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Preferential segregation of two allelic mutations for small leaf character in groundnut*

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Summary. Two radiation induced small leaf mutants were isolated in a Spanish Improved variety of groundnut. Both had more than a 50% reduced leaflet size which was associated with an increased number of imparipinnate leaves in one mutant and light yellow flower colour in the other. Genetic studies demonstrated that both mutants were allelic and controlled by recessive factors. Phenotypic and genotypic segregation ratios indicated a lower frequency of mutants. This was attributed to preferential segregation in favour of normal leaf size. Marker genes controlling krinkle leaf, virescent and chlorina characters showed independent assortment in crosses with the small leaf mutants. Absence of assortment of associated mutant characters viz., small leaf and light yellow flower colour, generally indicated pleiotropic effects. However, monohybrid segregation for flower colour in the cross between the two small leaf mutants showed that the two characters were independently induced and hence attributed to close linkage and not pleiotropy.

Key words: Groundnut – Small leaf mutants – Yellow flower allelic inheritance – Pleiotropy and linkage

Introduction

Variations in leaflet size of groundnut (*Arachis hypogaea* L., 2n=40) have been reviewed by Seshadri (1962) and Hammons (1973). Small leaf mutants obtained spontaneously in intervarietal crosses have shown monogenic (Bhide and Desale 1970) and digenic (Ashri 1970) inheritance. Induced small leaf mutants have also shown divergent segregations (Gregory 1968; Shchori and Ashri 1970).

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An induced small leaflet mutant designated imparipinnate (Patil 1966), had 25% imparipinnate leaflets and showed modified genetic segregations. Another mutant with leaflets of a similar size was isolated in a separate experiment. This mutant, had additionally light yellow flowers unlike the orange flower colour of the imparipinnate mutant and parent. Similarly, reduced leaflet size, differences in the number of imparipinnate leaves, and light yellow flowers were of interest for genetic studies. Comparative morphological characters and genetic behaviours of the two induced mutants are described in this paper.

Material and methods

Imparipinnate mutant (*imp*) with small leaflets was isolated in the M_3 generation in the Spanish Improved variety (SP) after 75 kR X-irradiation (Patil 1966).

Another small leaflet mutant having a few imparipinnate leaves was isolated independently in 1968. It was obtained in the M_4 generation and was derived from an M_2 fused pod by 25 kR γ-ray treatment to SP. It subsequently bred true and was designated as small leaf mutant (*sl*).

Both *imp* and *sl* were crossed to SP, krinkle leaf (Hammons 1964) and virescent (Patil 1969) mutants using a modified crossing technique (Patil 1971). In addition, the small leaf mutants were also crossed to each other. Thus, the genetic segregation of small leaf character was studied under different genetic backgrounds in order to understand the relationship of mutant characteristics. Phenotypes and recombinants were easily identifiable and are presented in Table 1.

Segregations for *sl* and *imp* were scored in the heterozygous progenies obtained by mutagenic treatments and hybridizations by adopting the plant-to-row method of cultivation. Due to limited facilities, only a small number of families having the maximum number of plants were studied for genotypic segregations. Phenotypes were scored in the field and their frequencies are summarised in Tables 3 and 4. Because of the small size of progenies and similar segregations in the reciprocal crosses, the results of different progenies were pooled to test chi-squares.

Table 1. Reciprocal crosses and resultant segregants.

	Parents crossed	F_2 Segregants
Small leaf	1. Spanish Improved	SP, *sl*
	2. Virescent	SP, *sl, v, sl-v*
	3. Krinkle leaf	*Kr,* SP, *sl, cl, Kr-sl, Kr-cl, sl-cl* and *Kr-sl-cl*
Imparipinnate	1. Spanish Improved	SP, *imp*
	2. Virescent	SP, *imp, v* and *imp-v*
	3. Krinkle leaf	*Kr,* SP, *imp, cl, Kr-imp Kr-cl, imp-cl* and *Kr-imp-cl*
	4. Small leaf	All plants had *imp* type leaves and segregated for flower colour

Parents and F_1 plants were grown together and the morphological characters summarised in Table 2 were studied according to established procedures (Patil 1966). Since the morphological characters of F_1 were generally uniform in different crosses, the observations on F_1 plants derived from SP × *sl* are only illustrated

Table 2. Comparative characters of mutants and hybrids

Characters	SP	*sl*	*imp*	F_1 hybrids	
				imp × *sl*	Sp × *sl*
Height (cm)	88±2.8	70±3.1	60±2.4	68±4.2	92±3.4
Branches:					
Primary	6±1.2	9±1.6	5±1.9	7±1.2	6±1.4
Secondary	8±2.3	16±3.5	6±1.4	12±2.8	6±1.3
Leaf colour	Green	Light green	Green	Green	Green
Leaf type (%)[a]					
i) Par	98.5	70.6	30.0	47.0	98.0
ii) Par + acc	1.5	18.6	36.2	20.5	2.0
iii) Imp	Nil	6.0	8.2	6.5	Nil
iv) Imp + acc	Nil	4.8	25.6	26.0	Nil
Leaflet size (cm)					
Lenght	7.4±0.12	3.6±0.06	3.8±0.06	3.9±0.11	7.3±0.20
Breadth	3.9±0.06	2.3±0.04	2.4±0.05	2.3±0.10	3.8±0.10
Flower colour	Orange	Yellow	Orange	Orange	Orange
Flowering pattern	S	Altered	S	S	S
No. of pods (1 + 2 seeded)	7+45	7+48	6+30	6+41	7+52
Maturity (days)	115	100	105	100	115
Shelling %	74.5	80.8	78.2	80.5	74.8
100 kernel wt (g)	51.3±0.41	24.8±0.62	34.7±0.56	36.5±0.50	50.4±0.38
Kernel colour	Rose	Rose	Flesh	Flesh	Rose

[a] Par = paripinnate, acc = accessories; Imp = imparipinnate; S = sequential

Results

Morphological characters

Small leaf mutant. This mutant was characterised by more than a 50% reduced leaflet size (Fig. 1), and a reduction of 20–25% in plant height compared to those in SP (Fig. 2). Its leaves appeared light green. In addition, flowering axils were absent on the stem, resembling the *Virginia* character (Gregory et al. 1951). However, on the primary branches there were 3–4 flowering axils followed by 2–4 vegetative axils, unlike *Virginia.* The altered flowering pattern in *sl* resulted in a greater number of branches (Table 2) which were thin (Fig. 2). The mutant had approximately 10% imparipinnate leaves and 20% leaves with accessory leaflets, which were found occasionally in SP. The flower colour in the mutant was light yellow unlike the orange found in SP and other parents. Pod setting in the mutant was slightly reduced. Pod size was smaller leading to 50% reduced kernel weight. The mutant had early maturity by two weeks.

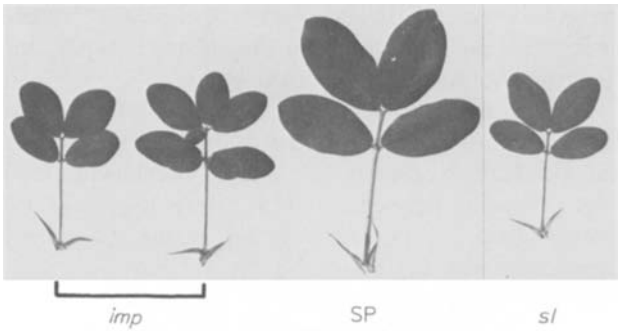

Fig. 1. (L-R) – Leaves of *imp*, SP and *sl*

Fig. 2. (L-R) – Plants of SP, *imp*, *sl*

Imparipinnate vs small leaf mutant. The imparipinnate mutant (*imp*) isolated in M_3 was also characterised by a similar reduced leaflet size as found in *sl* (Fig. 1). The two mutants differed in that *imp* had an increased number of leaves with accessories (36%) and imparipinnate types (34%) and *sl* had a light yellow flower colour (Table 2). The mutant was designated as imparipinnate to emphasize the increased number of imparipinnate leaves. The number of accessories per leaf varied and a rare leaf had as many as 10 leaflets (Fig. 3), six of them being accessories. Further, there was an altered flowering pattern in *sl* and smaller pod size (Fig. 4), leading to 23 g per 100 kernels unlike the 34 g found in *imp*.

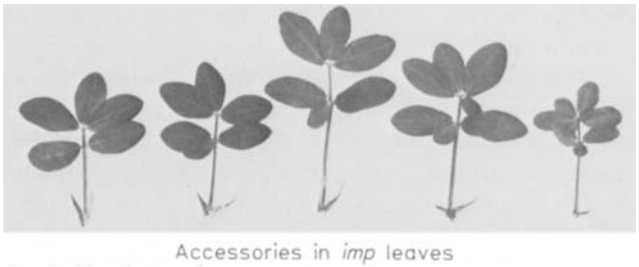

Fig. 3. Number and arrangement of accessory leaflets in *imp* leaves

Hybrid plants. F_1 plants derived from crosses, SP×*sl*, SP×*imp*, *v*×*sl*, *v*×*imp* and their reciprocals were not distinguishable from SP, indicating the dominant expression of normal leaflet size, plant height, flower colour and pod size. Similar expressions were observed in crosses with *Kr*, excepting krinkle leaves.

F_1 plants of crosses between *sl* and *imp* resembled the *imp* parent in all respects except for increase in number of branches and early maturity, as in *sl* (Table 2). The expression of small leaf character in the F_1 demonstrated that both *sl* and *imp* were allelic.

Segregation for small leaf mutant

Progenies following irradiation. Progeny from M_4 had 78 SP and 3 *sl* plants. In M_5, only 10 SP progenies were heterozygous and the mutants were true breeding. Pooled phenotypic segregation had 477 SP and 110 *sl* plants which did not fit either monohybrid or dihybrid segregation ratios. The average ratio of 5 : 1 was found to fit better ($\chi^2 = 1.76$, $P = 25$) than 6 : 1 ratio ($\chi^2 = 9.38$, $P = 0.5$) as in the case of *imp* (Patil 1966). Genotypic segregation was obtained by growing 158 progenies in the M_6. There were 60 homozygous SP, 70 heterozygous and 28 homozygous *sl* progenies; this provided a good fit to the 2 : 3 : 1 ratio ($\chi^2 = 2.10$, df 2, $P = 25$). The modified phenotypic and genotypic ratios were attributed to preferential segregation favouring normal leaf size. The expression of *sl*, therefore, was controlled by a pair of recessive genes whose transmission was influenced by preferential segregation.

Crosses. When SP and *sl* were crossed, two parental phenotypes appeared in the F_2. The frequencies of phenotypes in F_2 and F_3, summarised in Table 3, also indicated a better fit to the 5 : 1 ratio. Genotypic

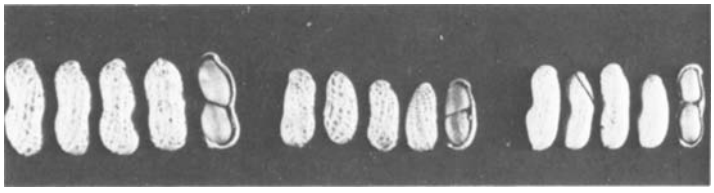

Fig. 4. (L-R) Pods and kernels of SP, *imp* and *sl*

segregation confirmed the phenotypic expression of the recessive nature of *sl* characters.

Crosses between *Kr* and *sl* produced eight phenotypes in the F_2 including four *chlorina* groups. The summarised frequencies of different phenotypes indicated tetargenic segregation (Table 4). Using modified segregation for *sl*, a ratio of 225 : 75 : 45 : 15 : 15 : 5 : 3 : 1 was computed based on monogenic inheritance of *Kr* (Hammons 1964) and digenic inheritance of *chlorina* (Patil 1973). The observations were in agreement with the expected ratio and hence independent assortment of *Kr, sl,* cl_1 and cl_2 factors.

Segregations of *v* × *sl* crosses produced four phenotypes in the F_2 (Table 4). Their frequencies showed an independent assortment of *v* and *sl* characters. However, parental frequencies in F_3 were less than expected. Only the pooled segregations for *sl*, were in accordance with 5 : 1 ratio.

Table 3. Segregations in crosses with small leaf mutants

Crosses	Generation	No. of progenies	Frequency of phenotypes				χ^2	P
			SP	*v*	*sl*	*sl-v*		
							(5:1)	
SP × *sl*	F_2	8	227		45		0.02	< 90
	F_3	45	2 350					
		56	3 145		664		0.28	50 – 75
		22			604			
Genotypic segregation, χ^2 (2:3:1) = 0.99, df 2, P = 50 – 75								
							(15:5:3:1)	
v × *sl*	F_2	3	110	29	17	10	3.11	25 – 50
	F_3	24	809	247	130	62	7.98	
			1 056		192		1.47	< 25
Genotypic segregation only for *sl*, χ^2 (2:3:1) = 0.56, df 2, P = 75								
			SP	*v*	*imp*	*imp-v*	(6:1)	
SP × *imp*	F_2	12	476		74		0.31	50 – 75
	F_3	73	3 285					
		71	3 920		684		1.20	25 – 50
		28			915			
Genotypic segregation, χ^2 (3:3:1) = 0.58, df 2, P = 75								
							(18:6:3:1)	
v × *imp*	F_2	4	126	40	16	7	1.03	75 – 90
	F_3	10	332	102	53	20	0.80	75 – 90
Genotypic segregation for *imp*, χ^2 (3:3:1) = 1.82, df 2, P = 25 – 50								

Table 4. Segregations of *sl* and *imp* in crosses with krinkle leaf

Cross	Generation	No. of progenies	Frequency of phenotypes								χ^2	df	P
			Kr	SP	*Kr-sl*	*sl*	*Kr-cl*	*cl*	*Kr-sl-cl*	*sl-cl*			
	(Expected ratio: 225:75:45:15:15:5:3:1)												
Kr × *sl*	F_2	4	56	26	12	3	5	2	1		3.06	7	75 – 90
	F_3	8	263	86	51	11	17	5	4	1	2.65	7	90
	(15:5:3:1)												
	F_3	6	164	48	32	12					0.66	3	90
			Kr	SP	*Kr-imp*	*imp*	*Kr-cl*	*cl*	*Kr-imp-cl*	*imp-cl*			
	(Expected ratio: 270:90:45:15:18:6:3:1)												
Kr × *imp*	F_2	11	264	84	39	10	16	6	4	1	3.55	7	75 – 90
	F_3	8	260	96	40	16	20	9	3	2	3.76	7	75 – 90
	(18:6:3:1)												
		12	418	165	64	18					5.27	3	10 – 25

Inheritance of imparipinnate mutant

Progenies following irradiation. Progeny of SP in M_3 had 17 normal plants and 3 small leaf mutants which had several imparipinnate leaves. The mutant progenies in M_4 were similar to *imp,* while 8 of the 17 SP progenies showed segregations. A pooled phenotypic frequency, 202 SP and 30 *imp,* provided an excellent fit to 6 : 1 ratio ($\chi^2 = 0.04$, $P = 75$–90) compared to a poor fit to the 5 : 1 ratio ($\chi^2 = 2.50$, $P = 10$). Phenotypic segregation in M_5 had 810 SP: 128 *imp* plants and did not agree with the 5 : 1 ratio ($\chi^2 = 6.25$, $P = 1$) thereby confirming the lower frequency of *imp* than *sl* types in the segregations. Genotypic segregation revealed 46 homozygous SP, 41 heterozygotes and 14 homozygous *imp* progenies, indicating a very good fit to the 3 : 3 : 1 genotypic ratio ($\chi^2 = 0.35$, df 2, $P = 75$–90). These were explained (Patil 1966) on the basis of preferential segregation for the dominant normal leaf character.

Crosses. Results of segregations from *v* × *imp* (Table 4) were similar to those reported earlier (Patil 1969) indicating a phenotypic segregation ratio of 18 SP : 6 *v* : 3 *imp* : 1 *imp-v.*

The crosses between *Kr* and *imp* had segregations similar to those of *Kr* × *sl* except for normal flower colour in all the phenotypes. The frequencies of the eight phenotypes were in agreement with the expected ratio, 270 *Kr*: 90 SP: 45 *Kr-imp*: 15 *imp*: 18 *Kr-cl*: 3 *Kr-imp-cl*: 1 *imp-cl* computed on the basis of independent assortment of mutant factors (Table 4). The number of progenies in the F_3 were inadequate to test genotypic segregations. However, pooled segregations for only *imp* confirmed the occurrence of 3 : 3 : 1 genotypic ratio.

Crosses between imp and sl. There was no segregation for leaflet size, confirming F_1 expression. However, segregation for flower colour was observed. Plants with orange and light yellow flowers numbered respectively 176 and 51 in the F_2 and 3,730 and 1,272 in the F_3. These observations were in agreement with a 3 : 1 phenotypic ratio ($\chi^2 = 0.78$, $P = 25$–50 in F_2 and $\chi^2 = 0.49$, $P = 50$ in F_3). In the F_3 there were 59 homozygotes for orange flowers, 117 heterozygotes and 51 homozygotes for light yellow flowers, indicating a good fit to the expected 1 : 2 : 1 genotypic ratio ($\chi^2 = 0.78$, df 2, $P = 50$–75). Thus, light yellow flower colour was controlled by a pair of recessive factors. The segregations demonstrated that the two mutations for leaflet size and flower colour in *sl* were independent and not due to pleiotropic effects.

Observations on the number of imparipinnate leaves on the stem (maximum expression 62% in *imp*) in F_2 showed a range of 10–70% expression. Their mean value in both orange and yellow flower colour groups were approximately 35% and hence similar to the mid-parental value, indicating a probable influence of quantitative genes controlling imparipinnate expression. It also suggested independent expression of flower colour and imparipinnate leaf characters.

Discussion and conclusions

The two induced small leaf mutants, *sl* and *imp,* were obtained in different experiments. Their genetic behavior indicated a deviation from monohybrid segregation due to reduced frequencies of the mutant types. Modified monohybrid ratios viz., 5 : 1 and 6 : 1 were found to fit *sl* and *imp* segregations (Table 3), presumably due to 'preferential' segregation favouring normal leaf size. Genotypic segregations, 2 : 3 : 1 and 3 : 3 : 1 respectively, were obtained, confirming 'preferential' segregation hypothesis. Their segregation patterns in different genetic backgrounds (Table 4) were similar, demonstrating independent assortment of krinkle leaf virescent and chlorina characters.

It may be emphasized that all the *sl* plants in the segregations had four associated characters viz., altered flowering habit, small leaf, light yellow flowers and reduced pod size (Table 2). The *imp* plants had reduced pod size in addition to the small leaf character. Hundred kernel weight varied between 20 and 55 g in the heterozygous progenies, suggesting the segregation of pod size. Plants with normal leaves having 30–55 g kernel weight were recovered; but small leaf plants with more than 35 g kernel weight were not recovered, indicating an influence of small leaf on pod size. Absence of assortment of light yellow flower colour and small leaf factors of *sl* could be due to either pleiotropism (Shchori and Ashri 1970) or close linkage. The occurrence of segregation for flower colour in the crosses between small leaf mutants were attributed to linkage and not pleiotropy.

When mutagenic treatment induces mutations in neighbouring genes, it is difficult to pin-point the change to be the result of pleiotropism, linked genes or loss of segment. The differences can be demonstrated only when similar mutants with different pleiotropic patterns (Gottschalk 1976) would be available as in the case of *sl* and *imp.*

Ashri (1970) proposed that sl_1 and sl_2 duplicate recessive genes were responsible for small leaf expression. Accordingly, the gene symbol for the present small leaf mutants would be *sl* because of the modified simple inheritance. In view of this, the earlier gene symbol *imp* is redesignated as *sl-imp* indicating simultaneous expression of imparipinnate leaf character.

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