Short communication

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

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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 P_2 to P_9 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 P_2 were evaluated for seed yield for seven generations (P_3 to P_9). Most of the selections were comparable to the control in seed yield. Seed oil content of protoclones in P_3 and P_4 was lower in comparison with the control.

Key words: *Brassica juncea* L., flowering time, Indian mustard, oil content, protoclones, 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 (P_1) 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 P_2 generation and were scored for visible mutations and seed yield. Six lines selected for lateness or earliness in flowering were carried forward to the P_4 generation to study their true breeding nature. Besides, seeds from 12 best yielding P_2 lines were yield tested for 7 generations (P_3 to P_9). In all the experiments, each treatment had two rows 4 m long (2.4 m^2) 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 P_2, 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 P_9

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Abbreviations: P_1 to P_9 - Protoclonal generations 1 to 9 respectively.

generation.

The seed yield of P_2 lines was very low compared to the parent variety Rai-5, since all the protoclones 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_5. In P_6 seven protoclones were comparable to the control in seed yield. One line showed significant increase in yield over the control in P_7 generation. In P_8, only three lines were at par with the control, while in P_9 eleven lines were comparable to the control. However, none of the protoclones 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 protoclones were inferior in oil percentage in comparison with the control (Table 1).

Table 1. Seed yield and oil percent of protoclones in Indian mustard from P_3 to P_9 generations (1988-1995).

Selection	Seed Yield (t ha^{-1})							Oil Per Cent	
	P_3 88-89	P_4 89-90	P_5 90-91	P_6 91-92	P_7 92-93	P_8 93-94	P_9 94-95	P_3 88-89	P_4 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 palnts derived from protoplasts and callus cultures and found similar somaclonal variants in both the populations, although they found more polyploids among protoclones. In the present study, the seed yield of the protoclones tested was almost comparable to the control in all the generations (P_3 to P_9), 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 form 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.

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