Indian J. Genet., 57(4): 395-400 (1997)

INHERITANCE OF SEED STORAGE PROTEINS IN *LATHYRUS SATIVUS* L.

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(Received : August 16, 1997; accepted: December 10, 1997)

ABSTRACT

SDS-polyacrylamide gel electrophoresis of F_2 seeds of crosses between lines of *Lathyrus sativus* showed that legumin mol wt 89 kD–80kD, 64 kD–57 kD and 45 kD–42 kD and vicilin polypeptides mol. wt. 78 kD–76 kD were controlled by mendelian genes showing codominance. These polypeptide patterns in different molecular weight regions were analysed for segregation of one with respect to the other. These were all seen to be inherited together in parental patterns and did not show any recombinants suggesting thereby that genes for different polypeptides were closely linked and thus behaved as a single locus.

Key words : *Lathyrus sativus*, Storage proteins, legumin, vicilin.

Lathyrus sativus L., commonly called as Khesari or chicling vetch, is an extensively grown legume of the Indian subcontinent. It is a hardy crop and grows very well under wide variety of soils and climatic conditions. In addition to the major interests towards elimination of the toxic factor - BOAA - in this crop [1], studies on biochemical characterization of its seed storage proteins for improved grain quality have also been carried out [2-4]. Like other legumes, it also has globulins, the salt soluble fraction, as dominating protein fraction in the seed. Globulins in legumes have been characterized as two major subfractions (i) the 11S fraction which is hexameric with disulphide-bonded subunits of mol. wt. 40 kd and 20 kD and (ii) the 7S fraction which is trimeric and lacks disulphide linkages [5-7]. In *Lathyrus sativus*, legumin subunit-pairs are known to vary from mol. wt. 32 kD to 89 kD with their large subunits varying from mol. wt. 74 kD to 25 kD [3]. The polypeptides of mol. wt. 78, 66, 54, 36.5, 34, 20 and 18.5 kD constitute the vicilin protein fraction [3]. Different globulin polypeptides in pea [8-10], soybean [11] and frenchbean [12] are reported to be controlled by simple mendelian genes showing codominance. In *L. sativus*, a number of lines showing variation for seed proteins on SDS-polyacrylamide gels when crossed, showed that their F_1s had polypeptide patterns as sum total of parental patterns. This pointed towards the occurrence of codominant genes controlling different polypeptide patterns [4]. The present paper deals with F_2 segregation data for genetical studies of a number of globulins polypeptides.

MATERIALS AND METHODS

F_1 and F_2 seeds of crosses P27 × EC21 and EC21 × EC51, were analysed for the polypeptide patterns by preparing seed protein extracts from a part of seed [8]. The seed was scratched in such a way that the embryo was not injured. The seed extracts were prepared by heating finely powdered seed meal in 0.125 M Tris-HCl buffer pH 6.8 containing 1% SDS. Polyacrylamide gel electrophoresis was carried out by the method employed by Matta *et al.* [13]. Fisher's chi-square (χ^2) test [14] was used to confirm the expected ratio wherein the F_2 segregation data fitted best.

RESULTS AND DISCUSSION

In an earlier communication [4], variation in polypeptide patterns of seed proteins of different *khesari* lines was reported in the range of mol. wt. 89 to 80 kD, mol. wt. 64 to 57 kD and mol. wt. 45 to 42 kD under non-reducing conditions and in the range of mol. wt. 75 to 63 kD, 45.5 to 41 kD and 20 kD to 18.5 kD under reducing conditions. Whereas polypeptides of mol. wt. 75 to 63 kD represented

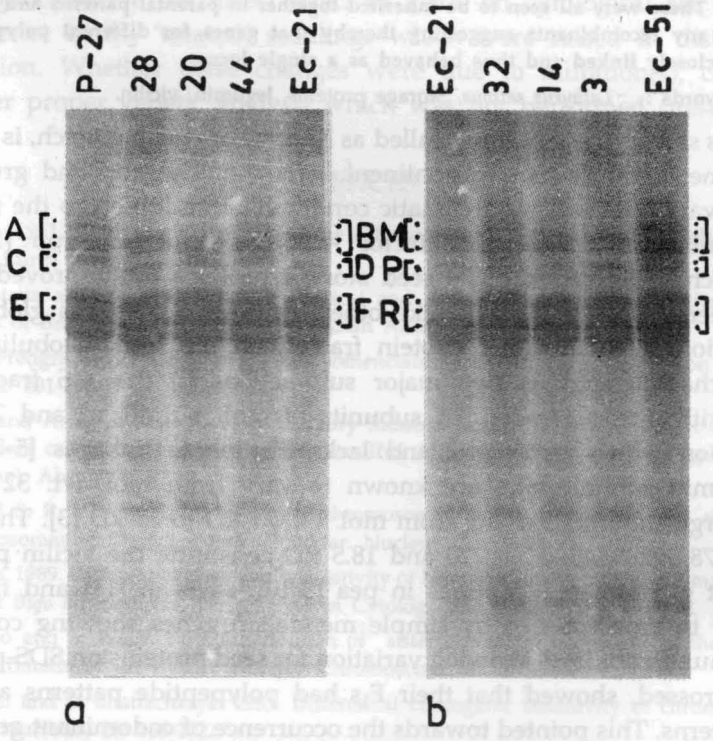

Fig. 1. SDS-Polyacrylamide gel electrophoresis of the seed protein extracts of F_2 segregants under non reducing conditions; a) of the cross between lines 'P-27' and 'EC-2I' and b) of the cross between line 'EC-2I' and 'EC-5I'

larger subunits of polypeptide pairs of mol. wt. 89 to 80 kD and polypeptides of mol. wt. 45.5 to 41 kD represented larger subunits of polypeptide-pairs of mol. wt. 64 to 57 kD, those of mol. wt. 20 to 18.5 kD represented smaller subunits of these subunit-pairs. Reciprocal crosses were made between lines 'P–27' × 'EC-21' and lines 'EC-21' × 'EC-51' and F_2 raised from these. Mol. wts. and codes used for polypeptide patterns for which the parental lines differed (Fig. 1) were as follows :

(i) *Cross 'P-27' X 'EC-21'*

	Polypeptide patterns (Line P-27)		*Polypeptide patterns* (Line EC-21)	
Sr.No.	*Code*	*Mol.wt.*	*Code*	*Mol.Wt.*
1.	A	89, 82, 80 kD	B	85, 83, 80 kD
2.	C	78, 76.5 kD	D	76 kD
3.	E	63.5, 61, 57 kD	F	64.5, 62.5, 57.5 kD
4.	G	45.5, 44.5, 43.5, 42 kD	H	45, 44, 43, 42 kD

(ii) *Cross 'EC-21' X 'EC-51'*

	Polypeptide patterns (Line EC-21)		*Polypeptide patterns* (Line EC-51)	
Sr.No.	*Code*	*Mol.wt.*	*Code*	*Mol.Wt.*
1.	M	85, 83, 80 kD	N	89, 86.5, 82, 80 kD
2.	P	76 kD	Q	78, 76 kD
3.	R	64.5, 62.5, 57.5 kD	S	63.5, 60, 57 kD

In the cross 'P-27' X 'EC-21', whereas polypeptide patterns A-B, E-F, and G-H represented legumin subunit-pairs, patterns C-D represented vicilin polypeptides [3]. The polypeptide pattern of each set (say A-B) in a given mol. wt. region was considered to represent a biochemical trait and the polypeptide pattern code (say A or B) which was constant for a particular line was assumed to be the allelic product of a particular locus. Thus, the polypeptide patterns of individual sets, say pattern 'A', and 'B' of set 'A-B' 'C' and 'D' of set 'C-D', 'E' and 'F' of set 'E-F' and 'G' and 'H' of set 'G-H', were assumed to be the product of allelic genes. The pea legumin is known to be synthesized as a precursor of the polypeptide-pair wherein cleavage occurs and disulphide linkages are established post-transcriptionally [15]. The precursor is thus, product of a single gene. The F_1s have been earlier [4] reported to show patterns which are sum total of the patterns of two parental lines.

F_2 seeds when analysed by SDS-PAGE under non-reducing conditions, segregated into three pattern-families of pattern codes for different sets. The three families were

represented by a) pattern of line 'P-27' (b) that of line 'EC-21' and c) additive pattern of these two parental lines [Fig. 1a]. The frequency of these three pattern-families for different pattern codes is given in Table 1. The chi-square value (2.86) for these three pattern-families a, b and c as above representing different polypeptide sets shows a good fit into the ratio 1a : 2c : 1b. This proves the occurrence of codominant simple mendelian genes controlling different polypeptide sets. Segregation of pattern code of one set with respect to pattern codes of the other sets was also studied. It was found that in a given F_2-segregant, variant patterns of four different sets belonging to the same line always occurred together i.e. as A, C, E and G belonging to line P-27' or as B, D, E and H of line 'EC-21' or patterns AB, CD, EF, GH as additive of the two lines. There were no recombinants of pattern codes for the four poly-peptide sets and thus the genes controlling different sets can be suggested as being closely located on the same chromosome.

Table 1. Frequency of polypeptide patterns in F_2 segregants and the chi- square values for crosses between different *Lathyrus* lines.

Cross	Polypeptide pattern-set	Frequency of F_2 segregant families				χ^2
			A	AB	B	
	A-B	Observed	18	23	18	2.86
		Expected	18	23	18	
			C	CD	C	
'P-27'	C-D	Observed	18	23	18	2.86
×		Expected	18	23	18	
'EC-21'			E	EF	F	
	E-F	Observed	18	23	18	2.86
		Expected	18	23	18	
			G	GH	H	
	G-H	Observed	18	23	18	2.86
		Expected	18	23	18	
			M	MN	N	
	M-N	Observed	17	26	19	1.21
		Expected	17	26	19	
			P	PQ	Q	
'EC-21'	P-Q	Observed	17	26	19	1.21
×		Expected	17	26	19	
'EC-51'			R	RS	S	
	R-S	Observed	17	26	19	
		Expected	17	26	19	1.21

In the cross 'EC-21' × 'EC-51', polypeptide patterns M-N and P-Q represented legumin subunit pairs whereas patterns R-S were the vicilin polypeptides. When analysed on SDS-polyacrylamide gels, F_1s showed the occurrence of polypeptide patterns which were sum total of the two parental patterns [4]. The frequency of polypeptide patterns as observed in F_2 segregants of this cross can be seen in Table 1. As is vivid, the polypeptide patterns found in F_2 seeds under non-reducing conditions [Fig. 1b] segregated into three families- two parental and one additive of the two parents. As worked out by using chi-square value (1.21), the segregation data fitted well into the ratio 1 ('EC-21' pattern) : 2 (additive of 'EC-21' and 'EC-51' patterns) : 1 ('EC-51' pattern) which is expected on account the codominant genes. Thus, the genes controlling these polypeptide sets show codominance and follow simple mendelian inheritance.

Analysis of data regarding the occurrence together of polypeptide patterns of different sets (M-N, P-Q, and R-S under non-reducing conditions) was also carried out. The only three combinations observed were (i) M, P and R (ii) N, Q and S (iii) MN, PQ, RS. The combinations described as (i) and (ii) were parental and (iii) represented sum total of two parents. Thus, there were no recombinants between polypeptide patterns of these different sets. These studies in cross 'EC-21' X 'EC-51' also show that the genes controlling polypeptides of different sets are all present on the same chromosome closely linked with each other and thus behave as a single gene.

All such polypeptide patterns as reported in various legumes 8-12 are controlled by simple mendelian genes. Also, the genes for different legumin and vicilin polypeptides as seen in present study are located on the same chromosome in *L. sativus*. Genes for different polypeptides in a number of other legumes have been reported as following independent segregation and hence are located on different chromosomes e.g. genes for legumin and convicilin in pea [8], the genes for glycinin and conglycinin in soyabean [11], and different legumin [10] and different vicilin genes [16] in pea. In some cases, however, the genes responsible for different polypeptides are linked together but with some distance between them [9, 12, 17]. And genes for various legumin and vicilin polypeptides under study are located together showing strong linkage in *L. sativus*.

ACKNOWLEDGEMENT

The authors convey thanks to Dr. R.B. Mehra, Division of Genetics, Indian Agricultural Research Institute, New Delhi, for kindly supplying the seeds of different *khesari* lines.

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