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Application of Auxins in Haploid Embryo Induction in Hexaploidy Wheat

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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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 utilized 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, Indole-3-acetic acid (IAA), phenylacetic acid (PAA), silver nitrate, 1-naphthaleneacetic acid (NAA), kinetin, 6-benzyladenine (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. 2,4-D found to be most effective auxin treatment both alone and with combination with other phytohormones.

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1. INTRODUCTION

Doubled haploid (DH) plant production has emerged as a crucial component of modern plant breeding initiatives [1,2]. Through traditional pedigree selection methods, it typically requires around a decade to develop a commercially viable wheat variety. Double haploid 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, 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 [3]. 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 [4].

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 [5]. The viability of these zygotes is limited, and the majority of them undergo abortion during the early stages of development [6]. 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 [7].

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) [8]. Studies conducted on bread wheat have demonstrated that both hormones exhibit comparable efficacy in inducing desired effects [8].

2. POTENTIAL OF HAPLOID EMBRYO INDUCTION IN HEXAPLOIDY WHEAT

Each cluster of wheat develops its own distinct set of haploid organisms. These haploids are sporophytes that possess the chromosome count specific to gametes (n). The haploid organisms derived from einkorn, emmer, and dinkel wheat have respective chromosome counts of n = x = 7, n = 2x = 14, and n = 3x = 21. These chromosomes carry the genomic compositions of A, AB, and ABD, respectively [9,10,11].

Wide crosses have been employed in crop improvement and genetic studies to generate haploids (Baum et al., 1992). Some of the bread wheat crosses were made by different intergeneric crosses *viz*., wheat x maize [7,12,13], wheat x pearl millet [12,14], wheat x teosinte [15], wheat x barley [16] and wheat x sorghum [12]. Multiple studies have shown successful production of doubled haploid plants by utilizing maize pollen on hexaploid wheat [17,18,19]. However, only a limited number of durum wheat genotypes exhibit comparable crossability with maize [20,21,22]. In recent times, the occurrence of polyhaploid embryo formation has significantly increased by manipulating Dicamba alone or in conjunction with 2,4-dichlorophenoxyacetic acid (2,4-D) [20,23,22], (Ahmad and Chowdhry, 2005).

3. 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 [24]. 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 [25]. Human intervention in auxin physiology has facilitated plant propagation and driven by artificial selection, has contributed to the development of current crop varieties [26,27].

4. TYPES OF AUXINS USED

The 2,4-D (2,4-dichlorophenoxyacetic acid) hormone has been used most and shown optimum seed setting and embryo formation followed by close results with dicamba (3,6-dichloro-o-anisic acid) [6,17,28,29,30].

Table 1. Concentration of auxin used with efficiency

S.no	Auxin	Concentration	Embryo Formation Efficiency	References
1	2,4 D	100 mgl^{-1}	48.00%	Ushiyama, T et al., [32]
2	2,4 D + ZEN	6 $\mu M \cdot dm^{-3}$	23.30%	Biesaga-Kościelniak, J., et al., [33]
3	NAA	1000 mgl^{-1}	3.40%	Ushiyama, T et al., [32]
4	IAA	1 mgl^{-1}	1.10%	Ushiyama, T et al., [32]
5	Kinetin	1-10 mgl^{-1}	0.90%	Ushiyama, T et al., [32]
6	6-benzyladenine (BA)	10 mgl^{-1}	1.50%	Ushiyama, T et al., [32]

Other auxins like picloram (4-amino-3,5,6-trichloropicolinic acid), IAA (indole-3-acetic acid), PAA (phenyl acetic acid) [31], silver nitrate [23], NAA (1-naphthaleneacetic acid), kinetin, BA (6-benzyladenine) [32] and zearalenone [33].

5. METHOD OF APPLICATION

For application of 2,4 D several methods have been tried by Kaushik et al., [34] such as spray method, tiller injection method, dipping method, and spikelet culture method. With 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. A total of 8 embryos recovered by this method.
2. **Tiller Injection Method:** The uppermost internodes of the wheat spike were injected with 1 ml 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. A total of 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 spikelet and positioned upright on a growth medium called *Murashige-Skoog* (MS) media, which contained 30mgl^{-1} sucrose and 0.2 mg/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 h of light. A total of 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 [34].

6. 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 mgl^{-1} 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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