# THE EFFECT OF CHILLING ON REGENERATION OF MICROSPORE DERIVED EMBRYOS OF *Brassica napus* L.

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## ABSTRACT

The effect of chilling on microspore-derived embryos' regeneration was studied in *Brassica napus* L. For this aim, $F_1$ seeds of PF7045/91×Quantum cross was planted in growth chamber under conditions of 19°C and 16/8-h photoperiod (200 $\mu$mol $m^{-2}$ $s^{-1}$). Small buds with 3-3.5 mm length, containing microspores at very late uninucleate stage were collected from racemes and cultured in modified NLN-13 medium in the dark at 32.5°C for 3 days. Cultures were then transferred to dark condition at 25°C and shaken at 70 rpm. Cell divisions were observed using an invert microscope 3 days after culture. After 14 and 20 days, tiny globular and heart-shaped embryos were visible with naked eyes, respectively. Finally after 28 days, cotyledonary embryos with big cotyledons were produced. Mature embryos were cultured on B5 medium supplemented with 0.1 g/lit $GA_3$ and pretreated at 4 °C for 3, 6, 9 and 12 days in the dark. The embryos regenerated at 25 °C were as control. Then, pretreated cultures were transferred to the light (16-h light/ 8-h dark) at 25 °C conditions. The experiment was carried out in a complete random design with 4 replication. There was no significant difference between the means of regeneration frequencies at 4 °C for 3, 6, 9 days and the control. Chilling for 12 days showed significant difference at 1% probability level compared to the control as the frequency of regeneration decreased.

**Key words:** microspore, embryogenesis, chilling, regeneration, *Brassica napus*

## 1. INTRODUCTION

The importance of microspore culture technique is completely obvious in genetic and plant breeding studies. This approach can shorten the period of breeding up to 3 years to produce pure lines compared to traditional breeding which takes 6-8 years. Oil seed rape (*Brassica napus* L.) is an amphiploid species and about 40% oil in seed. Microspore culture in *B. napus* was first reported by Lichter [1]). There are many reports about factors affecting microspore embryogenesis and its application in plant breeding [2,3,4]. Keller *et al.*[3] conducted that the growth of donor plants at low temperatures increases the freqency of embryogenesis in rapeseed. Heat shock at 32.5 °C or 30 °C is a key factor for induction of embryogenesis [3,4]. There are several reports about the effect of genotype on embryo production. In this study, the effect of chilling on mature embryos' regeneration at 4 °C was examined.

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## 2. MATERIALS AND METHODS

For microspore culture, $F_1$ seeds of PF7045/91×Quantum cross were raised in a controlled condition at 19°C under a 16-h photoperiod and 200 $\mu mol.m^{-2}.s^{-1}$ light intensity. Microspores isolated from 3-3.5 mm long buds cotaining late uninucleate microspores (Fig. 1) were cultured in liquid modified NLN-13 medium. Then they were treated at 32.5°C for 3 days in the dark. Then cultures were transferred to dark room at 25°C and shaken at 70 rpm until 28-35 days after culture. Normal embryos were transferred to solid B5 medium supplemented with 0.1 g/l $GA_3$ under white light and pretreated at 4 °C for 3, 6, 9 and 12 days in the dark. The embryos regenerated at 25 °C were as control. Then, pretreated cultures transferred to the light (16-h light/ 8-h dark) at 25 °C. The experiment was carried out in a complete random design with 4 replications.

## 3. RESULTS AND DISCUSSION

Cell divisions were observed using an invert microscope 3 days after culture. After 14 and 20 days, tiny globular (Fig. 2) and heart-shaped (Fig. 3) embryos were visible with naked eyes, respectively. Finally after 23 and 28 days, torpedo (Fig. 4) and cotyledonary embryos (Fig. 5) were produced. Embryos started regenerating three days after transferring to solid medium (Fig. 6). According to the following table, there was no significant difference

**Table 1.** Comparison of the means of regeneration frequencies (per cent)

| Days exposured | 4 °C | 25 °C |
|---|---|---|
| Control | | 98a |
| 3 | 96a | |
| 6 | 92ab | |
| 9 | 98a | |
| 12 | 87b | |

The same letters show no significant difference between treatments according to Duncans' test.

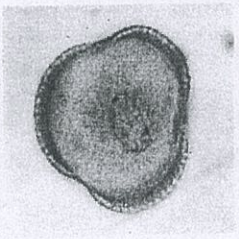

Fig. 1: Late uninucleate microspore

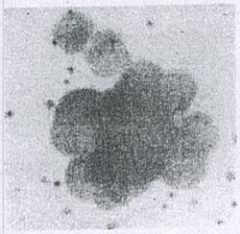

Fig. 2: Globular embryo

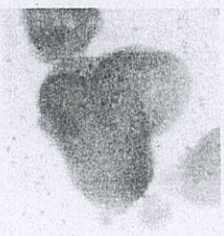

Fig. 3: Heart-shaped embryo

Fig. 4: Torpedo embryo

Fig. 5: Cotyledonary embryo

Fig. 6: Regenerated haploid plantlet

between the means of regeneration frequencies at 4 °C for 3, 6, 9 days and the control. Pretreatment at 4 °C for 12 days showed significant difference compared to the control at 1% of probability level. Kott *et al.* [5] increased the regeneration frequency of microspore-derived embryos by chilling at 4 °C against our result.

## 4. CONCLUSION

On the basis of obtained results, the effect of genotype can be considered as the cause of different regeneration frequecies. Choung et al [6] conducted that haploid production in *B. napus* is genotype dependent. Zhang *et al.* [7] statistically concluded that microspore embryogenesis is controlled by three genes.

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