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# Effects of Heat Shock and Culture Density on the Embryo Induction in Isolated Microspore Culture of *Brassica napus* L. Cv. Global

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**Abstract:** High yield and good quality embryos were obtained from cultures of isolated microspores of *Brassica napus* L. Cv. Global. The donor plants were grown in a growth chamber at 15/10°C (day/night) with a 16/8 photoperiod. Microspores were isolated from whole buds of 2.5-3.5 mm in length, containing late-uninucleate and early-binucleate microspores. Different heat shocks including, 30°C for 10, 14 and 18 days, 32°C for 2 and 3 days and 35°C for 18 h followed by 30°C for 10 days and various culture densities including 60,000, 40,000 and 20,000 microspores per mL were used. Results showed significant differences among the heat shocks, the culture densities and their interaction for embryo induction. The largest number of embryos was obtained from the microspores treated at 30°C for 18 days, 35°C for 18 h followed by 30°C for 10 days and 30°C for 14 days for a density of 60,000 microspores per mL.

**Key words:** *Brassica napus* L., microspore culture, embryogenesis, heat shock, culture density

## INTRODUCTION

The most effective way to produce haploids has been through androgenesis, by means of isolated microspores culture. This culture system could be utilized as a model system for somatic embryo induction, genetic manipulation and *in vitro* developmental studies[1]. Since the first report of isolated microspore culture in *Brassica napus*[2], there has been remarkable progress in developing this system. Numerous factors are required for high levels of embryogenesis from *Brassica* microspores. These factors include culture medium, growing condition of donor plants, genotype, microspore development stage[3,4] and the incubation of microspores at elevated temperature during the early stages of culture[5]. Culture density is another factor that may influence microspore culture in Brassica. A wide range of densities has been used, such as more than 100,000 microspore per mL[6], 50,000 microspore per mL[7] and 16,000 to 24,000 microspores per mL[8]. In this study we examined the effect of culture density and heat shock on efficiency of microspore embryogenesis in *Brassica napus* Cv. Global.

## MATERIALS AND METHODS

**Plant growth conditions:** A spring cultivar of rapeseed (*Brassica napus* Cv. Global) was examined in this study. Seeds of this experiment were kindly provided by Oilseed Research and Development Co. (Tehran, Iran). Donor plants were grown in a growth chamber with 16 h photoperiod and a day/night temperature of 15/10°C by sodium lights.

**Microspore culture:** Buds were selected on basis of size (2.5-3.5 mm), placed in baskets and surface sterilized in 5.25% sodium hypochlorite (Golrang) for 10 min on a shaker followed by two 5 min washes with sterile water. Up to 100 buds, the majority of which were at late-uninucleate and early-binucleate stage were blended with a cool blender in 30 mL of cold microspore isolation solution with 13% sucrose and pH 6[9]. The crude suspension was filtered through a 106 μm metal mesh followed by a 53 μm mesh, both cups and meshes were rinsed and a total of 50 mL was collected into two 50 mL centrifuge tubes and the microspore suspension was centrifuged at 200 g (1270 rpm) for 4 min, the supernatant removed and 25 mL of microspore isolation solution was added to each tube. This procedure was repeated twice, then 4-5 mL of filter-sterilized and modified NLN-13 liquid medium[2] supplemented with 13% sucrose but free of potato extract and growth regulators, was added to microspores. Then, the culture density was determined by a hemacytometer to achieve the desired density. Ten milliliter of microspore suspension was dispensed into each 120x20 mm sterile glass petri dish. Cultures were incubated in the dark and high temperature for the heat shock treatment. Then, the cultures were transferred to

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25°C and darkness on a shaker (40 rpm). In this study, three densities, 20,000, 40,000 and 60,000 microspore per mL and six heat shocks, 10, 14 and 18 days at 30°C and darkness, 2 and 3 days at 32°C and darkness and 18 h at 35°C followed by 30°C for 10 days was used. A factorial experiment on basis of Complete Randomized Design (CRD) with 2 factors and 8 replications (each replication was a petri dishe) was used in this study. The first factor (A) was the culture density with 3 levels and the second factor (B) was heat shock with 6 levels. After 30 days, number of embryos was determined.

## RESULTS

The analysis of variance showed significant differences between culture densities, heat shocks and their interaction for embryogenesis. The embryo yield differed according to the initial heat shock and culture density (Fig. 1). Global embryos were observed after 10 days of culture (Fig. 2A). These embryos continued to develop respectively to heart, torpedo and cotyledonary embryos (Fig. 2 B-D). The mass production of embryos was obtained in this experiment (Fig. 3). The largest number of embryos was obtained from the microspores treated at 30°C for 18 days, 35°C for 18 h followed by 30°C for 10 days and 30°C for 14 days for density of 60,000 microspores per mL. The incubation of microspores at 35°C for 18 h followed by 30°C for 10 days was the best temperature for densities of 40,000 and 20,000 microspores. Also 2 and 3 days incubation of microspores at 32°C and 10 days at 30°C for density of 40000 microspores per mL were the less effective heat shock for embryo production. Other heat shocks were also effective for embryo induction, but the yields were not as high as heat shocks described above.

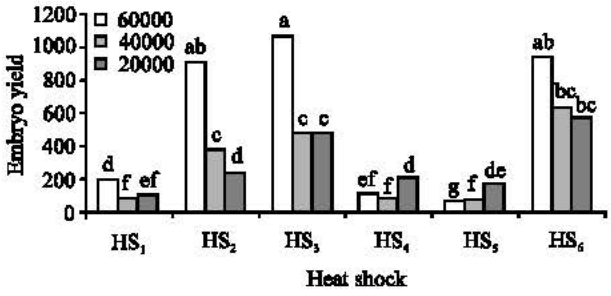


Fig. 1: Mean performance of heat shocks and culture densities interaction for embryo production in microspore culture of *Brassica napus* Cv. Global. HS: Heat shock, HS 1: 30°C for 10 days, HS 2: 30°C for 14 days, HS 3: 30°C for 18 days, HS 4: 32°C for 2 days, HS 5: 32°C for 3 days, HS 6: 35°C for 18 h followed by 30°C for 10 days

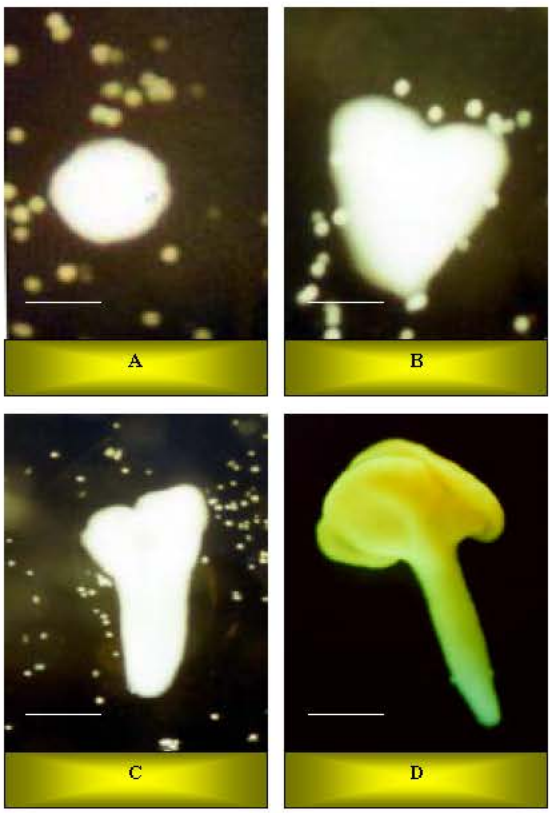


Fig. 2: Different stages of embryogenesis in microspore culture of rapeseed (*Brassica napus* Cv. Global). Globular stage (A). Heart stage (B). Torpedo stage (C). Cotyledonary stage (D). Bar: 1000 μm, Magnification: 40X

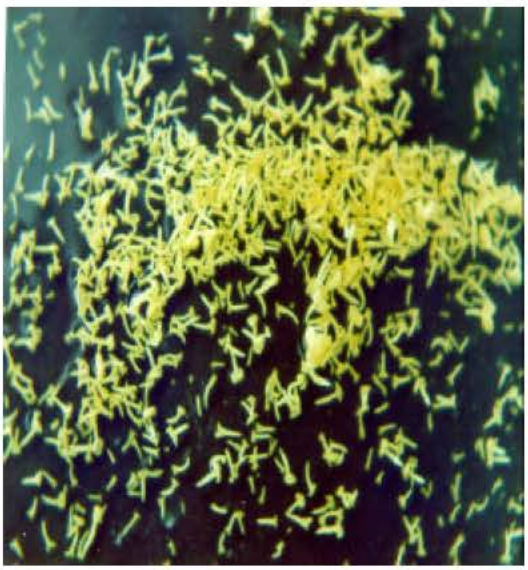

Fig. 3: The mass production of embryos in microspore culture of *Brassica napus* Cv. Global

## DISCUSSION

It has been suggested that the elevated temperature inhibits protein synthesis, leading to sporophytic induction. Alternatively, heat-shock may also cause destruction of pre-existing post-transcriptional control apparatus in the microspore. The destruction of these post transcriptional control mechanisms may contribute to the re-programming of microspore development toward embryogenesis.

In this study, the differences between the responses of microspores to heat shocks are consistent with the findings of Yang *et al.*[10], Takahata and Keller[11]. Previous studies on *Brassica napus* have also shown that a heat shock for 18 h at 35°C followed by 30°C for 10 days[3] and culture temperature of 30°C for 14 days[9] increased the number of embryos per bud considerably and our results are consistent with these studies. Thus, in *Brassica* species, an initial culture period of elevated temperature is required for embryogenesis[11,12]. Concerning the culture density in microspore culture. Huang *et al.*[1] found that in microspore culture of *Brassica napus* Cv. Topas embryo yields increased with culture density up to about 40000 microspores/mL. Also, the minimum density for induction of embryogenesis was 3000 microspores/mL. A much higher density (100,000 microspores/mL) appeared to be inhibitory to embryogenesis[1]. Kott *et al.*[13] suggested that the reduction in embryogenesis in higher densities resulted from a toxin which was released from microspores themselves. Also Duijs *et al.*[14] found no clear effect of the plating density except when there was a high proportion of binucleate pollen in cultures. In the present study the interaction of culture density with heat shock was significant. Thus, embryo yields for various culture densities differed according to the used heat shock.

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