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Full Length Research Paper

Allozyme variations in leaf esterase and root peroxidase isozymes and linkage with dwarfing genes in induced dwarf mutants of grass pea (*Lathyrus sativus* L.)

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Genetic basis of different leaf esterase and root peroxidase isozymes was investigated by analyzing their zymogram phenotypes in selfed and intercrossed progenies of two local varieties (used as control) and three induced true breeding *dwarf mutant* lines of grass pea (*Lathyrus sativus* L.). Two non-allelic genes, *df1/df2* and *df3* controlling dwarfism in grass pea were included in the present linkage studies with different isozyme loci. The dwarf mutants could be distinguished from one another and also from control varieties by the presence of unique allozyme/s coded by allele/s in different loci. A good fit to 1: 2: 1 in F_2 and 1:1 in back cross indicated monogenic segregation of isozyme loci with co-dominant expression of different alleles and the isozymes were functionally monomeric. Linkage analysis revealed closely linked associations among *Est-1*, *Est-2* and *df3* loci and also between *Est-3* and *Prx-1* loci. All five genes, however, assorted independently with *df1/df2* as well as with *prx-3*. Locus *prx-2* was monomorphic in all five parents.

Key words: Dwarf mutants, isozymes, linkage, *Lathyrus sativus* L.

INTRODUCTION

Growth habit has been considered an important yield related trait in legume crops and there is a considerable scope for improvement of species in this regard through isolation and characterization of novel genetic variants (Jain, 1975). Along with other leguminous crops, induced mutagenesis has been successfully used to develop different growth habit mutants in grass pea (*Lathyrus sativus* L.).

Recently, three dwarf mutant lines, *dwf1*, *dwf2* and *dwf3*, showing erect, determinate and compact habit were isolated and characterized as true breeding recessive mutations in this crop (Talukdar, 2009a). Development of high yielding and low seed neurotoxin, ODAP (β-N-oxalyl L α, β diamino-propionic acid) containing lines are two prime objectives in grass pea breeding for which detail knowledge on genetic linkage map is essential. Compared to other closely related members like *Pisum*, *Lens* and *Vicia* of tribe Viceae within family Leguminosae, construction of a linkage map in grass pea has been hindered due to lack of enough genetic markers including morphological as well as molecular variants.

Isozymes are widely used as molecular markers in saturated linkage mapping and have advantages over other markers due to their co-dominant expression, lack of pleiotropic and/or epistatic interactions and resilience to environmental influence (Wendel and Weeden, 1990). However, like many other crops a major problem has been encountered to find a sufficient number of polymorphic isozymes loci in existing germplasms of grass pea (Gutiérrez et al., 2001).

Limited information is available regarding inheritance and linkage association of morphological and molecular markers in grass pea (Chowdhury and Slinkard, 1999). Although a good num-ber of stable and distinct morphological mutants have been isolated in grass pea through induced mutagenesis, their potential in creating

isozyme polymo-rphism and their analysis through different cytogenetic stocks is being carried out only recently (Talukdar 2009b, 2010).

Considering the importance of dwarf mutations in grass pea breeding, a detailed investigation has been carried out on banding pattern of two widely used enzyme systems namely esterase and peroxidase in self and intercrossed progenies of dwarf mutant lines and their mother varieties in grass pea. The objectives of this study were to analyze;

(1) The zymogram phenotypes of two enzymes and detect any type of allozyme variant.
(2) Pattern of segregation and linkage of different isozyme loci along with two independent dwarfing genes, *df1* / *df2* and *df3* in grass pea.

MATERIALS AND METHODS

Plant materials

Five diploid parents (2n = 14) comprising of three dwarf mutants (*dwf1*, *dwf2* and *dwf3*) and two grass pea varieties, 'BioR-231' and 'Hoogly Local' (HL), were used in the present study. The two varieties were used as control throughout the experiment. The mutant lines isolated in mutagen treated progeny of two control varieties have been maintained in separate and well protected fields for the past eight years and all the three bred true for their characteristic dwarf phenotypes in successive self generations (Talukdar, 2009a). During hybridization, out-crossing pollination was prevented by covering the plants with cellophane bag before blooming. Artificial pollination from selected parents was performed at 7 am during winter months of November – January and identity of pollen parent was indicated on every pollinated flower.

Enzyme systems and electrophoresis

Horizontal starch gel electrophoresis was performed on 12% starch gels for leaf esterase (EST, E.C.3.1.1-) and root peroxidase (PRX, E.C.1.11.1.7) in three *dwarf mutant* lines and their diploid control varieties. For esterase, extraction buffer was prepared following the methods of Chowdhury and Slinkard (2000). This involves adding 1.0 g PVP-40, 20 mg Mgcl2, 8 mg KCl, 4 mg EDTA, 2 drops of Triton X-100 and 0.2 ml mercaptoethanol to 10 ml of 0.1 M of Tris-HCl buffer of pH 7.4. For peroxidase enzyme system, root tissue was chosen due to consistency of isozyme pattern in different developmental stages. Young healthy root tissue was extracted in buffer identical to that used by Gottlieb (1982) except for the use of 2-mercaptoethanol.

Exclusion of 2-mercaptoethanol adversely affected the staining quality of many other enzymes and thus, peroxidase was analyzed on separate gels. Samples extracted with Tris-HCl were placed on a pH 8.1 tris-citrate/lithium borate electrodes and gel buffer system (Selander et al., 1971). The esterase and peroxidase bands were assayed following the staining recipes of Wendel and Weeden (1990). In case of esterase, Fast Garnet GBC salt (1 mg/1 ml) was used instead of Fast Blue RR salt. The peroxidase assay contained 25 ml of 0.1 M acetate of pH 5.0, 20 mg 3-amino-9-ethylcarbozole (dissolved in 2 ml N,N dimethylformamide) and one drop of 30% H_2O_2. Isozymes and their loci were designated following the principles of Weeden (1988).

Inheritance and linkage analysis

The three *dwarf mutants* and their control varieties were selfed for several generations (M_2 - M_5) and zymogram phenotypes of leaf esterase and root peroxidase were analyzed to test the homozygosity of different loci. Following three generations of selfing, intercrosses including reciprocals were made between control varieties and three mutant lines (M_5) to raise F_1 and subsequently, backcross (BC) and F_2 progenies (Table 1). Inheritance of individual locus was discerned by monogenic segregation of isozyme bands on the gel and their good fit to the expected ratio of 1:2:1 in F_2 and 1:1 in corresponding back crosses for each cross (Table 1).

Linkage relationships of the segregating isozyme markers along with two morphological trait loci namely *df1* / *df2* (controlling *dwf1*/ *dwf2* phenotype) and *df3* (*dwf3* phenotype) were examined for pair-wise combinations of different isozyme loci and also between pairs of isozyme loci and loci controlling dwarfism for the expected ratio of 1:2:1:2:4:2:1:2:1 and 3:1:6:2:3:1, respectively, in the F_2 progeny. Test cross progeny was raised by crossing F_1 plant with the parent showing comparatively slow moving allozymes in case of isozyme loci and with recessive dwarf mutant lines. Chi-square test was employed to test the goodness of fit and significant deviation from the expected ratio was considered as linkage between the markers. Recombination fraction (r) was calculated from test cross data and was converted to map distance in centiMorgans (cM) through Kosambi's mapping function (Kosambi, 1944). Linkage between two loci was declared considering the recombination fraction, $r \leq 0.30$. Data from different families was pooled when homogeneous for analysis (Table 2).

RESULTS AND DISCUSSION

Inheritance of isozymes in self and intercrossed progenies

EST

Two control varieties and three *dwarf mutant* lines together generated 15 distinct bands which could be easily resolved into three separate zones of enzyme activity tentatively designated as EST-1, EST-2 and EST-3 from anodal side of the gel (Figure 1a). All the five parents bred true in successive selfed (M_2 - M_5) generations for the single-banded pattern in these three zones. At EST-1, three types of allozyme activity could be deduced of which the most anodal band (EST-1a) was common to both *dwf1* and *dwf2* and was followed by EST-1b in two control varieties. The third allozyme, EST-1c, was found specific to *dwf3*. Presence of a unique band (EST-2a) in EST-2 zone and another unique band in EST-3 zone (EST-3a) distinguished *dwf2* and *dwf3*, respectively, from all other parents (Figure 1a). Apparently, the EST-1 zone possessed three allozyme variants, while EST-2 as well as EST-3 contained two variants in each case. Identical banding pattern, however, was exhibited by control plants.

F_2 progeny derived from different intercrosses involving five different parents homozygous for their respective banding pattern indicated allelic segregation (single

Table 1. Single locus segregation of three esterase and two peroxidase isozyme loci in F_2 and back cross populations of different intercrosses among five parents in *Lathyrus sativus* L.

Cross++	Locus	Alleles	F2 phenotype			N	X^2 (1:2:1/1:1)
			FF	FS	SS		
Control×dwf1/dwf2	Est-1	ab	24	52	29	105	0.48*
F1× dwf2	Est-1	ab	12	10	-	22	0.18**
F1× control	Est-1	ab	-	17	19	36	0.11**
Control×dwf3	Est-1	bc	36	61	27	124	1.34*
F1× dwf3	Est-1	bc	-	17	21	38	0.42**
F1× control	Est-1	bc	10	14	-	24	0.67**
dwf1/dwf2×dwf3	Est-1	ac	28	61	30	119	0.14*
F1× dwf1/dwf2	Est-1	ac	18	22	-	40	0.40**
F1× dwf3	Est-1	ac	-	17	25	42	1.52**
Control/dwf1/dwf3×dwf2	Est-2	ab	37	78	41	156	0.21*
F1× dwf2	Est-2	ab	33	41	-	74	0.86**
F1× control/dwf1/dwf3	Est-2	ab	-	41	45	86	0.19**
Control/dwf1/dwf2×dwf3	Est-3	ab	38	80	41	159	0.12*
F1× dwf3	Est-3	ab	61	59	-	120	0.03**
F1× control/dwf1/dwf2	Est-3	ab	-	33	29	62	0.26**
Control/dwf3× dwf1/dwf2	Prx-1	ab	33	58	37	128	1.37*
F1× dwf1/dwf2	Prx-1	ab	-	21	29	50	1.28**
F1× control/dwf3	Prx-1	ab	38	29	-	67	1.21**
Control/dwf2× dwf1	Prx-3	ab	29	54	23	106	0.72*
F1× dwf1	Prx-3	ab	27	19	-	46	1.39**
F1× dwf2	Prx-3	ab	-	27	32	59	0.42**
Control/dwf2×dwf3	Prx-3	bc	31	59	33	123	0.27*
F1× dwf3	Prx-3	bc	-	33	40	73	0.67**
F1× control/dwf2	Prx-3	bc	30	23	-	53	0.92**
dwf1× dwf3	Prx-3	ac	17	40	21	78	0.46*
F1× dwf3	Prx-3	ac	-	25	19	44	0.82**

[a] FF-Homozygote of fast alleles, SS-Homozygote of slow allele, FS-Heterozygotes; * and ** consistent with 1:2:1 and 1:1 ratios, respectively at 5% level of significance; ++ pooled data of several crosses presented.

locus) in three separate anodal zones of esterase activity and accordingly, the gene symbols *Est-1*, *Est-2* and *Est-3* were tentatively assigned. Segregation of allozymes could not be detected in F_2 progeny of two control varieties, but a part of F_2 progeny plants were heterozygous for different alleles in crosses with *dwarf mutants*. Polymorphism for *Est-1* locus was observed in eight cross combinations except BioR-231 × HL and *dwf1* × *dwf2* where no allelic variation for this locus was present (Figure 1b). Considering the relative mobility of bands, three types of allelic activities were observed in *Est-1* in the segregating F_2 progeny. In the crosses BioR-231 × *dwf1* / *dwf2* and HL × *dwf1*/ *dwf2* the two-banded phenotype in each case was heterozygous for alleles *Est-1a* / *Est-1b*, while crosses between *dwf3* and *dwf1* or *dwf2* yielded double-banded phenotypes heterozygous for *Est-1a* / *Est-1c*. Individual heterozygous for *Est-1b* / *Est-1c* were detected in the F_2 progeny derived from crosses between control varieties and *dwf3*. On the other hand, crosses between *dwf2* and *dwf3* yielded plants

Table 2. Joint segregation of pairs of isozyme loci and *df3* gene exhibiting significant deviations from expected F_2 and back cross (BC) ratios of random assortment in *Lathyrus sativus* L.

Number of progeny with designated phenotypes (F2/BC generation)														
Loci (X)-(Y)	Progeny	XY	XH	Xy	H1Y	H1H	H1y	xY	xH	xy	Total	χ^2	r+	Map Distance (cM)
Est1-Est-2	F2	12	02	04	02	32	03	02	02	10	69	54.07**	-	-
Est1-Est-2	BC	-	-	-	-	30	02	-	03	54	89	83.09*	0.056	5.7
Est 3-Prx-1	F2	19	02	03	06	22	01	02	03	11	69	86.83**	-	-
Est 3-Prx-1	BC	-	-	-	-	35	04	-	03	37	79	53.61*	0.089	9.1
Est1-df3	F2	12	-	03	18	-	04	02	-	10	49	22.97***	-	-
Est1-df3	BC	-	-	-	-	39	06	-	04	42	91	55.68*	0.11	11.18
Est2-df3	F2	14	-	02	20	-	03	02	-	12	53	33.31***	-	-
Est2-df3	BC	-	-	-	-	24	02	-	03	30	59	41.95*	0.085	8.6

[a] H_1-Heterozygous for alleles at 'X' locus, H- heterozygous for alleles at 'Y' locus; r+ recombination fraction.
** values significant at 0.01% level for 1:2:1:2:4:2:1:2:, *** for 3:1:6:2:3:1in F_2 and * for 1:1:1:1 at back crosses.

which were heterozygous for the alleles *Est-2a* / *Est-2b* in *Est-2* and *Est-3a* / *Est-3b* in *Est-3* locus (Figure 1c). Allozymes showing segregations both at *Est-1* and *Est-3* loci for different alleles were detected in the F_2 progeny of the crosses involving *dwf3* and other parents (Figure 1d). Segregation of alleles both at *Est-1* and *Est-2* loci was found in *dwf2* × control varieties (Figure 1e). Allozymes in each isozyme segregated into three phenotypic classes showing good agreement with the expected 1 (homozygote of fast variant, FF): 2 (hetero-zygote, F/S): 1(homozygote for slow variant, SS) ratio in F_2. Backcross of one heterozygote (F/S) to one homo-zygote (FF or SS) showed goodness of fit to 1:1 ratio (Table 1).

PRX

Three mutant lines and two control varieties bred true for their respective single-banded phenotypes in successive self generations and allozyme variation in root peroxidase zymogram was exhibited by three *dwarf mutants* only (Figure 2a). Two anodal isozymes designated as PRX-1 and PRX-2 and one cathodal isozyme of peroxidase designated as PRX-3 were consistently resolved in gel. The most anodal band (PRX-1a) was shared by control varieties along with *dwf3* line and the slow moving band in this zone (PRX-1b) distinguished *dwf1* as well as *dwf2* from other parents. PRX-2 zone was monomorphic in all the five parents. In PRX-3 zone, *dwf1* was conspicuously different by the presence of a fast moving variant (PRX-3a) while the slowest one (PRX-3c) in this zone was resolved as unique band in *dwf3* line. PRX-3b was common to rest of the parents (Figure 2a).

In F_2 segregating progeny, three zones of enzyme activity were confirmed and accordingly, three different loci *Prx-1*, *Prx-2* and *Prx-3* controlling these three respective zones were assigned in the present material. Except F_2 individuals obtained from crosses between control varieties and *dwf3*, plants heterozygous for alleles (*Prx-1a*/*Prx-1b*) in *Prx-1* locus were identified in rest of the crosses. Crosses between *dwf1/ dwf2* × *dwf3* gave rise to heterozygous F_2 plants for both *prx-1* and *prx-3* loci (Figure 2b). In addition, in *Prx-3* locus, F_2 progeny at *Prx-3* locus while *prx-2* was monomorphic (Figure 2c). Allelic segregation in *prx-1* and *prx-3* loci showed good fit to the ratio of 1(FF):2(F/S):1(SS) and 1(F/S):1(FF or SS) in F_2 and back cross populations respectively (Table 1).

The results indicate that *dwarf mutants* were not only conspicuously different from control varieties but also differed from each other due to variant isozyme patterns and they were heritable and bred true for all the six loci in the following generations. The dwarf lines possessed some unique bands coded by specific alleles; *Est-1c, Est-3c and Prx-3c* in *dwf3*, *Est-2a* in *dwf2* and *Prx-3a* in *dwf1* line. Obviously, *Est-1* and *Prx-3* loci were triple-allelic and highly polymorphic. Enzyme activity was well resolved in the anodal portion of leaf EST, but root tissue extracts produced the clearest, consistent and more intense bands than leaf tissues in case of PRX and the result is in agreement with earlier reports in other legume crops (Weeden and Marx, 1987).

Mutation has been identified as one of the main sources of isozyme variation in higher plants. Creation of variant alleles in isozyme system through induced mutagenesis was reported in gamma radiation-induced *dwarf mutants* of rice (Yoshiba and Yamaguchi, 1988), in regenerated legumes (Amberger et al., 1992; Malaviya et al., 2006) and other plants also (Bartošova et al., 2005;

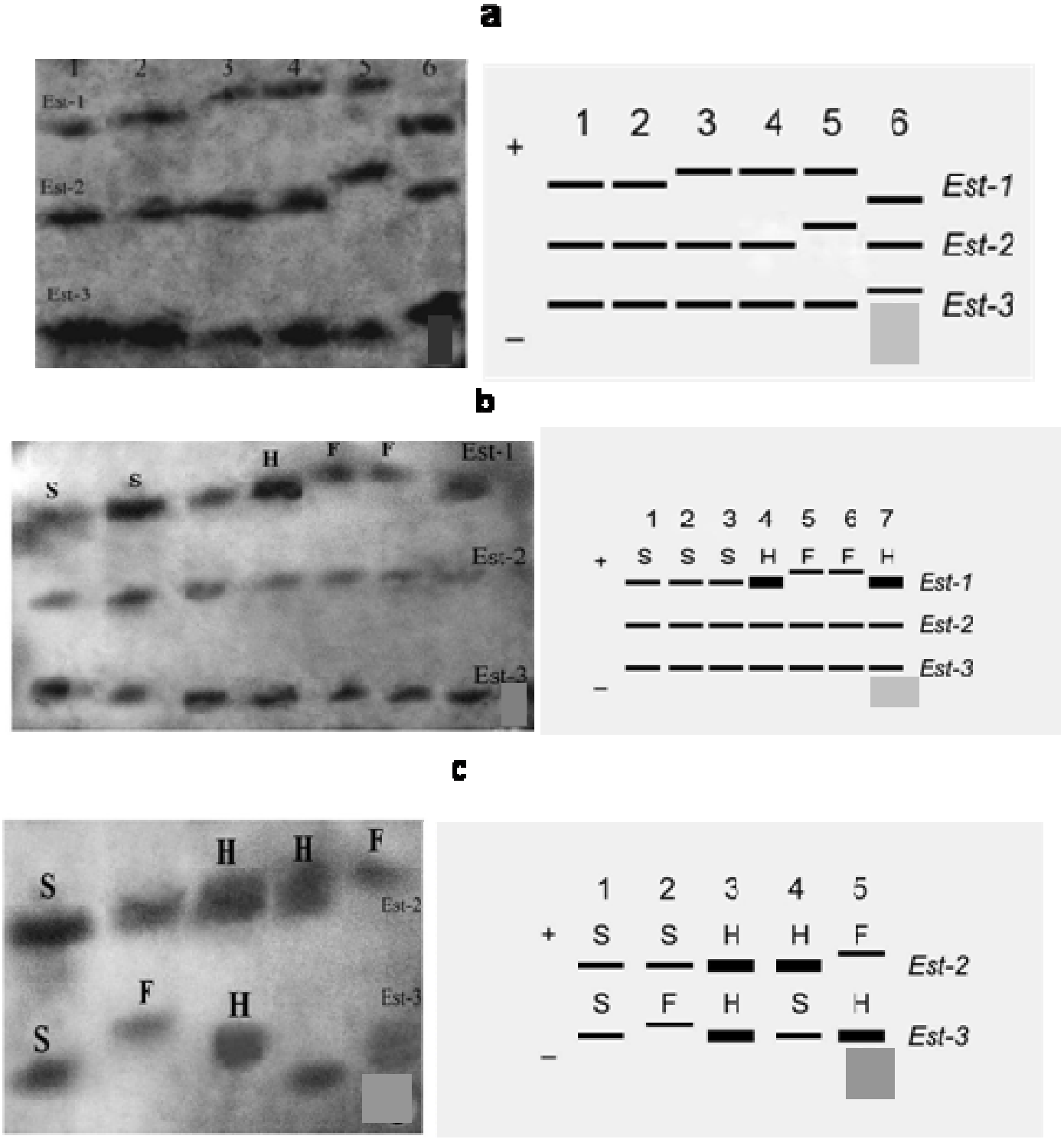

Figure 1. Zymogram of leaf esterase isozymes in parental and segregating F_2 populations of three *dwarf mutants* (*dwf1*, *dwf2* and *dwf3*) and two control varieties namely 'BioR-231' and 'Hooghly Local (HL)' in *Lathyrus sativus* L.
a. Esterase banding profile of five parents; lanes 1and 2-'BioR-231' and 'HL' respectively, lanes 3 and 4- *dwf1*, lane 5- *dwf2* and lane 6- *dwf3*.
b. A gel showing segregating allozymes at *Est-1* locus in F_2 population of BioR-231/HL × *dwf1*.
c. A portion of gel with segregation of different allozymes at *Est-2* and *Est-3* loci in F_2 generation of *dwf2* × *dwf3*.
F-homozygote of the alleles coding fast allozyme, **S**- homozygote of slow variant and **H**-heterozygotes (F/S).

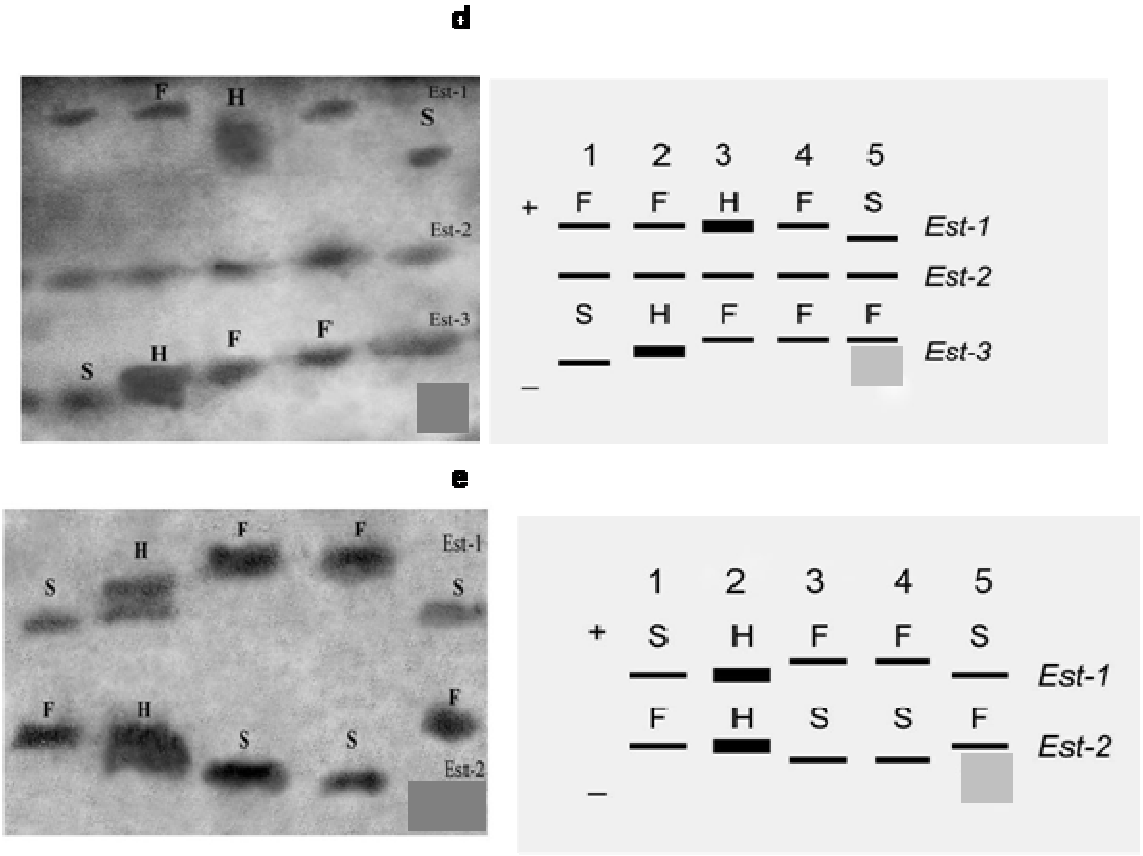

Figure 1. Zymogram of leaf esterase isozymes in parental and segregating F_2 populations of three *dwarf mutants* (*dwf1*, *dwf2* and *dwf3*) and two control varieties namely 'BioR-231' and 'Hooghly Local (HL)' in *Lathyrus sativus* L.
d. Segregation of variant allozymes in *Est-1* and *Est-3* loci in F_2 progeny of BioR-231/HL/*dwf1* × *dwf3*, *Est-2* was monomorphic.
e. Segregating allozymes in *Est-1* and *Est-2* loci in F_2 progeny of BioR-231/HL × *dwf2*.
F-homozygote of the alleles coding fast allozyme, **S**- homozygote of slow variant and **H**-heterozygotes (F/S).

Hossain et al., 2006). Recently, Talukdar (2009b) reported several polymorphic isozyme loci in 15 high yielding induced mutant lines of grass pea. However, in some accessions of grass pea (Gutiérrez et al., 2001) and in *Centrosema* sp (Penteado et al., 1997), occurrences of higher number derived from the crosses *dwf3* × control and *dwf2* × *dwf3* segregated for *Prx-3b*/*Prx-3c* in both cases. Similarly, crosses between *dwf1* (*Prx-3a*/*Prx-3a*) and *dwf3* (*Prx-3c*/*Prx-3c*) yielded plants heterozygous for *Prx-3a*/*Prx-3c* of alleles per locus was due to heterozygosity induced by significant out-crossing rate in these crops. In the present study, in addition to using out-crossing preventive measures during hybridization, effective isolation between lines and populations was maintained throughout the experiment to prevent intermixing and inheritance studies were carried out in advanced self generation (M_5) of different true breeding parental lines. Presumably, new alleles in EST as well as PRX isozymes in the present mutant lines resulted from the action of the recessive dwarfing genes originated through induced mutagenesis in grass pea.

Segregation of different alleles and their good agreement to 1:2:1 in F_2 and 1:1 in backcrosses suggests monogenic control of allozymes with co-dominant inheritance of alleles in both esterase and peroxidase isozymes. All the isozymes were functionally monomeric, as deduced by the presence of double-banded phenotype in heterozygotes of different crosses. The results are in agreement with earlier reports in different accessions of grass pea (Gutiérrez et al., 2001). Wendel and Weeden (1990) and Gour and Slinkard (1990) opined that co-dominant expression of isozyme coding genes allows accumulation of several to many isozyme loci in a single F_2 population, greatly enhancing the polymorphism and efficiency of gene mapping by facilitating the identification of heterozygotes and both homozygotes in F_2 generation.

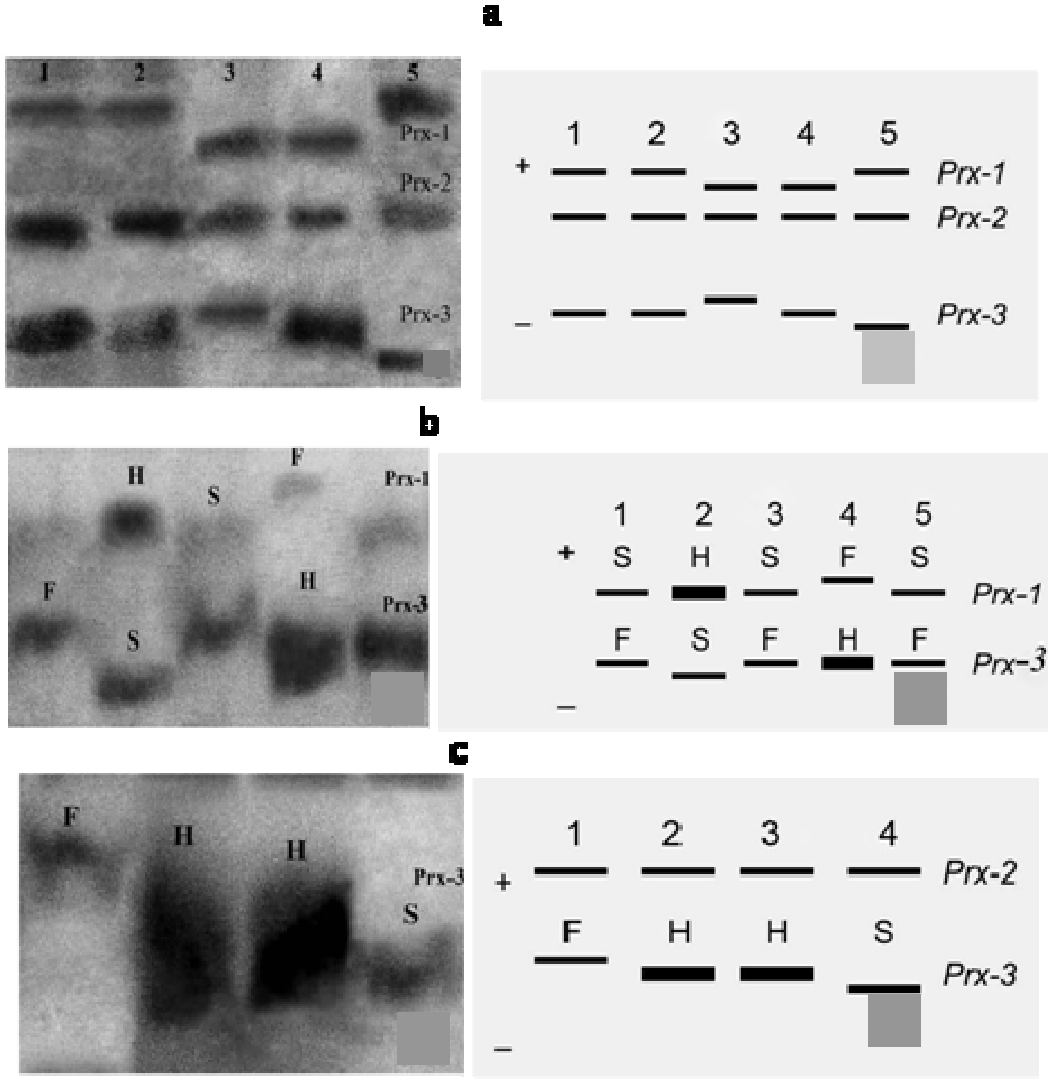

Figure 2. Root peroxidase banding profile in parental and segregating progenies.
a. Peroxidase banding pattern of five parents; lanes 1and 2-'BioR-231' and 'HL' respectively, lane 3-*dwf1*, lane 4- *dwf2* and lane 5- *dwf3*.
b. Portion of gel representing F_2 segregation of allozyme variants at the loci *Prx-1* and *Prx-3* in the crosses between *dwf1/dwf2* × *dwf3* lines.
c. A portion of F_2 zymogram phenotypes showing segregation of *Prx-3*.
F-homozygote of the alleles coding fast allozyme, **S**- homozygote of slow variant and **H**-heterozygotes (F/S).

Chowdhury and Slinkard (2000) earlier reported monomorphic banding at *Est-1* and *Est-2* loci, but detected polymorphism in *Est-3* in *Lathyrus sativus* L. Polymorphism in different esterase and peroxidase isozyme loci showing co-dominant expression has been observed in other legumes also (Weeden and Marx, 1987; Gaur and Slinkard, 1990; Zoro Bil et al., 1999). It was however, not possible to ascertain the inheritance pattern and structure of PRX-2 isozyme due to its monomorphic pattern in the present study.

Linkage analysis between isozymes and dwarfing genes

Two non-allelic dwarfing genes, *df1* / *df2* and *df3*, are inherited as monogenic (1:3 in F_2 and 1:1 in test cross) recessive mutations in grass pea (Talukdar, 2009a). In the present study individual isozyme locus exhibited normal Mendelian segregation (1:2:1 in F_2 and 1:1 in backcross) of alleles but joint segregation of *Est1-Est2*, *Prx1- Est3* and *df3-Est1*/*Est2* showed strong deviations

($P < 0.001$) from the expected ratios of independent assortment in F_2 (1:2:1:2:4:2:1:2:1 in first two cases and 3:1:6:2:3:1 in third case) and in back cross (1:1:1:1) progenies of different families, indicating linked association of the concerned loci. Using Kosambi's mapping function map distances of *Est-1- Est-2* and *Prx-1-Est-3* were calculated as 5.7 and 9.1 cM respectively (Table 2). Similarly, *df3* was found linked with *Est-1* by 11.18 cM, whereas *df3* and *Est-2* loci were mapped 8.6 cM apart (Table 2). No linkage however, was evident in rest of the cases (data not in table).

Limited information is available regarding the linkage relationship among esterase and peroxidase loci and between morphological and isozyme markers in grass pea. Gutiérrez et al. (2001) reported independent assortment between *Prx-1* and *Est-1* as well as *Est-2*, while Chowdhury and Slinkard (1999) mapped one flower colour locus with *Aat-2* in grass pea. This knowledge has now been extended with the detection of close linkage of two esterase loci with *df3* gene. The *df3* gene would be an excellent marker as dwarf phenotype was easily distinguishable in the present material and considered as a desirable criteria in plant breeding. Similar possibilities have been explored between a dwarf locus (*Le*) and *Tpi-p* isozyme in pea (Weeden and Hagens, 1998). Tight linkage between *Est-1* and *Est-2* and between *Est-1* and *Est*-3 was reported in *Pisum* and in *Lens* respectively (Weeden and Marx, 1987). In *Cicer*, close linkage between *Est-2* and *Est-3* appeared to be homologous to *Est-1* and *Est-2* in *Pisum* (Gaur and Slinkard, 1990). In the present study, linkage between *Est-1* and *Est-2* indicates possibility of homology of this group with that of *Pisum* and *Lens*, but further study is needed in this regard. Presence of very low number of recombinants recovered from various crosses in the present material suggests close linkage between *Prx-1* and *Est-3* also, but no linkage could be detected among any three of the *Prx* loci.

Earlier, *df1*/ *df2* and *df3* genes were mapped with other morphological markers on extra chromosome of trisomic-I and trisomic-III in grass pea respectively (Talukdar, 2009a). Close linkage among *Est-1*, *Est-2* and *df3* as deduced in present material indicates possible location of both esterase loci on extra chromosome of trisomic-III. Furthermore, close linkage between *Prx-1* and *Est-3* loci but their independent assortment with *Est-1*, *Est-2*, *Prx-3* as well as with *df1/df2* gene suggests either their distant location on the same chromosome or they were present in different linkage groups. On the basis of above results, four isozyme loci and two dwarfing genes could be tentatively mapped in cM as;

1) *Est-1*---5.7----*Est-2*----8.6-----*df3*
I----------11.18----------I

2) *Prx-1*---9.1---*Est-3*-not linked-*df1* / *df2*

Allozyme variation is essential for construction of saturated linkage map with other markers in grass pea (Chowdhury and Slinkard, 2000; Gutiérrez et al., 2001). Detection of different isozyme loci, variation in their allelic constituents (allozymes) and their linkage relationship with *dwarf mutations* for the first time in grass pea can be utilized in various fields of applied genetics and breeding of this crop. With this, the availability of primary trisomic stocks (Talukdar and Biswas, 2007) and a set of reciprocal translocations (Talukdar, 2010) will now be immensely helpful for location of different markers on definite linkage groups in grass pea.

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