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Anther Culture in Wheat (*Triticum aestivum)* and Induced Embryogenesis Study for Doubled Haploid Production

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ABSTRACT

Plants naturally have the ability to develop new plants from the microspores. The process of the complete regeneration of a new plant from the microspore culture under laboratory conditions is termed as Androgenesis. Differentiation of the numerous microspore cells leads to the development of embryos which further developed as haploid plants. Certain varieties of the hexaploid wheat (*Triticum aestivum)* also possess the androgenesis ability. There will be need of artificial treatments in order to flip the microspores to the sporophytic phase from the gametophytic pathway. Embryogenic microspores which are induced artificially contains unique phenotypic characters, also pass through cell division and differentiation in an organised manner. This leads to the embryoids development directly. As certain treatments are given in order to switch the gametophytic phase to the sporophytic phase, the events that occur during the embryoid development to the haploid plants can be easily predicted. Induced treatments help to isolate the microspores by controlling the embryogenesis process. This culture of the microspores isolated using the treatments has been so beneficial in studying the process of androgenesis clearly by understanding the events that occur within the cultured microspores. This further lead to the improvement in the production of the doubled haploids**.**

Keywords: doubled haploids, embryoids, embryogenesis, gametophyte, gametogenesis, microspores.

INTRODUCTION

Angiosperms life cycle undergoes two stages alternatively called as gametophytic phase which is haloid in nature and the other, sporophytic phase, diploid in nature. The microspore mother cells also called pollen mother cells present within the locules of the anthers undergo mitotic and meiotic cell divisions and leads to the pollen grains development. Due to alteration in the environmental conditions, the microspores also called immature pollen, enters the sporophytic stage by switching off the gametophytic phase. This switch leads to the development of Pseudo embryos called as Embryoids. (Reynolds, 1997). These embryoids (pseudo embryos) germinate and give rise to the haploid plants or doubled haploid plants (DHs).

The process of development of the doubled haploid/doubled haploid plants with the help of microspore culture is called as androgenesis/microspore embryogenesis. Within the single step, the individuals with the homozygous nature can be obtained through androgenesis and doubled haploid production. This method is very helpful for the crop improvement, also manipulation of the genes related to plant developmental biology. The homozygosity in conventional pure line breeding method is obtained after numerous generations. Comparatively, doubled haploid production leads to the homozygosity attainment within a single step. Thus, the doubled haploids are very useful in genome mapping, genetic transformation and so on.

Regeneration of wheat (*Triticum aestivum)* plants using (anther culture) microspore culture was done successfully in the early 1970s. During the early period, the culture method used to completely depend on the naturally occurred embryogenic microspores n certain genotypes. That was a great backlog for efficient growth. Also, agar is used as a solidifying media and increased levels of plant growth regulators for inducing the callus formation. The usage of increased levels of plant growth regulators give rise to chromosomal aberrations and also soma clonal variations. Later on, usage of liquid media has been started for the regeneration of the plants with the lowered levels of plant growth regulators. This increased the efficiency of regeneration of plants from the culture media.

Recently, the advancement in doubled haploid production in wheat has been achieved using the isolated microspore cultures (Hu et al). The efficiency of the method has been improved by artificial manipulation of the microspores directly. This review explains the microspore embryogenesis method in wheat and also different events that occurred during the regeneration of the plants from the embryoids.

Methodology

Automatic isolation of the microspores and their culture in order to regenerate the wheat plants has been started in the recent years. Even though the efficiency of this mechanical process of isolation is low, success was achieved in the wheat microspore culture development. This further lead to the improvement in the culturing method. Along with the efficiency of the microspore culture, the range of genotyped to isolate the microspores has been increased. Thus the microspores obtained through isolation from various genotypes are helpful in the production of doubled haploids sufficient enough to use them in wheat breeding.

Growing donor plants for microspores

The conditions provided for the growth of the plants plays a crucial role in affecting the quality of the plants. The growth conditions may be intensity of light provided, nutrition applied, photoperiod, temperature. Generally, the wheat plants show good response on the culture when low temperatures are maintained (12-18°C) (Simmonds). The effect of stress conditions as well as disease and pest attack must be avoided. Growth chambers are setup with the required conditions of growth balancing the changes for culturing the microspores. Protected conditions can also be used in order to obtain the healthy donor plants for microspore isolation. Harvesting the donor plants should be done at an appropriate stage of development for better microspore culture. Mostly the spikes are collected for the microspores at mid to late uni nucleate stage. At this stage, the induction treatment will be very easy on the microspores.

Switch to sporophytic phase

Even though there are various methods for wheat microspore culture, most of them use treatment that involve stress induction in order to induce androgenesis. Certain genotypes of wheat have the inherent capacity to start embryogenesis without any external treatment. But the efficiency of regeneration within the naturally inherent one is very low. Hence, in recent years, treatment inductions are most prevalent for the successful production of doubled haploids and further production of numerous plants from them. Major steps in the microsporogenesis involves firstly the treatment induction to switch the growth of microspore towards sporophytic phase. The treatments used may be cold treatment, heat treatment, reduction in sugar levels, nitrogen starvation, water stress, use of some radiations and some chemical inductions.

Treatment at a low temperature (1-5°C) is a very effective method in wheat for the better response in the anther culture. Treating the spikes at a very low temperatures in the wheat crop may interrupt the gametophyte stage and may nurse the effect of anther tissues on the microspores.

Isolation of microspores

There are various methods used for the isolation of the microspore and to purify them. Among them, four methods are prevalent to be used generally. They are shedding, magnetic bar stirring, maceration, blending. (Jahn and lorz, 1995). Under shedding method, the anthers obtained from heat will be cultured in a liquid media in which the microspores were shed. Treating the anthers with the 0.3M mannitol and macronutrients for at least 6-7 days before the shedding or before the magnetic bar stirring method is very effective. Magnetic bar stirring is similar to the stirring technique, where in this method a stirring force is used for easy shedding of the microspores. In wheat crop (*Triticum aestivum)* both the shedding and magnetic bar stirring methods are not much effective as they result in low yields of microspores. Whereas maceration technique of isolation is done by pressing the anthers against a mesh filter using a Teflon rod. Blending method involves sharp blades to cut the anthers into small pieces from which microspores will be isolated. Among the mentioned methods, blending method is very efficient as the viability of the microspores at initial stages is very high (75%) (Gustafson et al., 1995).

Embryoids germination and doubled haploid production

Embryoids with around 2mm diameter are shifted a media which is semi solid, free from PGRs and less carbohydrate content. The media for germination of embryoids in wheat is generally 190-2(Zhuang and Xu, 1983). Incubation in light at room temperature is done after the transfer. Within 5-7days, shooting and increase in size observed. Green plantlets can be obtained at 10-14 days, ready to transplant to the soil under greenhouse conditions.

The condition of the plants for ploidy can be checked from root tip or the size of the stomata. If the plants are haploid then colchicine treatment or caffeine treatment is applied to double the chromosome number. A regular colchicine treatment includes immersing the root tips of the haploid wheat plantlets in the colchicine solution (2g per litre) for 3hours and then washing under running water. Even though the colchicine treatment is very effective, it shows toxic effect on the plants. Comparatively, the caffeine treatment is used alternatively as it shows very low toxic effects on the plants but is less effective.

CONCLUSION

Huge progress has been taken place recently in the microspore culture development in the wheat crop. Factors that affect the microspore culture efficiency are genotype of the wheat, physiology of the plant donor, developing stage of the microspore, treatment conditions, purity of the microspores, conditions for inducing treatments, and ability of the plant to regenerate. First, the traits obtained through androgenesis are inherent in nature and so the difference in the genotypes in the culture is accepted. Second, plant which donates the microspores should be free from disease and pest attack and should be healthy to avoid misleading. Third, before the induction of the treatments the microspores should be at mid to late uni nucleate stage. Fourth, proper stress treatment is mandatory for conversion of gametophytic stage to sporophytic stage. Efficiency of microspore collection increased through the blending method of isolation. Lastly, the temperature and osmolarity are optimised for efficient germination of the embryoids. Further optimisation of the embryogenesis process has been in process with the use of markers.

REFERENCES

- Agache S, Bachelier B, de Buyser J, Henry Y & Snape J (1989) Genetic analysis of anther culture response in wheat using aneuploid, chromosome substitution and translocation lines. Theor. Appl. Genet. 77: 7–11
- Chu CC (1978) The N6 medium and its applications to anther culture of cereal crops Proceedings of Symposium on Plant Tissue Culture (pp. 43–50). Science Press, Beijing
- Gustafson JP(ed) Gene Manipulation in Plant Improvement II (pp. 213–235).
- Gustafson VD, Baenziger PS, Wright MS, Stroup WW & Yen Y (1995) Isolated wheat microspore culture. Plant Cell Tiss. Org. Cult. 42: 207–213
- Henry Y & de Buyser J (1989) Float culture of wheat anthers. Theor. Appl. Genet. 60: 77–79
- Hu TC, Ziauddin A, Simion E & Kasha KJ (1995) Isolated microspore culture of wheat (Triticum aestivum L.) in a defined media: I. Effects of pretreatment, isolation methods, and hormones. In Vitro Cell Dev. Biol. 31: 79–83
- Indrianto A, Barinova I, Touraev A & Heberle-Bors E (2001)
- Jahne A & Lorz H (1995) Cereal microspore culture. Plant Sci. 109: 1–12
- Kasha KJ, Ziauddin a & Cho UH (1990) Haploids in cereal improvement: anther and microspore culture.
- Konzak CF, Polle E, Liu W & Zheng YM (1999) Methods for generating doubled haploid plants. US Patent 6,362,393.
- Kyo M & Harada H (1986) Control of the developmental pathway of tobacco pollen in vitro. Planta 168: 427–432
- Mejza SJ, Morgant V, DiBona DE & Wong JR (1993) Plant regeneration from isolated microspores of Triticum aestivum. Plant Cell Rep. 12: 149–153
- Reynolds TL (1993) A cytological analysis of microspore of Triticum aestivum (Poaceae) during normal ontogeny and induced embryogenic development.
- Sunderland N & Roberts M (1977) New approach to pollen culture. Nature 270: 236–238
- Touraev A, Vicente O & Heberle-Bors E (1997) Initiation of microspore embryogenesis by stress. Trends Plant Sci. 2: 297–301
- Tuvesson IKD & Ohlund RCV (1993) Plant regeneration through culture of isolated microspores of Triticum aestivum L. Plant Cell Tiss. Org. Cult. 34: 163–167
- Vicente O, Benito-Moreno RM & Heberle-Bors E (1991) Pollen cultures as a tool to study plant development.
- Wei ZM (1982) Pollen callus culture in Triticum aestivum L. Theor. Appl. Genet. 63: 71–73
- Zhao JP, Simmonds DH & Newcomb W (1996) Induction of embryogenesis with colchicines instead of heat in microspores of Brassica napus L. cv. Topas. Planta 198: 433–439
- Zhuang JJ & Xu J (1983) Increasing differentiation frequencies in wheat pollen callus. In: Hu H & Veda MR (eds) Cell and Tissue Culture for Cereal Crop Improvement (pp. 431–432). Science Press, Beijing