Breeding 100: 73-82.





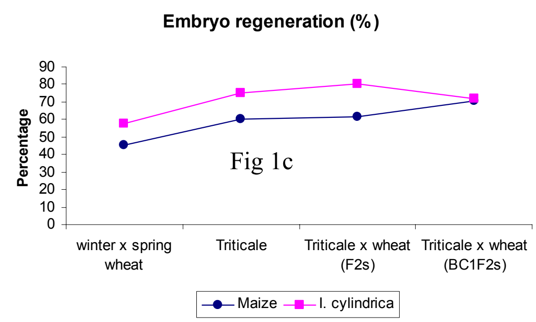


Fig 1. Relative frequencies of pseudo seed formation (Fig 1a), haploid embryo formation (Fig 1b) and their regeneration (Fig 1c) obtained from winter x spring wheat F_1s, triticale, triticale x wheat F_2s and BC_1 F_2s after hybridization with maize and *I. cylindrica*. Data pooled over the five spikes each of five genotypes of each group

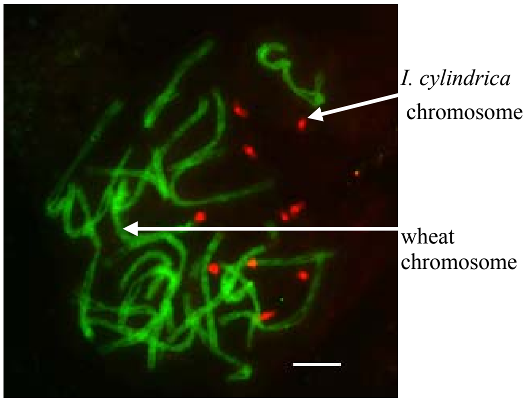


Fig 2. GISH analysis of first zygotic prometaphase of wheat x *I . cylindrica* hybrid. Bar represent 10 µm

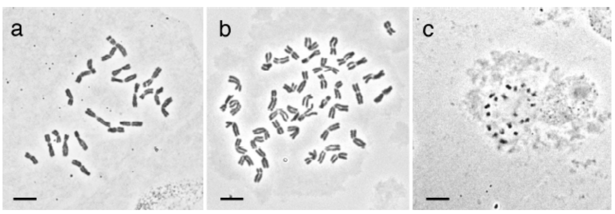


Fig.3 Metaphase chromosomes: (a) wheat haploid (n=21) generated through wheat x *Imperata cylindrica* hybridization (b) normal wheat (2n=42) and (c) *Imperata cylindrica* (2n=20). Bars represent 10 µ m

Fig 4. Anthesis in *I. cylindrica* spikes

Fig 5. Wildly growing *I. cylindrica* plants in the surroundings of wheat fields in North-West Himalayan regions