Review Article

Application of auxins in haploid embryo induction in hexaploidy wheat

Abstract:

Doubled haploid (DH) plant production plays a crucial role in modern plant breeding programs, offering an efficient means to generate homozygous lines from heterozygous parents within a single generation. Different types of auxins have been employed in wheat cross with maize DH production, with 2,4-D being the most widely used and effective hormone, followed by dicamba. Other auxins, including picloram, IAA, PAA, silver nitrate, NAA, kinetin, BA, and zearalenone, have also been tested for their potential role in haploid embryo induction. Various methods have been explored for the application of 2,4-D, such as spray, tiller injection, dipping, and spikelet culture methods.

Keywords: Double haploid (DH), auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), embryo recovery, haploid embryo induction, concentration, efficiency,

Introduction:

Doubled haploid (DH) plant production has emerged as a crucial component of modern plant breeding initiatives (Forster *et al.* 2007; Weyen 2009). Through traditional pedigree selection methods, it typically requires around a decade to develop a commercially viable wheat variety. DH technologies serve as effective techniques to expedite the generation of homozygous lines from heterozygous donor genotypes, eliminating the need for time-consuming selfing processes.

Doubled haploids (DH), which are presently utilized in various crop species such as wheat and triticale, allow breeders to obtain fully homozygous genotypes from heterozygous parents within a single generation. This advancement results in significant time savings of approximately 4 to 6 years in the breeding process. First report of wheat haploid production was reported by crossing wheat with maize.

The initial discovery of wheat haploid production was achieved through the crossing of wheat with maize (Barclay 1975). After fertilization, the process of zygote induction occurs, leading to the elimination of the male parent's chromosomes from the cells of the developing embryo (Laurie and Bennett 1989). The viability of these zygotes is limited, and the majority of them undergo abortion during the early stages of development (Laurie and Bennett 1988). The use of auxins, on the other hand,

stimulates the enlargement of the ovary and promotes the development of haploid embryos to a stage where they can be cultured on nutrient media (Laurie and Reymondie 1991).

Nevertheless, there exist numerous variations in the hormone treatments administered, encompassing factors such as the type and concentration of auxin, as well as the method and timing of hormone application. The hormone 2,4-dichlorophenoxyacetic acid (2,4-D) is extensively utilized and is considered the most prevalent, closely followed by dicamba (3,6-dichloro-o-anisic acid). Studies conducted on bread wheat have demonstrated that both hormones exhibit comparable efficacy in inducing desired effects (Matzk and Mahn 1994).

Auxins:

The notion of auxin as a mobile growth regulator was notably deduced by Charles and Frances Darwin, as documented in their renowned 1880 publication, *The Power of Movement in Plants*, where he observed the impact of a hypothetical substance that influenced the elongation of plant shoots, enabling them to exhibit tropic growth towards light (Darwin and Darwin, 1880). Auxin, being a crucial plant hormone, regulates a wide range of processes, including tropic responses to light and gravity, overall root and shoot architecture, organ patterning, vascular development, and growth in tissue culture (Davies, 1995). Human intervention in auxin physiology has facilitated plant propagation and driven by artificial selection, has contributed to the development of current crop varieties (Multani *et al.*, 2003; Salamini, 2003).

Types of auxins used:

2,4-D (hormone 2,4-dichlorophenoxyacetic acid) have been used most and shown optimum seed setting and embryo formation followed by close results with dicamba (3,6-dichloro-o-anisic acid) (Laurie and Bennett, 1988; Suenaga and Nakajima, 1989; Riera-Lizarazu and Mujeeb-Kazi, 1990; Kisana *et al.*, 1993; Matzk and Mahn 1994).

Other auxins like picloram (4-amino-3,5,6-trichloropicolinic acid), IAA (indole-3-acetic acid), PAA (phenyl acetic acid) (Warchol *et al.* 2016), silver nitrate (O'Donoughue and Bennett 1994), NAA, kinetin, BA (6-benzyladenine) (Ushiyama, T *et al.*, 2007) and zearalenone (Biesaga-Kościelniak, J., *et al.*, 2003).

Method of application:

For application of 2,4 D several methods have been tried by Kaushik *et al.*, 2004

such as spray method, tiller injection method, dipping method, and spikelet culture method. 240 florets pollinated in each method.

1. Spray method: The spikes were subjected to spraying with a concentration of 100 ppm of 2,4-dichlorophenoxyacetic acid (2,4-D) at three different time intervals: one, two, and three days after pollination. 8 embryos recovered by this method.

2. Tiller injection method: The uppermost internodes of the wheat spike were injected with 1ml of 2,4-dichlorophenoxyacetic acid (2,4-D) at a concentration of 100 ppm, starting one day after pollination. This injection process was repeated for three consecutive days. 9 embryos recovered.

3. Dipping method: The spikes that underwent pollination were immersed in an aqueous solution of 2,4-dichlorophenoxyacetic acid (2,4-D) for three consecutive days. 14 embryos were recovered.

4. Spikelet culture method: On the second day after pollination, the spikes were extracted and subjected to surface sterilization before being dried on filter paper. The rachis (central axis) was then divided into individual spikelets and positioned upright on a growth medium called MS media, which contained 30mg/l sucrose and 0.2mg/l 2,4-dichlorophenoxyacetic acid (2,4-D). The cultures were subsequently incubated for a duration of three weeks under continuous light at a temperature of 20°C, with a daily exposure of 16 hours of light. 27 embryos were reported to be recovered by this method.

Applying 2,4-dichlorophenoxyacetic acid (2,4-D) through spray, dipping, and tiller injection methods resulted in low embryo recovery rates. In contrast, when 2,4-D was administered using the spikelet culture method, significantly higher embryo recovery rates were achieved (Kaushik *et al.*, 2004).

Table 1. Concentration of auxin used with efficiency

S.no	Auxin	Concentration	Embryo Formation Effeciency	Refrences
1	2,4 D	100 mg/l	48%	Ushiyama, T *et al., 2007*
2	2,4 D + ZEN	6 µM·dm-3	23.30%	Biesaga-Kościelniak, J., *et al.*, 2003
3	NAA	1000 mg/l	3.40%	Ushiyama, T *et al., 2007*
4	IAA	1 mg/l	1.10%	Ushiyama, T *et al., 2007*

5	Kinetin	1-10 mg/l	0.90%	Ushiyama, T *et al., 2007*
6	6-benzyladenine (BA)	10 mg/l	1.50%	Ushiyama, T *et al., 2007*

Conclusion:

Auxins, including 2,4-dichlorophenoxyacetic acid (2,4-D) and dicamba, play a crucial role in inducing haploid embryo development in wheat. The application of these auxins has shown effective results, with 2,4-D being the most widely used hormone. The method of application varies, with techniques such as spray, tiller injection, dipping, and spikelet culture being employed. Among these methods, the spikelet culture method has shown the highest embryo recovery rates.

Different concentrations of auxins have been tested, and their efficiency in inducing embryo formation varies. For instance, 100 mg/l of 2,4-D has been reported to have a 48% embryo formation efficiency. Other auxins such as NAA, IAA, kinetin, and BA have also been studied, each with varying levels of effectiveness in promoting embryo formation. This advancement has significantly contributed to the acceleration of plant breeding programs and the development of improved crop varieties.

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