# EMBRYOGENIC RESPONSIBILITY OF SELECTED GENOTYPES OF *BRASSICA CARINATA* A. BRAUN TO MICROSPORE CULTURE

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***Abstract***

*Microspore culture protocol has been modified to produce satisfactory amount of embryos from six genotypes of Brassica carinata. The highest frequency of cell division and embryo yield was obtained from 2.5 mm to 3.25 mm long buds in cultivar Dodolla, whereas other genotypes of B. carinata utilized in the experiment were favourable when microspores from 3 - 3.5 mm long buds were cultured under continuous 30°C temperature treatment. Significant difference in frequency of microspore embryogenesis was also determined between genotypes. Cultivation of microspores in the liquid NLN medium with 0.05 mg/l colchicine for 18 hours was more efficient for the embryo production than the control.*

**Key words**: *brassica carinata*, haploids, doubled haploids, microspore embryogenesis

## INTRODUCTION

Ethiopian mustard (*Brassica carinata*, A. Braun) is an amphidiploid with the BB genome derived from *Brassica nigra* and CC genome from *Brassica oleracea (*Snowdon et al., 199) . It is mainly self-pollinating *Brassica* oilseed crop constitute the third most important source of vegetable oil in the world **,** as well as in Ethiopia after noug (*Guizotia abyssinica,* Cass**)** and linseed *(Linum asitatisimum* L.) both in terms of area and production (CSA 1998).

The oilseed crop *Brassica carinata* is grown in the highlands of the Ethiopia plateau and its cultivation believed to date back in the 4$^{th}$ to 5$^{th}$ Millennia BC (Nigussie, 1999). Traditional utilization of this crop embraces quite array of purposes. The young leaves are eaten as a vegetable; the seed oil is primarily for human consumption, but also for a range of non edible applications, such as lubricants, plasticizers and detergent ingredients.

Ethiopian mustard plays a significant role in the farming system of Ethiopian agriculture. It is a break crop of cereals in different agro ecology highlands of Ethiopia, used as green manure. Besides this, grown under semi-arid conditions it has several desirable agronomic characteristics compared with other *Brassica* crops, the root system is much more developed than in *B. napus* and the crop is resistant to drought, wide range of diseases and pests **(**Malik, 1990; Getinet *et al.,* 1996**).** Ethiopian mustard is particularly suitable for organic farming, acting as a biopesticide. Despite these positive attributes all cultivars developed so far however, are avoided, because of high erucic acid in oil and glucosinolates in meal **(**Nigussie A and Becker, 2001**)**. However, accelerated improvement of the species may be aided by more recently developed biotechnological techniques such as the use of doubled haploids (DH) in breeding process.

Microspore culture protocol was first reported in *B. napus* **(**Lichter, 1982**).** The method was adopted and modified for Czech cultivars and breeding lines by Vyvadilova and Zelenkova(1992**)** and later applied in the Czech breeding programs of different *Brassica* species.

The doubled haploid methodology is now employed in breeding programs of many crops around the world as alternative/ supplement to conventional methods of homozygous line production (Barro and Martin, 1998; Kucera *et al.,* 2002); The production of doubled haploids through *in vitro* microspore culture to improve the *Brassica* oilseed breeding has several advantages against classical breeding methods. *In vitro* haploid production provide rapid development of homozygous lines and has also potential for more applications as a versatile genetic manipulation tool for evaluation of desirable genotypes **(** Barro and Martin, 2001**)**.

In the country like Ethiopia where the main economy activity is agriculture, biotechnology has revolutionary potential for the enhancement of crop production. Biotechnology approach towards the production of doubled haploids by means of microspore culture could contribute to both high yield and desired seed quality. Therefore, high frequency of microspore embryogenesis and production of DH plant regeneration is essential for effective and routine utilisation in breeding programmes.

This work was aimed at development an efficient and reliable microspore culture protocol for the generation of a large number of microspore derived embryos and their rapid development into doubled haploid plants in different *B. carinata* genotypes.

## MATERIAL AND METHODS

### Plant material

Five *Brassica carinata* genotypes provided by Czech University of Life Science, Institute of Tropics and Subtropics developed by Doc.Ing.Miroslav Bechyne and high productive but high erucic acid *B. carinata* cultivar Dodolla received from Ethiopia Genebank were used for experiments.

Donor plants for microspore culture were cultivated in the growth room under controlled environmental conditions with 16/18 hrs photoperiod and at day/ night temperature 15/10°C. Mineral fertilizer "NPK" was applied weekly.

**Microspore culture and plant regeneration**

Microspore isolation was carried out according to Coventry *et al.* (1988) with some modifications. After purifying procedures the clean microspore suspension was divided to equal parts and rsuspended in 10 ml of particular doubling agent solutions (0.05 mg/l colchicine, 10 µmol/l of trifluraline or 20 µmol/l of oryzaline). Each microspore suspension was then dripped to 9 cm diameter plastic Petri dishes and cultivated for 18 hrs in the thermostat at 30°C in the dark. After 18 hrs treatment, the suspensions were purified, resuspended in fresh NLN medium and cultured at the same conditions as described above. After three weeks of cultivation, embryos were counted at heart and torpedo stage (at least 2 mm in length) and the Petri dishes were placed on the shaker (70 rpm). Further cultivation carried on according to Klíma *et al.* (2004). Well established plantlets were transferred to a rooting MS medium without growth regulators. Plantlets with roots were then transferred to the soil.

## RESULTS AND DISCUSSION

The best embryogenic response was achieved in Dodolla cultivar with microspores isolated from buds 2.5 mm to 2.25 mm in length, whereas in four breeding lines of *B. carinata* utilized in the experiment were favourable for embryogenesis buds from 3 mm to 3.5 mm long (Table 1).

The highest embryo yield in all investigated genotypes was obtained from a culture density between 60,000 to 124,000 microspores/ml of NLN media. The best yield of embryos at globular and heart stage per Petri dish were achieved in cultivar Dodolla (Table 2). Similar results in bud size and density were observed by F. Barro and A. Martin (1998) and Deepak P..(2004)

Frequency of embryogenesis and microspore embryo yield in microspore cultures treated with individual antimitotic chemicals were higher than in control cultures with conventional NLN medium. The most positive influence was shown especially with 0.05% colchicine in both tested genotypes (Table 3). It was obvious that lower concentration of oryzalin and higher microspore culture density resulted in higher frequency of embryo production. Similar observations were made by Jiping Zhao and Daina H. Simmonds (1995).

We observed that cultivation of microspores in presence of trifluralin and oryzalin for 18hrs slowed down the embryogenesis and embryo formation at least one week, whereas NLN with colchicine enhances embryogenesis and never suppress formation of embryos. The most important factor affecting microspore culture efficiency and direct microspore embryos development in our experiment was genotype responsibility to microspore culture. Commercial cultivar Dodolla, genotype IV, VI and II respectively, showed the best embryogenic responsibility, whereas genotype I and III had very low efficiency for androgenic embryo production (Table 2). But even in responsive genotypes there were differences in embryo yield between individual plants. Therefore the genetic factor plays significant role in embryo development.

## CONCLUSION

An effective microspore culture technique is essential for high embryo yield and development of homozygous diploid lines of *Brassica carinata*. In our experiments (utilized different genotypes of *B.carinata*) identified that the highest frequency of microspore embryogenesis was obtained from the late uninucleate microspores from bud sizes 2.5 mm to 3.5 mm irrespective of genotype and planting densities of 60 000- 124 000 microspores/ml cultured under continuous 30°C temperature treatment. But even among responsive genotypes there were differences in embryo yield between individuals. Therefore the genetic factor plays significant role in embryo development.

Production of doubled haploid lines through the application of microspore culture is more rapid and effective method than conventional breeding with 6- 8 generations of inbreeding. Further more the application of doubled haploid lines is important for study the genetic control of traits. In general In vitro (tissue culture) techniques are more labour intensive, time saving and some times low cost Therefore application of microspore culture technique to small scale agriculture has a big potential to solve the most pressing problems of agriculture in my country Ethiopia.

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**Tab. 1. :** Optimal bud size of selected *Brassica carinata* genotypes under continuous temperature treatment (30°C)

| Genotype | Bud length [mm] |
|---|---|
| Dodolla | 2.50–3.25 |
| Line I. | 3.00–3.50 |
| Line II. | 3.00–3.50 |
| Line III. | 3.00–3.50 |
| Line IV. | 3.00–3.50 |

**Tab. 2. :** Number of embryos at globular and heart stage per Petri dish

| ***B. carinata*** genotype | **Embryos per Petri dish** | | | Embryos transferred to DM | | |
|---|---|---|---|---|---|---|
| | Replication | | Mean | **Replication** | | Mean |
| | **1.** | **2.** | | **1.** | **2.** | |
| Dodolla | 51 | 73 | 62.0 | 35 | 42 | 38.5 |
| Line I. | 15 | 30 | 22.5 | 0 | 17 | 8.5 |
| Line II. | 35 | 47 | 36.0 | 24 | 22 | 23.0 |
| Line III. | 4 | 21 | 12.5 | 3 | 7 | 5.0 |
| Line IV. | 67 | 52 | 59.5 | 29 | 33 | 31.0 |
| Line VI. | 55 | 43 | 49.0 | 27 | 25 | 26.0 |

**Tab. 3. :** Influence of various antimitotic agents upon microspore culture of selected *Brassica carinata* genotypes

| Chemical | Density of microspores [$1 \times 10^3$/ml] | **Genotype** | | | |
|---|---|---|---|---|---|
| | | **Line IV.** | | **Dodolla** | |
| | | **Mean number of embryos** | | | |
| | | Per Petri dish (globular stage) | Transferred to DM | Per Petri dish (globular stage | Transferred to DM |
| **Colchicine** | 97 and 124* | 107 | 63 | 147 | 72 |
| | 70* | – | – | 63 | 32 |
| Trifluraline | 70 and 100* | 61 | 41 | 25 | 15 |
| Oryzalin I | 70 and 100* | 65 | 43 | 72 | 36 |
| Oryzalin II | 70 and100* | 50 | 39 | 57 | 48 |
| Control | 70 and 100* | 54 | 34 | 64 | 45 |

Key…* Density of microspores of genotype Dodolla

**Tab. 4. :** Optimal microspore culture density of different B. carinata genotypes (haemocytometer counting)

| Genotype | Microspores/ml | **Average embryo yield per Petri dish** |
|---|---|---|
| Dodolla | 124,000 | ++++ |
| Line I. | 100,000 | +++ |
| | 164,000 | + |
| Line II. | 60,000 | +++ |
| | 172,000 | + |
| Line III. | 70,000 | +++ |
| Line IV. | 97,000 | ++++ |

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