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PRELIMINARY CHARACTERIZATION OF SOME PHYSIOLOGICALLY IMPORTANT MUTANTS IN *BRASSICA JUNCEA* L. COSS. & CZERN. VARIETY RLM 198

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ABSTRACT

This is a preliminary report of some EMS induced morphological mutants in *Brassica juncea* variety RLM 198. In this paper we report mutants in which the angle formed by the primary branches to the main shoot was 50% less than control plants. Such mutants can be used for obtaining higher plant population in the field which is not possible with existing higher yielding cultivars. We report some 'Early flowering' mutants in which flowering was advanced by 20 days increasing the effective seed filling duration. Total seed weight per siliquae increased by 78% over control. We also report 'waxy' mutants in which SEM studies revealed thick waxy deposits on leaf surface and some variants that show branching from the base.

Key words : Brassica, branching, EMS, Early Flowering, Waxy mutants.

The yields of brassicas are very low in India and it has been realized that there are some inherent defects in brassica plant that put constraints on productivity. Some of these defects have been summarized by Bhargava and Tomar [1]. To achieve a 'plant type' suited for monoculture [2] under Indian agricultural conditions mutagenesis can be used to induce variability in plant architectural characters. This variability can be used in conventional breeding programe. In this paper we describe certain EMS induced mutants which have desirable traits that can be exploited towards obtaining a suitable 'plant type'.

MATERIALS AND METHODS

Alkylating agent Ethyl Methane Sulfonate (EMS) was used for chemical mutagenesis. Different concentrations of EMS were prepared and seeds were treated as described previously [3]. The plants obtained in M_1 and in subsequent generations

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were studied for following morphological traits :

Plant architecture characters :

(i) Plant height (cm), (ii) Plant spread (cm): recorded as the maximum distance between the two branches at flowering, (iii) I branch node : The node from which the first primary branch originated from the main axis, (iv) Branch angle (degrees): The angle made by the primary branches to the main shoot.

Leaf shape and size :

The plants were screened for any change in leaf shape and size. Following leaf characters were studied: (i) Leaf length (cm) was recorded from the base of the lamina to the tip, (ii) Leaf Width (cm) was recorded as the maximum width of the leaf, (iii) Main Lobe length (cm), (iv) Main lobe width (cm), (v) Number of lobes: Total number of lobes in the leaf was counted as the number of leaflets with atleast 10% of the main lobe length, (vi) Number of 45 degree angles or sharp edges per unit main lobe length were recorded.

Leaf surface characteristics

Plants were screened for leaf surface differences using Scanning Electron Microscopy (SEM).

Reproductive behavior

M_1 and M_2 plants were studied for their reproductive behavior. Following characters were studied :

(i) Days to Flower : Days taken to first flower were recorded for all mutants.

(ii) The main branch length was recorded in cm

(iii) Siliquae Characters : The main shoot was divided into three regions : Top, middle and bottom. From each region five representative pods were selected. Each of the following characters was recorded as an average of these five pods :

Structural components : Peduncle length (cm), beak length (cm), pod length (cm).

Yield Components : number of pods on main branch, pod weight (mg), number of seeds/pod, Seed weight (mg)/pod.

Measurement of Photosynthetic rates

Variants obtained for leaf surface characteristics were studied for their rates of photosynthesis as the light perception and therefore the rates of photosynthesis may be different as compared to control plants. The measurements were made at 3rd, 5th, 7th and 9th nodes at 97, 104, 114 and 127 days after sowing using an Infra red gas analyzer (IRGA, LI 6200, LUI-COR Instruments, Nebraska, USA).

Mean, standard deviation, standard error of mean, coefficient of variation and regression coefficients were calculated as per standard statistical procedures for the data obtained.

RESULTS AND DISCUSSION

In this study EMS was used for mutagenesis and the mutant population in M_1 and M_2 were screened for morphological differences.

Branching Angle Acute (BAA) mutants : One mutant was obtained in M_1 generation wherein the angle formed by the primary branches to the main axis (13°) was only half that of the control plants (26°). In M_2 generation raised from this mutant, the average angle formed by the branches to the main stem at 19° was almost half that in control (37°). This gave an almost appraised appearance to the mutant plants (Fig. 1). These mutants also had different plant architecture and leaf phenotype as compared to control.

Table 1. Plant architecture characters in some morphological mutants of *Brassica juncea var.* RLM 198

Plant type		Plant Height (cm)	Plant Spread (cm)	First Branch Node
BAA mutants	Mean	187* ± 4.53	55.79* ± 4.09	4.10 ± 0.48
	Range	127-257	10-138	1-9
	CV (%)	18.7	56.36	48.45
BB mutants	Mean	215.33* ± 2.36	69.48* ± 3.25	0
	Range	172-251	28-127	0
	CV (%)	7.76	33.07	0
Control	Mean	226.83 ± 1.58	79.76 ± 2.43	4.86 ± 0.35
	Range	175-270	37-150	1-12
	CV (%)	6.97	30.47	72.8

*Significant change over control

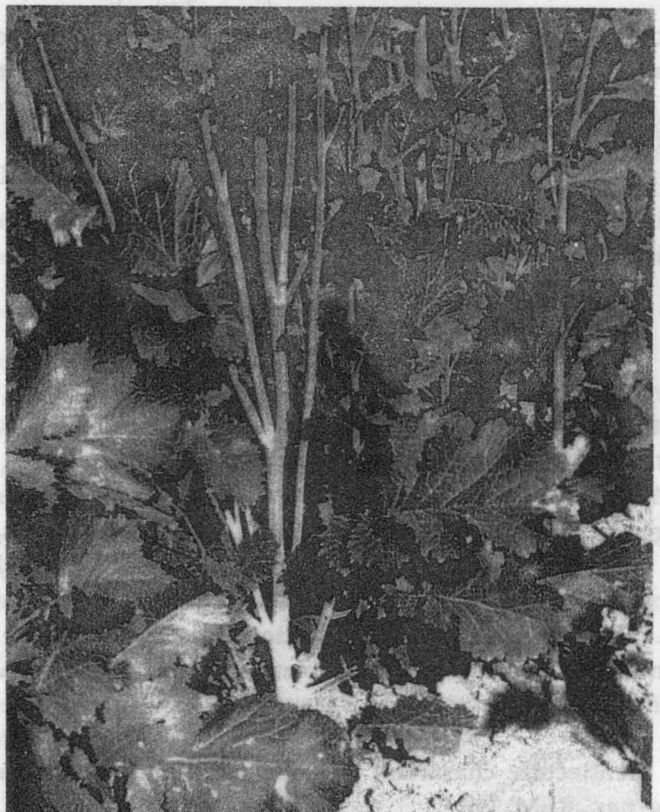

Fig. 1. Branching angle acute (BAA) mutants. The angle formed by the Primary branches to the mainshoot in the mutants (in foreground) was less than 50% of that in control plants (in background)

Plant architecture characters : The average plant height in control was 227 cm as compared to that of the mutant (188 cm). In these mutants the mean maximum spread was also significantly less as compared the control plants. The mean node from which the first branch arose was fourth in mutants as against fifth in control plants (Table 1).

Leaf Phenotype : In the mutant the average leaf length and main lobe length was significantly less than control. The average number of lobes in mutants was 10 as against 11 in control. The leaf though smaller was sharply serrated. The mean number of sharp edges (45 degree angles) in leaf was 7.8 per unit of main lobe length as against 6.8 in control leaf (Table 2).

Table 2. Leaf phenotype in some morphological mutants of Brassica juncea var. RLM 198

Plant type	Characters	Leaf length (cm)	Leaf width (cm)	Main lobe length (cm)	Main lobe width (cm)	Number of lobes	No of 45° angles per unit of main lobe length
BAA mutants	Mean	33.06*±0.90	16.63*±0.40	19.44*±0.68	16.63*±0.40	9.60*±0.37	7.82*±0.50
	Range	23-44	12-21	11-27	12-21	7-14	4-13
	CV (%)	13.61	12.02	17.38	12.02	19.27	31.71
Efl mutants	Mean	30.90*±0.66	14.31*±0.32	19.61*±0.32	14.31*±0.32	6.18*±0.26	5.10*±0.26
	Range	19-40	11-21	15-26	11-21	3-10	3-10
	CV (%)	15.05	15.44	11.58	15.44	29.29	36.28
Control	Mean	48.70±0.77	22.48±0.55	32.43±0.50	22.48±0.55	10.56±0.27	6.77±0.24
	Range	39-54	17-27	28-38	17-27	8-13	4-9
	CV (%)	7.88	12.32	9.07	12.32	12.6	18

*Significant change over control

Siliquae characters : The BAA mutants were divided in four classes on the basis of branch angle formed by primary branches to the main axis. In class I branch angle was 10°, in class II branch angle was between 10°-20°, in class III branch angle was between 20°-30° and in class IV, branch angle was > 30°. Flowering was delayed in these mutants. The average days taken to first flower were 78 as compared to 74.5 in control.

Structural Components : Main branch length in all classes of BAA mutants showed an increase over control. Beak length got reduced significantly in comparison to control in BAA mutant class II, III and IV and peduncle length was reduced in BAA mutants class II and IV. Also a reduction in pod length was observed in all classes of BAA mutants (Table 3).

Yield Components : An increase in the number of pods on the main branch was observed in class II and III mutants. In middle and upper positions of the main shoot a reduction in pod weight was observed in all classes. A dramatic reduction

Table 3. Structural components for siliquae on main shoot in M_2 generation of BAA mutants of *Brassica juncea* var. RLM 198

Plant type \ Components		Main branch length (cm)	Beak length (cm)	Peduncle length (cm)	Pod length (cm) Upper	Middle	Lower	Mean
BAA class I mutants	Mean	63.81*±5.12	0.40±0.02	1.43±0.04	2.68±0.21	3.51±0.13	3.23*±0.23	3.14
	Range	51.5-82.5	0.4-0.5	1.4-1.6	1.9-3.2	3.1-3.8	2.5-3.8	
	CV (%)	25.36	15.80	8.84	24.76	11.70	22.50	
BAA class II mutants	Mean	61.79*±4.89	0.36±0.04	1.33*±0.10	2.58±0.14	3.36*±0.11	3.32*-0.10	3.09
	Range	33.5-87.0	0.27-0.58	1.3-1.6	1.9- 3.3	2.6-3.8	2.7-3.9	
	CV (%)	25.00	35.12	23.76	17.15	10.34	9.52	
BAA class III mutants	Mean	69.18*±5.95	0.36±0.09	1.44±0.07	2.74±0.08	3.46*±0.29	3.34*±0.16	3.18
	Range	59.5-80.0	0.3-0.9	1.3-1.5	2.6- 2.9	2.9-3.3	3.1-3.6	
	CV (%)	27.18	79.00	15.36	9.24	26.49	15.14	
BAA class IV mutants	Mean	61.76*±4.97	0.30*±0.02	1.32*±0.03	2.53±0.12	3.48*±0.10	3.2±0.11	3.07
	Range	42.0-84.5	0.3-0.5	1.2-1.4	1.8-2.9	3.0-3.9	2.8-3.8	
	CV (%)	25.43	21.07	7.18	14.99	9.08	10.89	

*Significant change over control

in seed number was observed in class I and IV. The total seed weight got reduced in all classes of these mutants. A drastic reduction was observed in seed weight in BAA class I (60% less than control) mutants. Mean seed weight per pod for pods borne lower, middle or upper portion got reduced (Table 4).

This BAA type of mutants could be useful for understanding the development of branches and branch angles. Besides this, these mutants could also be useful in maintaining higher plant population in the field. The recommended plant spacing in India is either 60 × 15 cm, 45 × 15 cm or 30 × 15 cm which gives a plant population of 11, 15 or 22 plants m^{-2}. This is exceptionally low in comparison to the plant population of 200-300 plants m^{-2} maintained in western countries [5]. The existing higher yielding Indian cultivars are not suitable for growing in a crowded community because of morphological constraints [6]. BAA mutants could prove to be valuable for introgression of this character in these cultivars.

Table 4. Yield components for siliquae on main shoot in M_2 generation of BAA mutants of *Brassica juncea* var. RLM 198

Plant type	Components	Number of pods on main shoot	Pod weight (mg)			Seed number/pod			Seed weight (mg)/pod		
			Upper	Middle	Lower	Upper	Middle	Lower	Upper	Middle	Lower
BAA class I mutants	Mean	43.36±6.35	18.46* ± 2.94	47.11* ± 6.40	41.63* ± 8.74	5.68* ± 1.28	90.00* ± 0.75	6.40* ± 1.53	6.66* ± 2.08	15.40* ± 2.90	15.40* ± 0.37
	Range	29.0-66.0	10.1-28.3	33.8-68.2	23.8-74.6	1.8-9.0	7.4-11.8	3.0-11.6	2.16-14.0	3.4-32.7	5.26-12.3
	CV (%)	46.28	50.33	42.93	66.34	71.21	26.34	75.54	98.69	59.51	7.59
BAA class II mutants	Mean	55.17 ± 4.24	28.17 ± 3.10	58.53 ± 6.48	59.31* ± 4.44	7.66* ± 1.00	9.16* ± 0.93	8.76 ± 0.79	11.1* ± 3.00	25.5* ± 3.78	26.7* ± 3.10
	Range	24.0-72.0	11.1-66.0	31.9-90.2	41.0-83.3	2.6-12.0	6.0-12.6	5.0-12.6	2.0-32.4	3.7-42.5	12.0-40.5
	CV (%)	24.32	34.77	34.99	23.66	41.25	32.08	28.50	85.41	46.84	36.64
BAA class III mutants.	Mean	59.00* ± 6.08	38.50 ± 1.29	77.64* ± 1.66	6.681* ± 3.39	10.34 ± 0.37	12.14 ± 1.12	10.70* ± 1.79	21.48 ± 3.04	36.3 ± 2.33	33.5* ± 2.33
	Range	48.0-69.0	36.0-40.3	74.4-80.0	62.8-74.8	9.8-11.0	12.6–13.8	8.4-14.2	19.5-23.1	30.2-393	29.8-37.8
	CV (%)	32.56	10.59	6.76	16.03	11.31	29.50	52.89	44.69	20.28	21.98
BAA class IV mutants	Mean	51.10 ± 3.77	25.00* ± 3.71	58.52* ± 6.63	60.1* ± 5.18	4.98 ± 0.72	9.38* ± 0.85	8.02* ± 0.73	9.20* ± 1.86	27.6* ± 2.94	28.0* ± 2.76
	Range	33.0-64.0	14.9-50.6	14.8-80.5	35.2-82.5	1.8-8.0	5.6-2.4	4.0-11.0	40.0-19.6	12.9-37.5	12.3-40.4
	CV (%)	23.31	46.89	35.80	27.24	45.69	28.64	28.76	63.89	33.66	31.14

*Significant change over control

Basal Branching variants (BB) :

54 plants (2% of the total population) were obtained in M_1 generation in which the branches arise from the very base of the stem (Fig. 2). The mean spread in the variant was 48 cm as against 90 cm in control. In M_2 and subsequent generations it was not possible to purify plants with this character. The plants with basal branching appeared at random in the population. In M_2 generation also about 1% of the population showed branching from the very base.

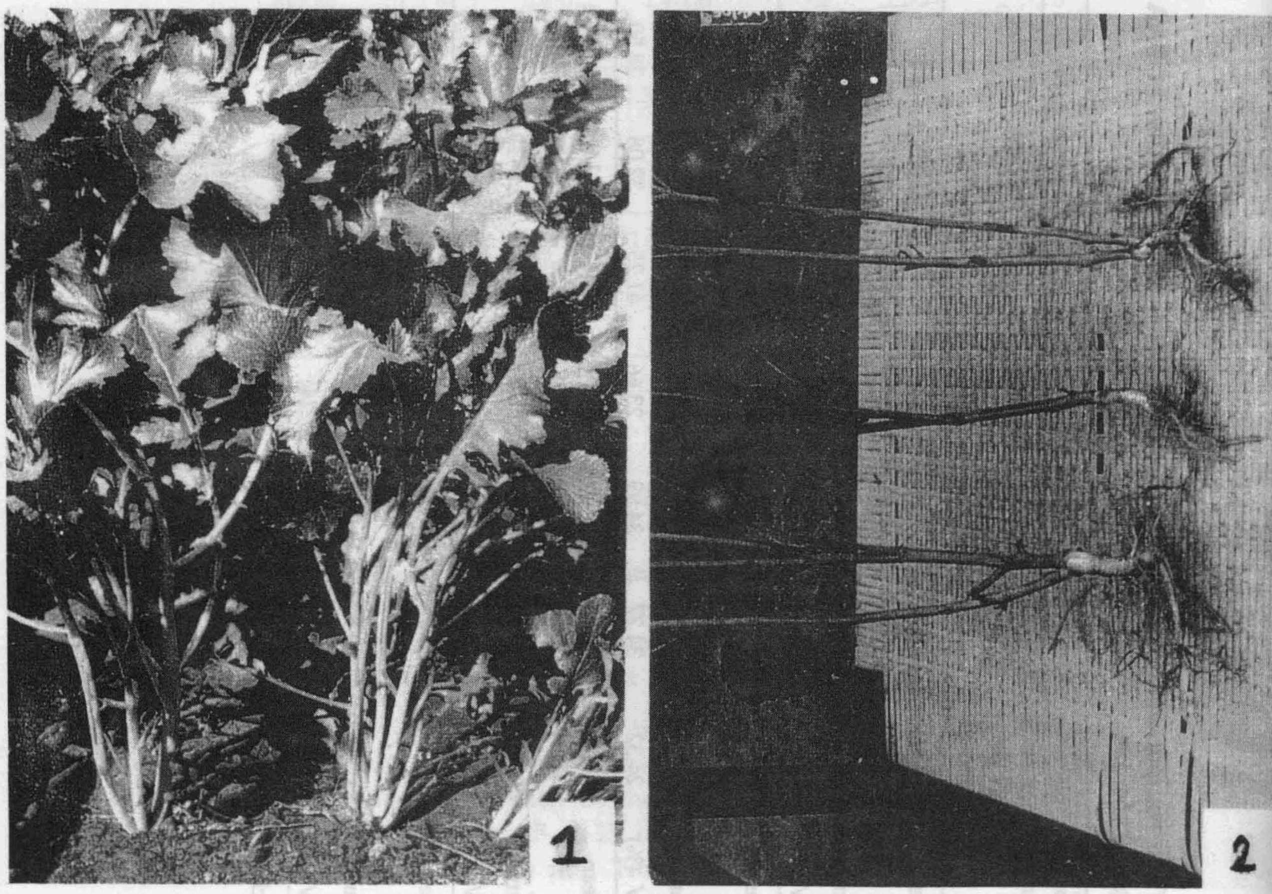

Fig. 2. Basal branching variants. In these plants the stem branched from the very base unlike control (in center) (Fig. 2). The number of branches originating from the base varied from one to many (Fig. 1).

Table 5. Structural components for siliquae on main shoot in M2 generation of BB and Waxy mutants of Brassica juncea var. RLM 198

Plant type		Main Branch length (cm)	Beak length (cm)	Peduncle length (cm)	Upper pod length (cm)	Middle pod length (cm)	Lower pod length (cm)	Mean pod length (cm)
Waxy mutants	Mean	54.34±5.48	0.49±0.02	1.41±0.07	1.7±2.9	2.8±4.1	2.4±4.3	2.03
	Range	40.0-76.0	0.4-0.6	1.2-1.7	1.7-2.9	2.8-4.1	2.4-4.3	
	CV (%)	24.71	9.39	12.7	21.9	14.99	25.52	
BB mutants	Mean	51.02±2.06	0.53*±0.03	1.56*±0.07	2.77±0.28	4.18±0.20	3.88±0.24	3.61
	Range	43.5-65.0	0.4-0.7	1.3-2.0	1.4-4.1	3.2-5.4	3.1-5.6	
	CV (%)	12.5	17.36	14.81	31.77	14.82	19.07	
Control	Mean	48.90±3.73	0.43±0.03	1.43±0.04	3.07±0.17	4.19±0.13	4.17±0.12	3.81
	Range	28.5-62.0	0.3-0.6	1.2-1.6	22.0-4.1	3.6-4.8	3.7-4.6	
	CV (%)	24.13	22.07	8.85	17.51	9.81	9.1	

Table 5 (Contd.)

Plant type	Components	No. of pods on main shoot	Seed weight (mg)/pod		
			Upper	Middle	Lower
BB variants	Mean	54.6 ± 1.81	30.63* ± 1.92	32.89 ± 2.52	13.38* ± 2.10
	Range	49.0 - 67.0	22.0-430	25.0-47.0	3.3-36.0
	CV (%)	10.48	19.21	24.21	49.6
Waxy	Mean	56.67 ± 6.52	3.55* ± 1.21	10.82* ± 3.38	12.04* ± 4.05
	Range	40.0-78.0	2.0-9.2	3.8-25.9	4.7-30.2
	CV (%)	28.19	83.66	81.52	82.3
Control	Mean	50.40 ± 3.48	18.6 ± 2.14	36.1 ± 1.90	42.5 ± 2.75
	Range	26.0-62.0	6.4-28.5	30.0-48.9	32.9-59.7
	CV (%)	21.82	36.37	16.63	20.45

*Significant change over control

Plant architecture characters : The average maximum height in the mutants at 215 cm was about 5% less than control at 227 cm (Table 1). In mutants the mean maximum spread at 70 cm was also less than control at 80 cm. Branching started from the very base of the stem in mutants unlike in control where the first branching node ranged from 1-12. Reduced plant height is a useful trait in Brassicas. Most *Brassica juncea* varieties grown in India are excessively tall with heavy concentration of pod bearing branches on the top. The top heavy branches are a major cause of lodging due to heavy winds which are usual around maturity time. Branching from the very base as seen in these mutants cannot only affect the canopy structure but also provide an advantage as far as lodging is concerned.

Siliquae characters : No difference in flowering time was observed between the variant and control population.

Structural components : Beak length increased from 0.43 cm in control to 0.53 cm in the mutant. Peduncle length also showed a slight increase in the mutant (Table 5).

Yield components : Pod weight decreased significantly in middle and lower pod positions. The seed number per pod decreased significantly in lower pod position. Total seed weight per pod was less in the mutants as evident from a decrease in seed weight in lower, middle and upper pod positions (Table 6).

Table 6. Yield components for siliquae on main shoot in M2 generation of BB variants and waxy mutants of Brassica juncea var RLM 198

Plant type	Components	Pod Weight (mg)			Seed number/pod		
		Upper	Middle	Lower	Upper	Middle	Lower
BB variants	Mean	68.04*±4.47	71.11*±4.68	27.28*±5.15	11.54±0.81	14.50±0.52	9.29*±0.73
	Range	58.0-80.8	47.0-103.0	8.9-64.0	9.4-15.6	11.2-16.4	1.4-16.4
	CV (%)	20.76	20.79	59.66	22.18	11.33	24.83
Waxy	Mean	17.83*±2.96	37.57*±6.35	43.94*±8.95	3.44*±0.87	774*±1.52	6.60*±1.32
	Range	10.8-28.7	21.6-63.8	23.8-83.2	1.6-7.2	2.0-11.8	2.0-11.0
	CV (%)	40.61	41.42	49.91	49.09	48	49.09
Control	Mean	41.90±3.16	95.0±7.1	86.90±3.87	11.5±0.98	13.9±0.70	17.5±0.80
	Range	19.4-50.0	59.5-90.4	69.3-113.2	4.6-13.0	8.0-15.8	10.0-20.0
	CV (%)	23.83	23.62	14.07	26.93	15.92	14.45

Early Flowering Mutants (efl) :

Two mutants were obtained in EMS treated M_1 population which flowered much earlier than control plants. The days taken to first flower were 55 for the mutant as against 75 for control. Since the maturity remained the same but flowering was advanced by 20 days, the effective seed filling duration increased.

Leaf Phenotype : The leaf shape in these mutants was almost conical with very sharp and pointed edges. The average leaf length, leaf width and main lobe length was

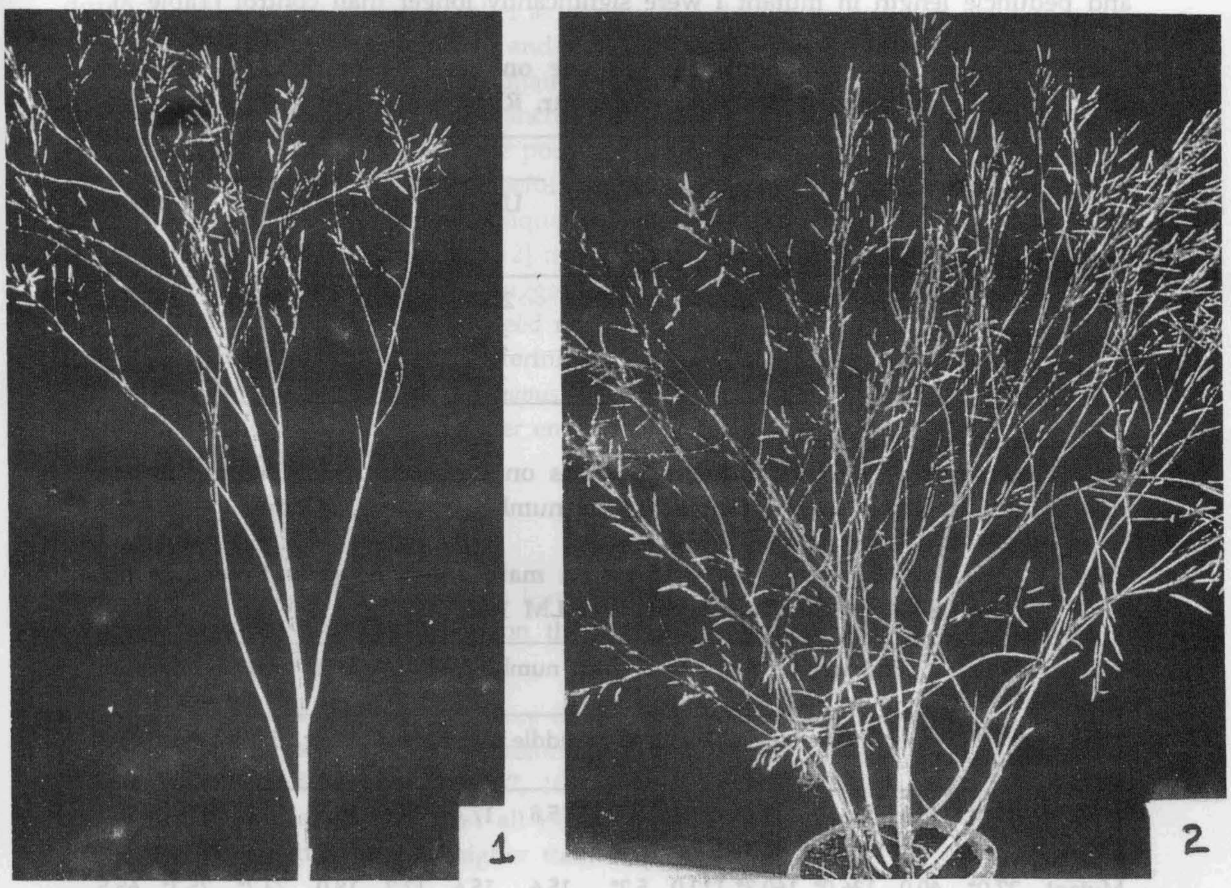

Fig. 3. Early flowering mutants (Efl mutants): Early flowering mutant at the time of harvest (Fig. 2.). The total number of pods on the main shoot were less in the mutants but the pod weight and the total seed weight per pod was more than control (Fig. 1.)

significantly less as compared to the control (Table 2). The average number of lobes at 6.2 was significantly less (42% less) than control and the 45 degree angles (sharp edges on leaf margins) were fewer in mutants. In Brassicas the lower leaves are very big and eventually become parasitic because of reduced availability of light in the canopy. The reduced leaf size as seen in these mutants can have profound effect on canopy structure and on interception of the incident light and therefore yield.

Siliquae Characters : In general the pods on the main shoot were fewer but were longer and heavier in mutants (Fig. 3).

Structural components : The main branch length and beak length in both mutants and peduncle length in mutant I were significantly longer than control (Table 7).

Table 7. Structural components for siliquae on main shoot in early flowering (efl) mutants of *Brassica juncea* var. RLM 198

Components / Plant type	Main branch length (cm)	Beak length (cm)	Peduncle length (cm)	Pod length (cm)			
				Upper	Middle	Lower	Mean
Mutant I	65.0*	0.60*	1.68*	2.60*	5.80*	6.50*	4.97
Mutant II	370*	0.80*	1.36*	2.96	5.10*	5.36*	4.47
Control	48.9	0.43	1.43	3.07	4.19	4.17	3.81

*Significant change over control

Yield Components : The total number of pods on the main shoot in mutants was significantly less than control (Table 8). The number of seeds in upper pods was

Table 8. Yield components for siliquae on main shoot in early flowering (efl) mutants of *Brassica juncea* var. RLM 198

Components / Plant type	Pods on main shoot	Pod weight (mg)				Seed number/pod				Seed weight (mg)/pod			
		Upper	Middle	Lower	Mean	Upper	Middle	Lower	Mean	Upper	Middle	Lower	Mean
Mutant I	45.0*	38.0	126.0*	199.4*	121.1	6.2*	15.8	17.4	13.1	16.5	62.3*	100.2*	59.7
Mutant II	27.0*	40.0	136.0*	160.3*	112.0	5.2*	15.6	15.6	12.2	18.0	73.2*	75.3*	65.5
Control	50.4	41.9	95.0	86.9	74.5	11.5	13.9	17.5	14.3	18.6	36.1*	42.5	32.4

*Significant change over control

also significantly less (41%) in mutants. Pod weight in the lower and middle positions was significantly more than the control. The total seed weight/pod in lower and middle pod positions in the mutants was 107 % and 88% more than control.

The increased pod filling duration was finally reflected in total seed weight per siliquae which showed an increase of 78% over control. Selection for flowering time has been positively correlated with a number of quantitative characters such as primary and secondary branches, number of siliquae on the main shoot, seeds per siliquae and seed yield [7-9]. Early flowering has been proposed to be especially beneficial for biomass under Indian conditions as it allows for longer seed filling duration [10]. Decreased pod number as observed in these mutants is also a desirable trait in Brassica spp. Decline in pod and seed weight especially on apically positioned nodes have been shown to be due mainly to setting of more pods than that can be supported [11]. Therefore, scope for increasing yield by reducing the number of pods was suggested. In these mutants the pod number decreased but the pod weight and seed weight increased. Another useful character observed in these mutants was reduction in number of seeds per siliquae on the main shoot (Table 8) and increased pod length (Table 7). Asana *et al.* [12] made a comparative study of yield in diploid and induced autotetraploids and suggested that yield of autotetraploids could be improved by selecting for reduced seed number per siliquae. The long pod character, which has been shown to be determined by two dominant genes acting in a complimentary manner in *Brassica napus* [9], has been shown to increase seed yield as longer pods could provide a better environment for a higher proportion of ovules surviving as mature seeds.

Waxy Mutants

A few plants with thick waxy leaves were observed in M_2 generation. These plants had thick waxy deposition on the stem also (Fig. 4). The flower size was almost 50% more than control.

Photosynthetic behavior : Since the waxy deposit on the leaf surface in these mutants may affect light absorption and therefore their photosynthetic rates were measured at 3rd, 5th, 7th and 9th nodes at 97, 104, 114 and 127 days after sowing (Fig. 5). Except at 114 day at 3rd node at all other days of observation at all other nodes the rate of photosynthesis was higher than control.

Siliquae characters : Structural components : Main branch length, beak length and peduncle length did not show any significant change from control. Pod length at upper, middle and lower pod positions got significantly reduced in mutants as compared to control (Table 5).

Fig. 4. Waxy mutants: From left : a control plant and a waxy mutant. In the mutant the leaves and the stem were covered with waxy deposit

Yield components : Pod weight, seed number per pod and seed weight per pod got significantly reduced at upper, middle and lower pod positions (Table 6).

SEM Studies : Scanning electron microscopy revealed the presence of thick waxy pointed deposits of wax on leaf surface around stomata (Fig. 6).

Epicuticular wax has been reported to be directly associated with resistance to *alternaria* blight in oil seed Brassica [10, 13]. However, precise mechanism of resistance and its genetic control are still least understood for which these mutants can be especially useful. It is interesting to note that although the rate of photosynthesis were higher in mutants the seed yield was not. This needs further investigation.

These mutants need further investigation but they can provide a source of variability for conventional breeding objective of obtaining a suitable plant type. Besides that the study of these mutants can also shed light on basic plant processes such as branching that affect the canopy structure (BAA mutants), development of leaf surface characteristics that affect the microenvironment of the plants (Waxy mutants) and flowering (efl mutants).

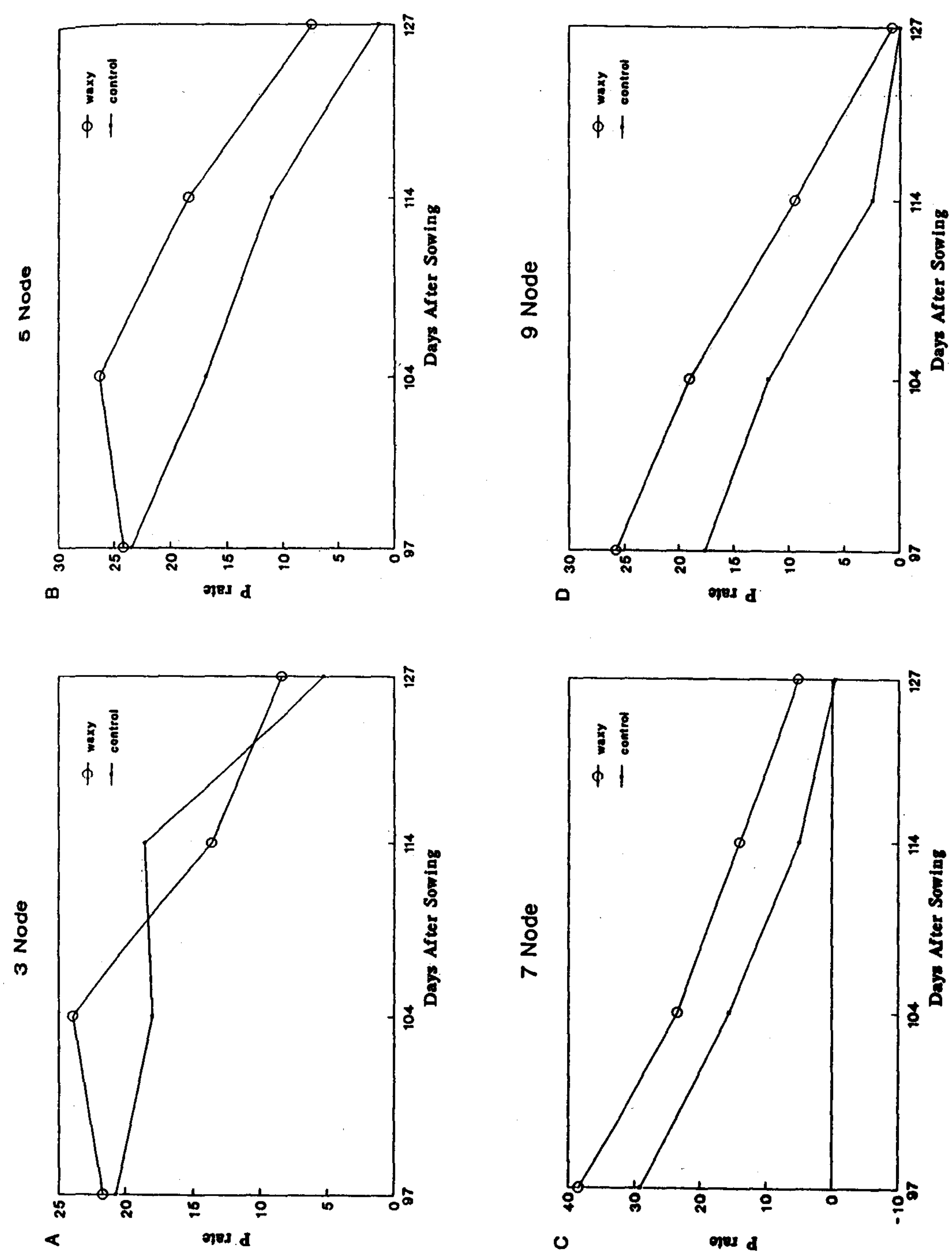

Fig. 5. Photosynthetic (P) rate (μ mols m^{-2} sec^{-1}) in leaves at different nodes in waxy mutants (average over three mutants).

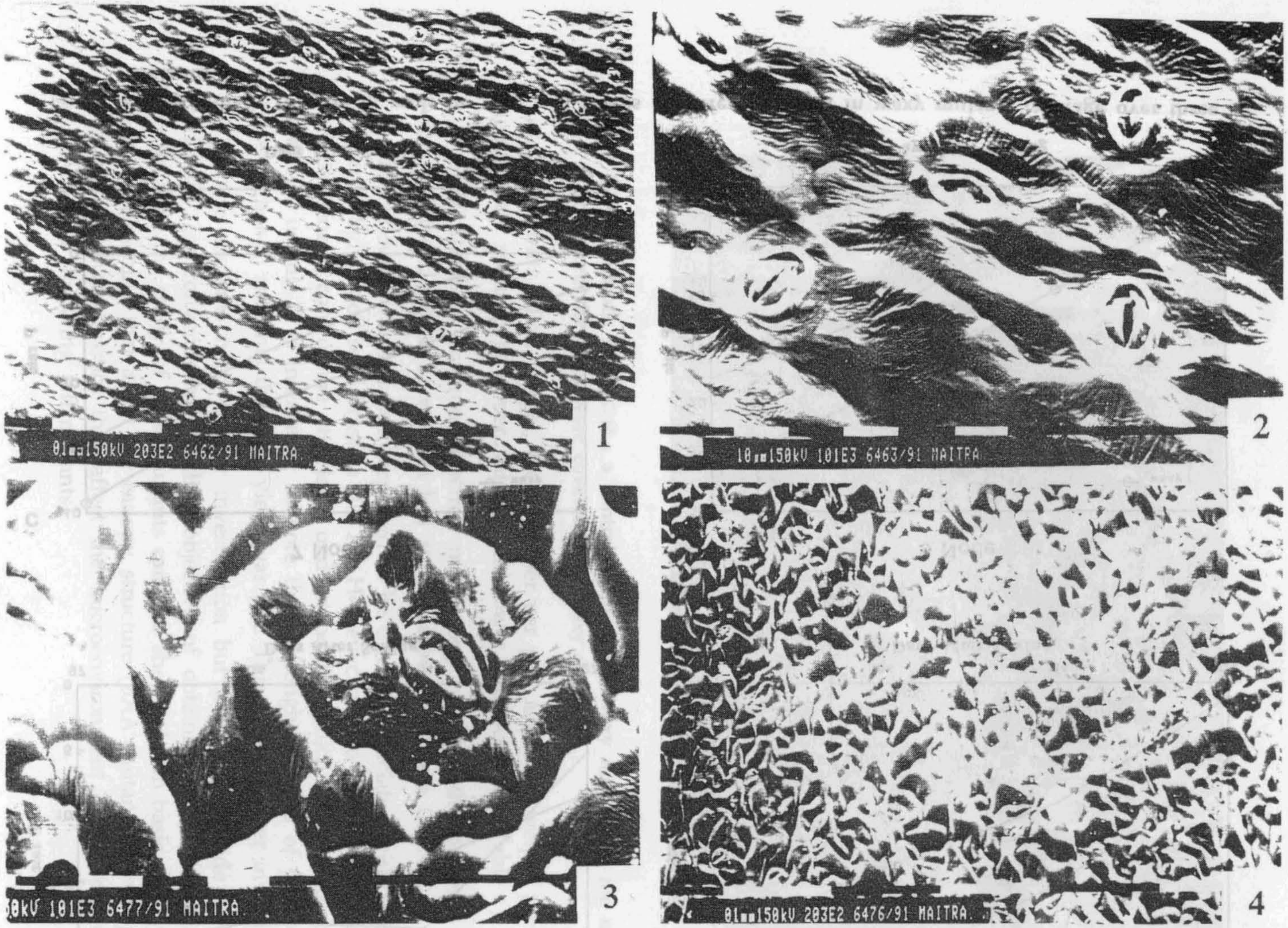

Fig. 6. SEM studies. Scanning electron micrographs of smooth leaf surface (1 & 2) and abrasive/waxy leaf surface (3 & 4)

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