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DOUBLED HAPLOIDY BREEDING IN WHEAT AND BARLEY

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Abstract: The production of doubled haploids (DHs) in wheat and barley has proven to be a valuable tool for plant breeders, allowing the release of a number of new cultivars and is an ultimate choice for genetic and QTL analysis and linkage maps production. Myriad protocols have been established for an efficient production of haploids and DHs, of which chromosome elimination and *in vitro* anther culture is the most effective and widely used one in wheat and barley. The production of haploids and DHs through these methods results in the development of complete homozygous lines from heterozygous genotypes in a single-step, thereby shortening the time required for homozygous genotypes development when compared to the conventional breeding. This paper highlights the influence of genotype on the anther culture protocol for generating callus in barley and DHs plant development *via Imperata cylindrica* mediated chromosomal elimination approach in wheat. These optimized techniques can be used as a potential tool for genetic improvement of wheat and barley by dint of haploids and DHs development.

Keywords: *Imperata cylindrica*, Anther culture, Barley, Wheat, Doubled haploids.

INTRODUCTION

Doubled haploid production is an eminent biotechnological tool for accelerating plant breeding programmes *via* achieving a homozygosity and allowing transgene or mutation stabilization in the genome within a considerably shorter time. Whilst the conventional breeding programmes *au contraire* requires more generations for the development of homozygous lines. In wheat, chromosome elimination doubled haploidy breeding having large-scale practical application has been studied by researchers (Patial *et al.,* 2021). The technique was introduced in wheat with the efforts of Barclay (1975), but it was genotype specific due to the presence of the dominant crossability inhibitor genes *Kr1* and *Kr2* (Alfares*et al*., 2009). Later, Laurie and Bennett (1987) identified wheat × maize system (maize pollen being insensitive to the activity of these genes) but, the unsynchronized flowering of these two species flummox the hybridization process. Thereafter, wheat haploid development following *Imperata cylindrica* (Chaudhary *et al*., 2005, Patial 2015a) was found to be more efficacious (Chaudhary *et al*., 2005; Patial *et al*, 2017). *I. cylindrica* grass is easily available winter season perennial (2n=20) which coincides well with wheat flowering. Due to its perennial nature, there is no need of growing this grass in polyhouse or green house as is incumbent on maize mediated system. Also, the frequency of haploid embryo formation and regeneration through *I. cylindrica* is significantly higher than Maize mediated system (Patial, 2020).

Haploids in barley can be produced through several techniques. But of all techniques, only two have been used for large-scale application *viz*., andogenesis, including anther and microspore culture, and wide hybridization involving *Hordeum bulbosum* followed by chromosome elimination technique. The *in vitro* technique of

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anther culture is the most successful and quite useful method to produce doubled haploid lines in barley. DH plants derive from divisions of haploid microspores that have undergone a developmental switch under the appropriate conditions. The successive divisions lead to the formation of an embryo or callus rather than the formation of mature pollen grains. However, the anther technology has overall low green plant production, more number of albino plantlets and high genotypic dependency. Therefore, the efficiency of DH plant production is highly variable depending on the genotype of the source material. Despite this limitation, DH plants have been widely used in breeding and research programs. This paper describes the induction of haploids in wheat and callus in barley using *Imperata cylindrica* and *in-vitro* androgenesis approach, respectively.

MATERIALS AND METHODS

Doubled haploid induction in wheat *via Imperata cylindrica* mediated approach

The present study was conducted in wheat F_1's. The florets of F_1 plants were hand emasculated 2-3 days before anthesis. The fresh pollen from *I. cylindrica* was applied to the feathery stigma of the emasculated florets. The uppermost internodes of the pollinated wheat spikes were injected with 100ppm of 2,4-D solution 24 hours after pollination for two consecutive days. At 18-20 days after pollination, the spikes were harvested and sorted for the presence of embryo. The pseudoseeds containing embryo were excised and cultured on a nutrient MS medium. The cultured embryos were incubated in dark at 20± 2°C and when the first regeneration appeared, they were transferred to a culture room under a 10/14 hours light/ dark regime and light intensity of 3000 Lux until they developed healthy. For doubling, the roots of haploid plants at 4-5 tiller stage, were treated with colchicine (0.1%) + 1.5% DMSO for six hours and then rinsed with distilled water. The plants were transferred in pots, placed in growth chamber and allowed to grow at 16/12°C day/night temperature for one month and then transferred to the field.

Callus induction in barley *via* anther culture

The six barley genotypes (BHS400, BHS380, BHS352, BHS 482, BHS 474 and DWR 150) were used for callus induction. Tillers having spikes for anther culture were collected when the microspores were at mid- or late-uninucleate stage. The stage indicator was assessed with the inter-ligule length of the tip of the tiller. Cultured anthers were incubated at 27°C in the dark. After 21 and 28 days, pollen embryoids and plantlets were counted.

3. RESULTS AND DISCUSSION

3.1. Development of Doubled Haploid lines in wheat F_1

Imperata cylindrica is an important source for substituting/supplementing maize mediated doubled haploid production in wheat. In present study, the abundant pollen along with synchronized wheat flowering highlighted the potential of the technique for its use in wheat improvement programme. A total of, three hundred and ninety-six florets of wheat F_1 were pollinated with *I. cylindrica* which resulted in 89.06 percent of pseudoseed formation, 15.35 percent of embryo formation and 13.13 percent of haploid plant regeneration. The haploid embryos resulted from crossing of wheat F_1 with *I. cylindrica* lacked endosperm, thereby, highlighting the haploid nature of the embryo. After colchicine treatment for doubling the haploid plants, six Doubled Haploid plant survived and developed seeds. Chaudhary *et al*. (2013) and Patial *et al*. (2015 b) have also highlighted the potential use of *I. cylindrica* for production of wheat Doubled Haploids.

3.2. Development of callus in barley genotypes

The callus formation from anthers of six barley cultivars (BHS 400, BHS 380, BHS 352, BHS482, BHS 474 and DWR 150) were investigated. The anther culture response of these barley cultivars was tested on MS media. A considerable difference in callus induction was obtained in relation to the genotypes cultured. Anther culture is one of the most popular and widely employed techniques for development of haploids. However, the low embryogenic capacity and the high albinism rate

of some genotypes are still open fields for further improvements in these protocols. The study reported genotypic specificity for the callus induction in barley with only BHS 482 and BHS 474 showed the callus induction (Table 1).

Table 1: Development of callus from anther culture

Cultivar	*Development of callus*
BHS 400	No response
BHS380	No response
BHS352	No response
BHS482	Few anthers responded (20%)
BHS474	Few anthers responded (30%)
DW150	No response

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