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Pollen-embryogenesis and chromosomal variability in anther culture of *Brassica hirta* Moench (*Sinapis alba* L)

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ABSTRACT

The anther cultures of Brassica hirta underwent poIlen-embryogenesis and callusing, which showed a wide range of chromosome numbers varying from 9 (n=12) to a highly polyploid. For embryogenesis, pretreatment of floral buds in 0.4M sucrose solution for 72 hrs at 4℃ was superior to freshly cultured anthers. Culture temperature of 30℃ for 14 days before maintenance of cultures at 25℃ was significantly beneficial for embryo yield in comparison to cultures conlinuously incubated at 25℃. Dark treatment during culture was more effective for pollen-embryo yield.

Key words: *Pollen-embryogenesis, anther culture, Brasia hirta chromosomal variability.*

INTRODUCTION

White mustard is an important oilseed crop. The in vitro induction of haploids, and the genetic variabiliby would considerably help to widen the genetic base for its further incorporation in other oilseed crop improvement programs. The significance of the in vitro induction of haploids [1, 2] and their utilization in oilseed Brassica breeding have been fairly emphasized [3, 4, 5]. In an earlier study the frequency of induced pollen embryos was very low E6], however it is necessary that the frequency of such events should be sufficiently high to enable their incorporation into breeding programs. The present investigation therefore deals with some of the factors enhancing pollen embryogenesis, and the induction of chromosomal variation in callus cultures.

MATERIALS AND METHODS

The seeds of white mustard, Brassica hirta Moenoh syn Sinapis alba L. were grown in the oilseed fields of the Punjab Agricultural Univeristy, Ludhiana during October-November, and normal agronomic practices were followed to raise the crop. The first produced young inflorescenees were collected from the healthy plants, and twe types of pretreatments were given, either (i) the excised inflorescences were inserted in a beaker of water, and kept in a refrigerator at 4℃ for 7 days in the dark, or (ii) the flower buds were

kept in 0.4M sucrose solution and cold treated for 3 days in dark. The freshly colleoted anthers were cultured as the controls.

Flower buds (2-3 mm) were sterilized in chlorine water for 4-5 minutes, and washed thoroughly in sterilized water. The anthers were excised from the buds and about 20 of them were cultured per test-tube. Most of the anthers were at the uninucleale stage. Three media were used for the culture i.e. (i) modified B5 medium [7] containing NAA (0.1 mg/1) and 2, 4-D (0.1 mg/1), (ii) modified B_5 with NAA (1 mg/1) +2, 4-D (1 rag/l), and (iii) MS + NAA (0.1 rag/l) + BAP (0.1 mg/l). All manipulations were carried out aseptically in a laminar flow chamber.

The cultured anthers were incubated in both light and dark at 25℃ continuously, and at 30℃ for a period of 14 days prior to maintenance at 25℃. The anthers were examined at weekly intervals for 8 weeks. The anthers and the calli were fixed periodically in acetic alcohol (acetic acid: alcohol, 1: 3), and acetoearmine squashing technique was followed to study embryogenesis and chromosome counts in the calli.

RESULTS AND DISCUSSION

The observations on the in vitro growth response of the excised anthers, effect of' various pretreatments on pollen embryogenesis, and the induction of variability in chromosome numbers are shown in Figures 1-3, and Table 1.

The excised anthers cultured on B_5 medium initiated callus within 3 weeks (Fig. 2A), and in some cases they burst open and a mass of callus was formed in 5 weeks (Fig. 2B). If the callus was not transferred to other media, it had a tendency to undergo rhizogenesis, and sometimes the whole surface of the callus was covered with small and very fine roots (Fig. 2C).

A glance at Table 1 and Figure 2 shows the beneficial effect of various pretreatments of the floral buds on the frequency of embryogonesis. The cold treatment caused an increase in embryogenic anthers from 1.28% in the untreated anthers to 3.24% in the cold-treated ones. The cold treatment, though enhanced the frequency of pollen embryos, at the same time it reduced the in tensity of callusing. Whereas 20% of the anthers callused in the control, in the pretreated anthers it was reduced to only 8.4%. The microscopic examination showed pollen at various stages of division of the nuclei (Fig, 2 D--F, Table 1). The frequency of uninucleate pollen was as high as 30.08%, followed by binucleate (5.27%), trinucleate (1.28%), tetranucleate (0.89 %), multinucleate (0.79%) and multicellular structures (0.39%), the rest either degenerated or the nuclei could not be identified.

The beneficial role of temperature shocks on the induction of pollen-embryos is established [8, 9]. The coldtreatment slows down the regular development of pollen and prevents their degeneration,which further depends on the temperature, duration of the cold treatment and varies wibh bho genotypes. In B. campestris, for instance 5℃ for 7 days reduced embryo yield [5], whereas continuous treatment at 4℃ for several days in B. napus was stimulatory [10]. Klimaszwski and Keller [6] reported that high temperature pretreatment (35℃) for two days prior to maintenance at 25℃ induced embryogenesis in B. hirta. We combined these two manipulations and pretreated the anthers at 47℃ for 3-7 days, followed by their

culture at 30℃ for 14 days, and Short incubating them at 25℃. By doing so considerable increase was noticed. Various stress pretreatments have been suggested to increase embryogenosis [11]. In the present study prefreating the floral buds with 0.4 M sucrose solution for 3 days at 4℃ before culture greatly helped the embryo yield (Table 1).

Tab. 1. Effect of various pretreatments of the floral buds an the frequency of androgenic anthers in Brassica hirta

Pretreatment condition	Duration of treatment	Number of anthers cultured	Number of embryogenic anthers	Frequency of embryogenic anthers()%
1. Control (no treatment)	-	262	1	1.28
2. 4℃ in water	7 days	340	6	1.76
3. 4℃ in sucrose solution (0.4M)	3 days	432	14	3.24

The cytological evaluation of anther-derived calli established the existance of wide chromosomal variability. In addition to haploid (n= 12), largo number of hypo-, and hyperhaploid cells, along with aneuploids and polyploids were observed (Fig. 1, 3). The callus cells were predominantly diploid (up 6o 59%) followed by haploids (26--47 %), the numbers less than haploids or more than tetraploids varied between 1--4%. Recently alomst similar observations have been made on anther culture of various grain legumes [12], and cotton [13].

The wide range of chromosomal variability in the anther-derived callus could possibly be utilized by raising plants from them. Since the callus cultures of Sinapis alba undergo differentiation to form shoots and somatic embryos [14, 15], there is a possibility of the regeneration of variant plants from cell cultures.

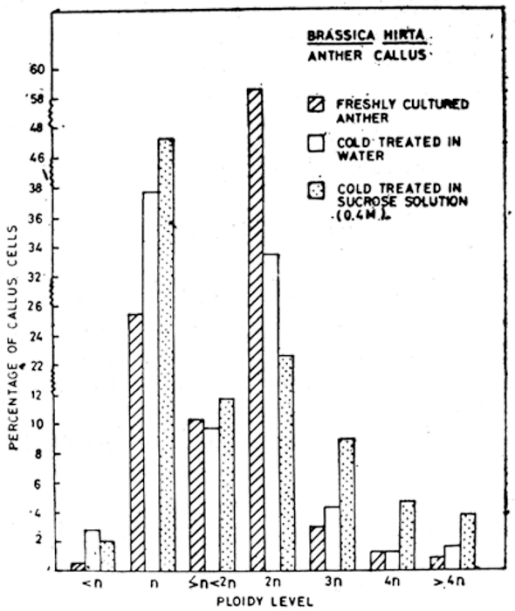

Fig. 1 Histogram showing the extent of genetic variability in anther-derived callus of B. hira after various treatments i.e. control (freshly cultured anthers), cold treated (4℃) anthers in wa~er, and cold treated in 0.4M sucrose solution for 3 days.

To conclude, the manifold increase in pollen-embryos by the cold treatment, and the wide range of chromosome numbers in the anther-derived callus would be helpful for the production of increased numbers of haploids and variant plants, and for their further incorporation into oilseed Brassica improvement programs.

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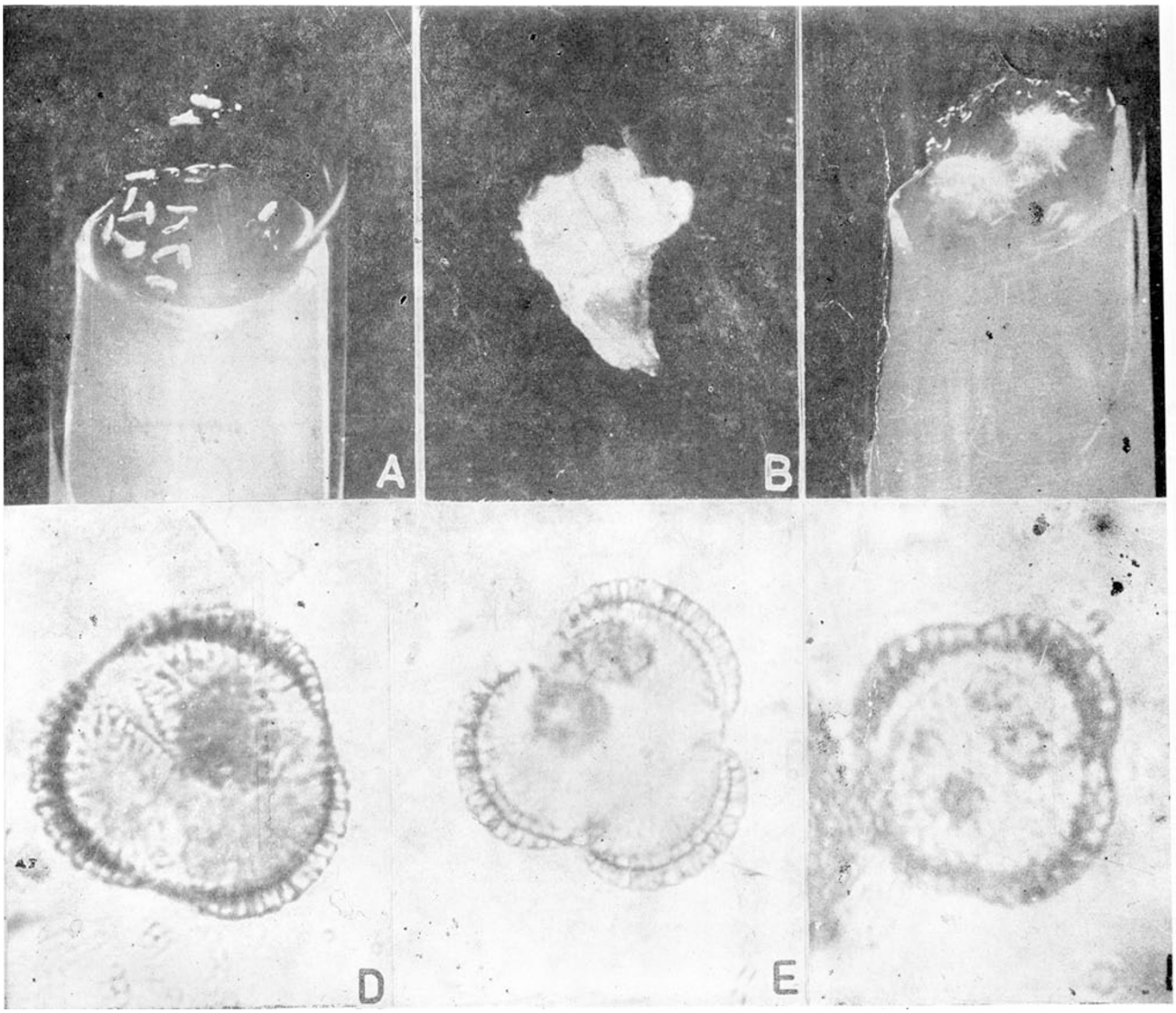

Fig.2 A-F. In vitro response of excised anthers of Brassica hirta cultured on modified B_5 medium. A. Anthers showing callus induction after 21 days of culture. B. Profuse callus, produced from the anther after 36 days of culture. G. Anther-derived callus producing roots after 40 days of culture. D--F. Uni-, hi-, and tetranucleate pollen from anthers of 21-day-old culture.

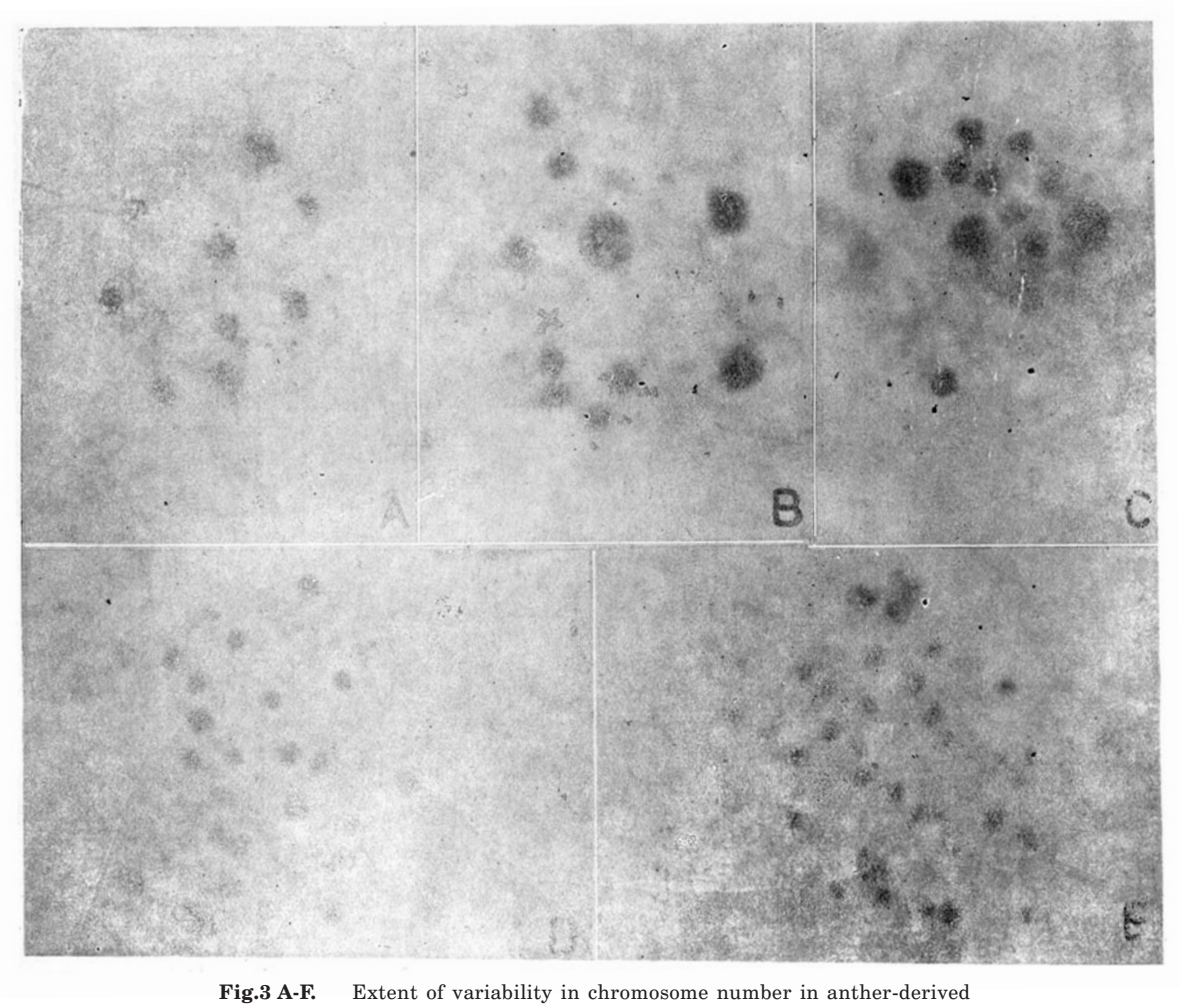

Fig.3 A-F. Extent of variability in chromosome number in anther-derived callus of B. hirta (n=12), Cell showing 9(A), 10(B), 12(C), 24 (D) and.35(E) chromosome numbers.