

Short communication

Somaclonal variation in indian mustard (*Brassica juncea* L.) plants derived from protoplasts

V. Abraham¹, V.T. Srinivasan², and Susan Eapen^{1*}

¹Nuclear Agriculture and Biotechnology Division, ²Radiation Biology and Biochemistry Division, Bhabha Atomic Research Centre, Trombay, Mumbai - 400 085, India.

Accepted 08 May 2000

ABSTRACT

Seeds collected from 53 plants regenerated through direct somatic embryogenesis in Indian mustard (*Brassica juncea* L.) Cv. Rai 5- from mesophyll protoplasts were grown from P₁ to P₉ to study the somaclonal variation for different agronomic characters. Three lines showed 3-5 days early flowering while another three lines showed late flowering compared to the control. Twelve lines selected for best yield in P₁ were evaluated for seed yield for seven generations (P₁ to P₉). Most of the selections were comparable to the control in seed yield. Seed oil content of protoclonal lines in P₁ and P₉ was lower in comparison with the control.

Key words: *Brassica juncea* L., flowering time, Indian mustard, oil content, protoclonal lines, seed yield, somaclonal variation.

INTRODUCTION

Indian mustard is an important oil seed crop of the tropics and is a model plant for transformation experiments. In general, for majority of transformation procedures, cell and tissue culture processes are indispensable. Somaclonal variation appears to be frequent in plants regenerated from somatic tissues and callus cultures of Indian mustard (George and Rao 1983; Katiyar and Chopra 1990). The occurrence of somaclonal variation will complicate the expression of introduced foreign genes and is one of the major problems in experiments on transformation. Although plant regeneration from protoplasts has been achieved in Indian mustard (Chatterjee *et al.* 1985; Eapen *et al.* 1989), detailed information is not available on the performance of the regenerants and their progenies except one preliminary report (Eapen *et al.* 1989). Therefore a study was undertaken to evaluate plants obtained from mesophyll protoplasts of Indian mustard for nine generations.

MATERIALS AND METHODS

In an earlier paper, we reported direct somatic embryogenesis and plant regeneration from mesophyll protoplasts of Indian mustard, *Brassica juncea* (L.) Czern and Coss Cultivar Rai - 5 (Eapen *et al.* 1989). Sixty plants (P₁) obtained from mesophyll Protoplasts of a single plant origin from cultivar Rai-

5 with black seeds were transplanted to pots, of which 53 survived to produce seeds. Seeds from these 53 plants, all of them with black seeds were used to raise P₂ generation and were scored for visible mutations and seed yield. Six lines selected for lateness or earliness in flowering were carried forward to the P₃ generation to study their true breeding nature. Besides, seeds from 12 best yielding P₂ lines were yield tested for 7 generations (P₂ to P₉). In all the experiments, each treatment had two rows 4 m long (2.4 m²) with 30 cm spacing between rows and 10 cm between plants arranged in a randomized block design with 3 replications. Rai-5 was used as the control. Urea (50 kg N ha⁻¹) was applied 20 days after sowing. The crop was irrigated and the pests were controlled by 0.07% malathion as and when required. Seed yield was evaluated for seven years and oil content for two years. For estimation of oil content, seeds were harvested, bulking 2 rows of each selection and from the general bulk 10 gm seeds in three replications were used for oil estimation. The oil content in the seeds was estimated using Pulsed Nuclear Magnetic Resonance method (Srinivasan *et al.* 1985).

RESULTS AND DISCUSSION

In P₂, three lines started flowering early (31-34 d) in comparison with 36-43 d for the parent line. Three other lines were late flowering taking more than 45 Days for the first flowers to appear. These six lines were true breeding for this character up to P₉.

Abbreviations: P₁ to P₉ - Protoclonal generations 1 to 9 respectively.

*Corresponding author.

Fax-92 22 5560750,

Email - biotech@magnum.barc.ernet.in

generation.

The seed yield of P_2 lines was very low compared to the parent variety Rai-5, since all the protoclonal lines were found to be more susceptible to pests. Out of 12 P_2 lines selected for the highest seed yield, two lines in P_3 were found superior to the control in seed yield. However, in P_4 all 12 lines were inferior in seed yield (Table 1). Eight out of 12 lines were statistically at par with the donor parent in P_3 . In P_4 seven protoclonal lines were comparable to the control in seed yield. One line showed significant increase in yield over the control in P_3 generation. In P_5 , only three lines were at par with the control, while in P_6 eleven lines were comparable to the control. However, none of the protoclonal lines showed a consistent increase in seed yield over the control in all generations tested. Analysis of oil content (P_3 and P_4) showed that all protoclonal lines were inferior in oil percentage in comparison with the control (Table 1).

Table 1. Seed yield and oil percent of protoclonal lines in Indian mustard from P_3 to P_6 generations (1988-1995).

Selection	Seed Yield (tha*)								Oil Per Cent	
	P_3 88-89	P_3 89-90	P_4 90-91	P_4 91-92	P_4 92-93	P_5 93-94	P_5 94-95	P_3 88-89	P_3 89-90	
Sel. 3	0.54	0.46	0.53	0.73	0.31	0.63	0.96	30.4	28.4	
Sel. 5	0.75	0.52	0.75	0.107	0.41	0.58	0.91	31.3	34.6	
Sel. 12	0.81*	0.41	0.34	0.114	0.31	0.65	0.101	31.5	32.7	
Sel. 14	0.65	0.58	0.69	0.86	0.45	0.77	0.94	28.9	32.0	
Sel. 19	0.96*	0.59	0.52	0.79	0.53	0.78	0.103	30.9	33.9	
Sel. 21	0.78	0.38	0.36	0.70	0.43	0.62	0.88	30.1	33.6	
Sel. 28	0.78	0.41	0.61	0.114	0.39	0.37	0.88	30.1	31.4	
Sel. 29	0.72	0.48	0.54	0.45	0.29	0.38	0.59	30.9	32.6	
Sel. 30	0.64	0.41	0.32	0.113	0.46	0.80	0.104	31.1	33.8	
Sel. 37	0.74	0.57	0.38	0.105	0.34	0.59	0.93	29.8	33.4	
Sel. 39	0.58	0.51	0.89	0.12	0.70*	0.68	0.92	32.1	32.9	
Sel. 43	0.51	0.43	0.50	0.116	0.37	0.79	0.115	31.0	34.5	
Rai-5	0.60	0.82	0.77	0.129	0.35	0.105	0.102	34.0	36.8	
L.S.D.(0.05)	0.194	0.198	0.292	0.417	0.194	0.306	0.222	0.46	0.64	

*Significantly higher at 5% level.

In a previous study we isolated protoplasts from the leaves of a single *in vitro* plant (one month old) of Indian mustard and regenerated plants by direct somatic embryogenesis without an intervening callus phase. Even under such a short exposure to culture conditions, stable mutations for earliness or lateness could be isolated. These characters bred true up to nine generations. Tissue culture itself is mutagenic and is known to lead to genomic rearrangements (Phillips *et al.* 1994) leading to stable mutations.

Using callus cultures, Katiyar and Chopra (1990) also isolated somaclones for earliness in Indian mustard. Our results indicate that isolation and culture of protoplasts also leads to similar mutations as compared to callus cultures. Yamagishi *et al.* (1996) compared somaclonal variation in rice plants derived from protoplasts and callus cultures and found similar somaclonal variants in both the populations, although they found more polyploids among protoclonal lines. In the present study, the seed yield of the protoclonal lines tested was almost comparable to the control in all the generations (P_3 to

P_6), while oil content was lower. Abraham *et al.* (1988) conducted yield and oil analysis of somaclones of Indian mustard obtained from cotyledon explants and observed that seed yield of the yellow seeded mutants from tissue culture was equal to control while oil content was higher. However, the black seeded somaclones were inferior in yield and oil content in comparison with the control.

The occurrence of somaclonal variation that complicates the introduced foreign genes is one of the major problems in plant transformation. The present studies have shown that it is very difficult to avoid somaclonal variation when protoplasts are used for plant regeneration. Therefore, when transformed plants are generated starting from protoplasts, it is essential to screen them in the field and only plants that are comparable to control in all other agronomic qualities should be selected.

REFERENCES

- Abraham V, George L and Srinivasan VT 1988 Seed yield and oil content of mustard somaclones (*Brassica juncea* Linn. Czern and Coss.). *Curr. Sci.* 57: 1019.
- Chatterjee G, Sikdar SR, Das S and Sen S.K. 1985 Regeneration of plantlets from mesophyll protoplasts of *Brassica juncea* (L) Czern and Coss. *Plant Cell Rep.* 4: 245-247.
- Eapen S, Abraham V, Gerdemann M and Schieder O 1989 Direct somatic embryogenesis, plant regeneration and evaluation of plants obtained from mesophyll protoplasts of *Brassica juncea*. *Ann. Bot.* 63: 369-372.
- George L and Rao PS 1983 Yellow seeded variants in *in vitro* regenerants of mustard (*Brassica juncea* Coss. var Rai-5). *Plant Sci. Lett.* 15: 1-6.
- Katiyar RK and Chopra VL 1990 Somaclonally induced earliness in *Brassica juncea* germplasm accession with field resistance to important diseases. *Plant Breeding.* 140: 262-264.
- Phillips RL, Kaeppler SM and Olhopt P 1994 Genetic stability of plant tissue culture: Breakdown of normal controls. *Proc. Natl. Acad. Sci. USA.* 91 (12): 5222-5226.
- Srinivasan VT, Singh BB, Chidambareswaran PK and Sundaram V 1985 Estimation of cottonseed oil by pulsed nuclear magnetic resonance technique. *J. Am. Oil Chem. Soc.* 62: 1021-1025.
- Yamagishi M, Otani M and Shimada T 1996 A comparison of somaclonal variation in rice plants derived and not derived from protoplasts. *Plant Breeding.* 115: 289-294.