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REPORT OF SOME LEAF PHENOTYPE MUTANTS IN *BRASSICA JUNCEA* L. COSS. & CZERN VARIETY RLM 198

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ABSTRACT

Chemical mutagenesis of seeds with 0.5% EMS has yielded some leaf shape mutants in *Brassica juncea* L. Coss. & Czern. variety RLM 198. These mutants are designated as *sl* type (simple leaf), *dil* type (deeply incised lobe) and *G* type. Morphological characteristics of these mutants are discussed in this paper. The F_2 data suggests the involvement of two genes in *sl* type and only one in *G* type mutants. Significance of such chemically induced leaf shape mutations in the same species are discussed in terms of understanding the generation of leaf as well as plant forms. Also since these mutants develop different canopy structures their importance in attaining a suitable plant type in Brassica is also discussed.

Key words : Brassica, EMS, leaf shape, mutant

In recent years mutants defective in almost every aspect of development have been identified [1] and it has dramatically expanded the field of developmental genetics. These mutants can provide valuable information that will eventually help in identification and characterization of these genes in higher plants which play a critical role in cell division, metabolism and differentiation. Identification of several floral mutants of *Arabidopsis* and *Antirrhinum* [2] have led to considerable progress in identifying genes with direct role in morphogenesis of floral structures [3, 4]. Similarly several embryonic [5] and root shape mutants [6] have been identified and their study may eventually provide valuable information regarding development and differentiation of these organs.

Leaf shape is an important characteristic that finally governs plant form. However, not much is known about the genetics or the morphological and the biochemical events leading to the development of its final shape. In recent years some leaf shape mutants have been identified [7] and their analysis may eventually lead to the

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understanding of the production of leaf shape and finally the plant form. In this paper we describe certain EMS induced leaf shape mutants in *Brassica juncea* L. Coss & Czern variety RLM 198. Availability of these leaf shape mutants will not only provide an opportunity to understand the generation of leaf shape at anatomical, biochemical and molecular level but also to modify the plant canopy by introgression of the desired characteristics.

MATERIALS AND METHODS

Alkylating agent ethyl methane sulfonate (EMS) was used for chemical mutagenesis. Different concentrations of EMS were prepared in 0.1 M phosphate buffer, pH 7.0 [8, 9]. 3000 seeds of almost uniform size were selected and divided in three lots. Each lot was presoaked in distilled water for 12 h and then treated with 0.3, 0.4 and 0.5% EMS for 6 h. The seeds were washed thoroughly and then transferred to field of Water Technology Center, Indian Agricultural Research Institute, New Delhi. Irrigation and fertilizer was provided according to the recommended agronomic practices and the plant spacing was maintained at 30 × 40 cm.

ISOLATION AND CHARACTERIZATION OF MUTANTS

The plants obtained in M_1 and in subsequent generations were studied for variation in leaf shape or size. Following morphological characteristics of leaf shape were studied:

(i) Leaf length (in cm) (ii) Leaf width (in cm) (iii) main lobe Length (in cm) (iv) Main lobe width (in cm) (v) Number of lobes: Any leaflet with length equal to or greater than 10% of the main lobe length was taken as a lobe. (vi) Number of 45^o angles per unit lobe length : The plants were also characterized for number of sharp edges (45^o angles on leaf margins per unit of main lobe length).

Crosses were attempted between some of the mutants and their parental lines. Mutants were taken as female and the parental line as pollen parent. Flowers were emasculated prior to anthesis and pollination was effected when the stigmas were in receptive condition.

RESULTS AND DISCUSSION

Three distinct leaf shape mutants were identified in *Brassica juncea* variety RLM 198 and designated as - Simple leaf (*sl*), G-Type and Deep incision lobe (*dil*).

MORPHOLOGY OF THE MUTANTS

The *sl* mutant: The *sl* mutants was characterized by the presence of a single lobe in the leaf. In this mutant unlike the normal compound leaf the lamina was entire (almost simple leaf) (Fig. 1). The leaf length and leaf width were significantly reduced (40% and 30% reduction respectively) as compared to the parental line (Tables 1, 2). The number of 45° angles per unit of main lobe length in the mutant leaf were also significantly less than the parental line (Table 1). The

Table 1. Leaf Phenotype in different morphological mutants of Brassica juncea variety RLM 198

Mutant type	Char-acters	leaf length (cm)	leaf width (cm)	main lobe length (cm)	main lobe width (cm)	Number of lobes	No of sharp edges/ main lobe length
sl	mean	29.50*+0.43	15.84*+0.24	29.50*+0.43	15.84*+0.24	1.00*+0.00	4.34*+0.15
	range	24-35	12-21	24-35	12-21	0	2-10
	cv (%)	10.34	10.7	10.34	10.7	0	24.85
dil	mean	30.72*±0.70	18.16*±0.48	17.34*±0.37	15.26*±0.32	8.20*±0.28	9.24*±0.36
	range	25-42	13-27	13-25	12-22	5-15	5-16
	cv(%)	16.2	18.56	15.11	14.68	23.78	27.27
G	mean	54.52*±1.13	25.52*±0.59	31.18*±0.70	25.52*±1.04	12.68*±0.43	5.57±0.35
	range	43-66	19-32	23-40	19-32	9-17	4-14
	cv (%)	10.4	11.6	11.23	20.46	16.8	31.24
Control	mean	48.70*±0.77	22.48*±0.55	32.42±0.59	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

frequency distribution curves for leaf length, leaf width, main lobe length, main lobe width and number of sharp edges per unit of main lobe length were negatively skewed (Fig. 2).

The *G* mutant. The average leaf length and width in mutant was significantly higher than parental lines (Tables 1, 2). Although this type of leaf had several lobes but most of these were very small and vestigial giving the leaf a lyrate appearance (Fig. 1). Also the edges of the leaf were smooth (less serrated). The frequency distribution curve showed positive skewness for leaf length whereas it was negatively skewed for the number of sharp edges per unit of main lobe length (Fig. 1).

The *dil* mutant. This mutants had deeply divided or pinnatisect leaves (Fig. 1). The average leaf length and width in the mutants was significantly less than parental

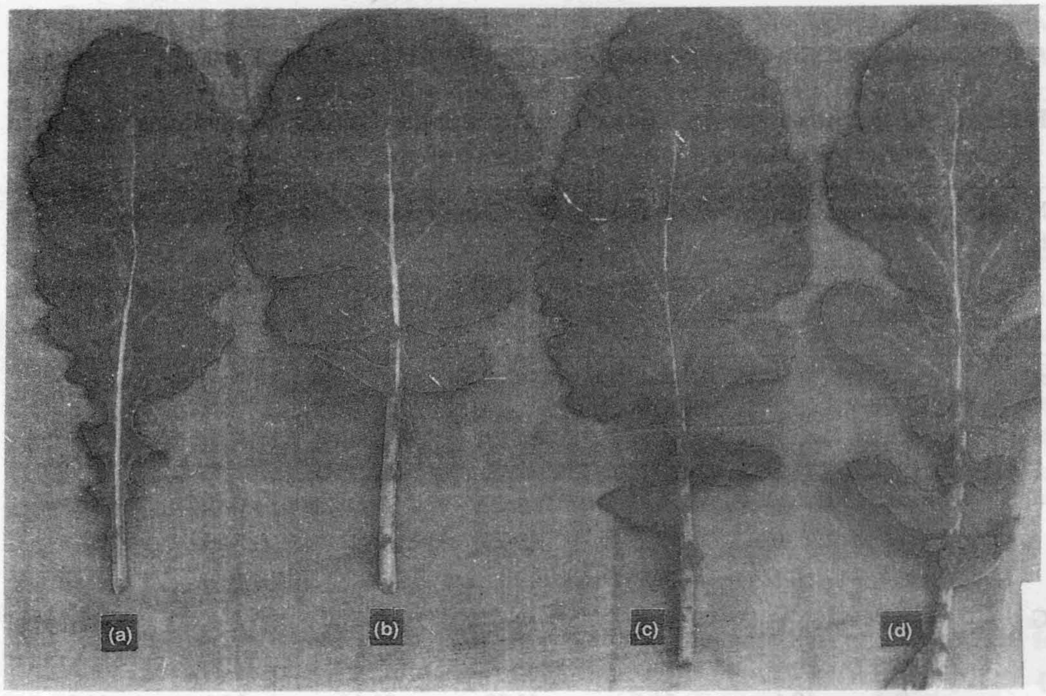

Fig. 1. Variation in leaf morphology in leaf shape mutants in Brassica juncea (from left): (a) simple leaf (*sl*) mutant; (b) G type mutant; (c) Control leaf and (d) deep incision lobe (*dil*) mutant

line (37% and 19% respectively Table 1, 2). Lamina had very deep incisions because of which the main lobe was smaller (47% less than control). The average main lobe length in the mutant was 17 cm as against 32 cm in parental line (Table 1). In all other mutants the main lobe width was also the maximum leaf width. However, in this particular mutant the main lobe width (at 15 cm) was less than the maximum leaf width (at 18 cm). Also despite the presence of very deep incisions in the leaf, the number of lobes in the mutants were significantly less than that in the parental line.

Leaf had very sharp and pointed serrations and the number of serration per unit of main lobe length were more (Tables 1, 2). A negative skewness was observed in leaf length, leaf width, main lobe length, main lobe width and number of lobes (Fig. 1).

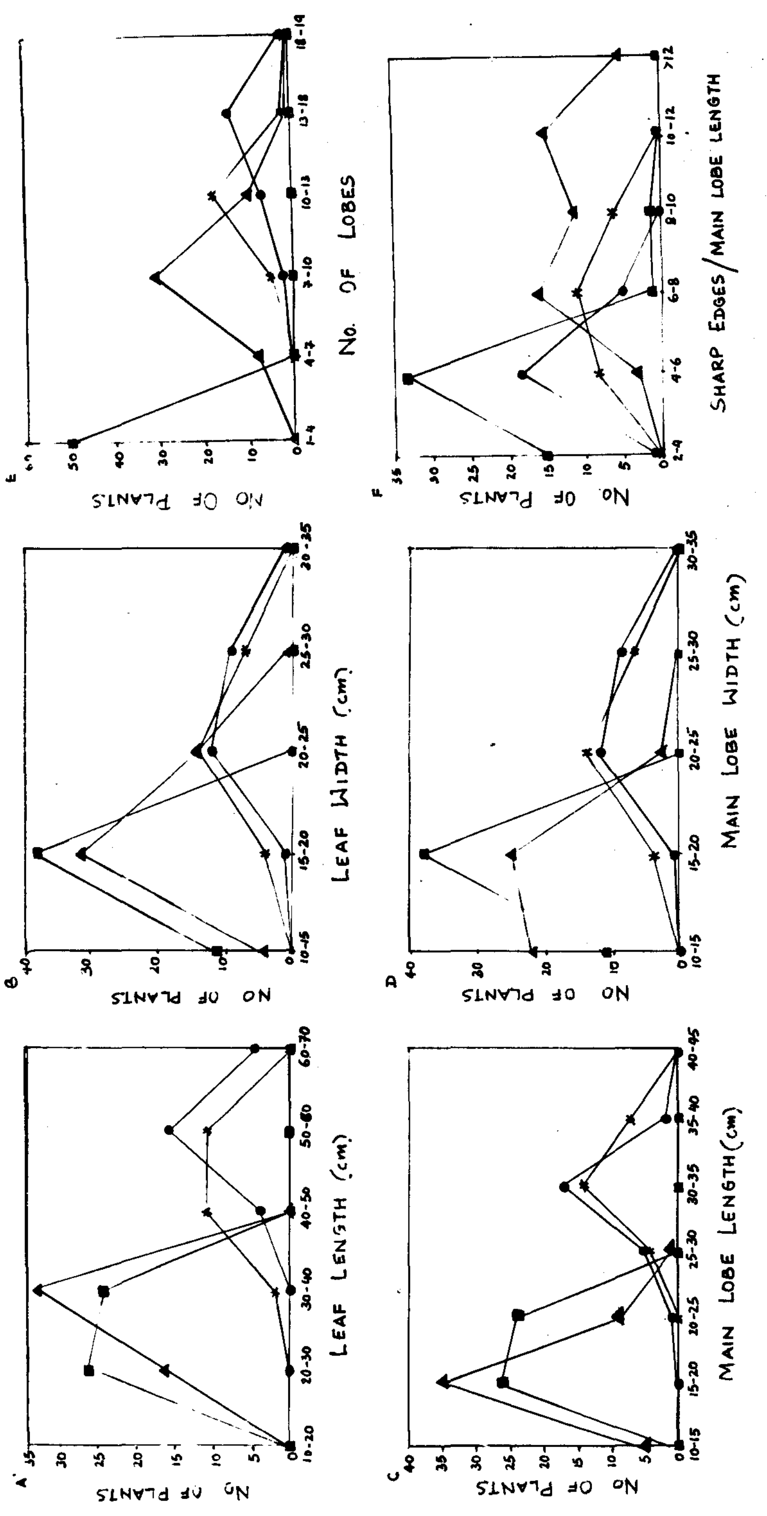

Fig. 2. Leaf characteristics in control(*) G Type (•), deeply incised lobe (*dil*) (▲)and simple (*sl*) (■) leaf mutants in *Brassica juncea* variety RLM 198

Table 2. Morphological changes in leaves of mutants of Brassica juncea variety RLM 198

Mutant type	Leaf shape	Increase or decrease over control			Serrations	Number of lobes
		leaf length	leaf width	main lobe length		
sl	Simple nearly entire	40% decrease	30% decrease	30% decrease	Fewer blunt	1
dil	Deeply divided or pinnatisect	37% decrease	19% decrease	47% decrease	More, sharp, pointed	8
G	Lyrate	12% increase	13% increase	13% decrease	Fewer, blunt	13*
Control	Pinnati divided				Sharp	11

*Significant change over control

GENETICS OF LEAF SHAPE

Genetics of leaf shape was studied in *sl* and *G* mutants. Crosses were attempted with mutants as female parent and parental wild type leaf as the pollen parent.

The sl mutants: In F_1 generation all the seven plants had normal wild type leaves. In F_2 generation, out of a population of 95 plants, 14 had simple leaf while 81 showed normal leaf (Table 3). This suggested a two gene segregation (one basic and one inhibitory) ratio of 13 normal : 3 simple leaf. The chi-square value of 1.00 for this fit had a probability range of 0.30-0.50 (Table 3). Such epistatic interactions have previously been reported in pea leaf [10] and Trapeaolum [11].

The G mutants: In F_1 generation all plants had normal leaves. In F_2 out of 103 plants, 82 showed normal while 21 showed *G* type leaf. The chi-square for goodness of fit corresponded to 3 normal : 1 *G* type leaf showed a value of 1.168 for 1 degree of freedom with corresponding probability range of 0.2-0.3 (Table 4). Involvement of a single gene in controlling leaf shape has been reported recently in pearl millet [12] and sunflower [13]. In *Brassica campestris* acute apex was found to be partially dominant over obtuse apex [14]. No information is available on the number of genes controlling leaf shape in brassicas.

In the tribe *Brassicae* considerable natural variation exists for leaf shape in different genotypes and between wild type and cultivated species. Four basic types of leaves can be distinguished in the tribe *Brassicae* and the genus *Brassica* has leaves of each of this kind showing intra - generic heterogeneity [15]. These four basic types of leaves are - (a) simple, entire to shallowly lobed (b) lobed to partite with sinuses not reaching the nerviation (c) divided with sinuses reaching the medium nerviation and (d) with reduced number of segments (Lyrate). In the present paper we report the generation of some distinct leaf shape mutants in *Brassica juncea* variety RLM 198. These are simple leaf (*sl*) mutants, *G* type mutants (almost Lyrate leaf) and deep incision lobe (*dil*) mutant (deeply divided or pinnatisect leaf).

Table 3. F2 segregation behaviour for cross Simple(sl) × Normal leaf in Brassica juncea

	Frequencies			Probability
Leaf type	Observed	Expected	Chi-square	range
Simple (sl)	14	17.81	0.81	
Normal	81	77.19	0.19	0.30-0.50
Total	95	95.00	1.00	

Table 4. F2 segregation behaviour for cross G Type × Normal in Brassica juncea

	Frequencies			Probability
Leaf Type	Observed	Expected	Chi Square	range
G Type	21	25.75	0.876	
Normal	82	77.25	0.292	0.20-0.30
Total	103	103.00	1.168	

Although naturally existing variation can be used in understanding the generation of variation in leaf shape, mutants within the same species provide a very powerful tool as they provide the possibility of cloning the genes. Another advantage of using induced mutants over naturally existing variation is that the difference can be attributed to a few genes as has been done in Trapeaolum, pea, maize, and tomato [10-11, 16]. Although they need further investigation, these leaf shape mutants are ideally suited for genetic analysis of generation of leaf shape. In recent years some other leaf shape mutants have also been identified [17, 18]. The analysis of these mutants should eventually lead to the understanding of leaf shape determination in particular and plant form generation in general.

The Brassica plants has been suggested to have some inherent physiological constraints that limit productivity [19, 20] and one of the major concerns has been the large almost parasitic lower leaves in the mature plant. Since these mutants have different shapes and sizes of leaves they provide an opportunity to study the effect of different type of plant canopies on productivity in *Brassica juncea* and may also prove to be useful in obtaining a desired plant type by introgression of the desired traits.

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