RESEARCH ARTICLE

Dwarf mutations in grass pea (*Lathyrus sativus* L.): origin, morphology, inheritance and linkage studies

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Abstract

Induction of mutation has been used to create additional genetic variability in grass pea (*Lathyrus sativus* L.). During the ongoing investigations on different induced-morphological mutants, the author detected three types of dwarf mutants in grass pea. One mutant, designated as *dwf1* type was earlier identified in colchicine-treated C_2 generation of grass pea variety BioR-231 while the other two, designated as *dwf2* and *dwf3* were isolated in 250 Gy and 300 Gy gamma ray irradiated M_2 progeny of variety 'BioR-231' and 'Hooghly Local', respectively. As compared to their parental varieties (controls), all the three mutants manifested stunted, erect and determinate stem, early maturity and tolerance to pod shattering habit. The mutants differed from each other, as well as with controls, in number of primary branches, nature of stipules and internodes, length of peduncle, leaflet and seed coat colour, seed yield and seed neurotoxin content. The three dwarf mutants were monogenically recessive and bred true in successive generations. F_2 segregation pattern obtained from the crosses involving the three mutants indicated that dwarf mutation in grass pea was controlled by three independent non-allelic genes, assigned as *df1* (for *dwf1* type), *df2* (for *dwf2* type) and *df3* (for *dwf3* type), with the *df1* locus showing multiple allelic. Primary trisomic analyses revealed the presence of *df1/df2* locus on the extra chromosome of trisomic type I, whereas *df3* was located on the extra chromosome of type III. Linkage studies involving five other phenotypic markers suggested linked association of *df1/df2* locus with *lfc* (leaflet colour) and *wgn* (winged internode) and *df3* locus with *cot* (seed coat colour). Both the loci; however, assorted independently with flower colour and stipule character. The dwarf types can be utilized as valuable tools for further cytogenetic research and breeding of grass pea.

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Introduction

Grass pea (*Lathyrus sativus* L.) is an annual winter pulse crop in India occupying an area of about 1.6 million ha (FAO 2002). Of the nearly 200 species and subspecies of the genus *Lathyrus* belonging to family Leguminosae, *L. sativus* is the only member used as a grain legume (McCutchan 2003; Biswas 2003). Although cultivated for more than 8000 years, little evolutionary progress and improvement of this crop was made as a pulse crop mainly due to narrow genetic variability and unavailability of marker genes with prominent morphological manifestations, suitable for comprehensive genetic studies and breeding for high yield of low seed neurotoxin (ODAP) lines (Smartt 1984; Waghmare and Mehra 2000). Induction of mutation in recent times yielded some useful morphological variants and the author successfully isolated and studied inheritance patterns of different flower colour, seed coat colour and stipule mutations in grass pea (Talukdar *et al*. 2001a; Talukdar and Biswas 2005a,b). The author was also able to establish a complete set of seven different primary trisomics and three different dwarf types in grass pea and, since 2003, work has been going on to ascertain the genetic nature of these three dwarf types. In this communication my objectives are to: (i) characterize the three dwarf mutants, (ii) determine their mode of inheritance and gene action, and (iii) conduct linkage tests with primary trisomics as well as with five other distinct phenotypic markers isolated and characterized in grass pea.

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Materials and methods

Origin and morphology of dwarf mutants

Fresh and dry healthy seeds of grass pea varieties 'BioR-231' and 'Hooghly Local' (HL) were treated with different doses (50, 100, 150, 200, 250, 300, 350 and 400 Gy) of gamma rays to induce mutations. M_1 seeds were sown treatment-wise in randomized block design with uniform distances of 20 cm and 30 cm between plants and rows, respectively to raise M_2 progenies. Untreated plants of respective varieties were maintained as the control under identical conditions. In M_2 generation, three plants in 250 Gy irradiated progeny of variety 'BioR-231' were detected by their characteristic dwarf erect and determinate stem habit (mean plant height 25.04 cm), modifications in leaflet length (mean 3.12 cm), breadth (mean 0.69 cm) and colour (dark green), stipule (mean length 0.64 cm) and internode morphology (mean length 1.69 cm, winged) while stunted but erect habit (mean plant height 20.65 cm) in association with complete absence of stipule distinguished two plants in 300 Gy induced progeny of variety 'HL' at early vegetative stage during winter season of 2002–2003. Both the variant types matured 15–20 days earlier than their respective control varieties in M_2 and they were harvested separately to raise subsequent generations. Beside these two variant plant types isolated from gamma ray induced progeny, another plant showing similar stunted phenotype was earlier isolated from 0.25% colchicine treated (6 h, 3 days) populations of variety BioR-231 and primarily characterized as 'dwarf mutant' in C_2 generation (Talukdar *et al*. 2001b). All the three variant types were maintained through selfing in separate and well protected fields for the past few years and no segregation within type was observed for the respective characters. The progeny of three types showing characteristic dwarf habit were true breeding in the advanced generations and characterized as *dwarf mutant 1 (dwf1), dwarf mutant 2 (dwf2) and dwarf mutant 3 (dwf 3)* for the type derived from colchicine treated, 250 Gy and 300 Gy gamma ray induced populations respectively. As compared to M_2, progeny mean values of different morphological and yield related traits of control varieties and the three dwarf types were recorded and statistically analysed in advanced (M_3) generation. Colchicine treated *dwf1* was also cytologically analysed at meiosis-I to study chromosome number and configuration. Seed ODAP content was estimated following the procedure developed by Rao (1978).

Inheritance studies and test of allelism

All the three dwarf mutant lines in M_4 generation were reciprocally crossed with their respective parental cultivar and F_1 progeny was raised during winter season of 2004–2005. In the following season F_2 population in each cross was grown and segregation data for concerned traits were recorded. Utmost care was taken during harvesting of F_2 plants individually of every cross combination, and F_3 progeny were raised subsequently. Dwarf F_3 types were separately harvested at maturity. Segregation data was tested by χ^2 to determine goodness-of-fit between observed and expected ratios for dwarf habit. Reciprocal crosses were conducted to trace the involvement of any type of cytoplasmic inheritance. To study the allelic relationship of gene(s) controlling dwarfism, intercrosses were made among *dwf1, dwf2* and *dwf3* in all possible combinations. The F_1, F_2 and F_3 generations were evaluated for segregation of normal plant type and dwarf type in normal field condition.

Linkage test: primary trisomic analyses of dwarf mutants

Simultaneously, a detailed crossing programme was initiated from the year 2003 involving *dwf1, dwf2* and *dwf3* and different primary trisomic lines (Talukdar *et al*. 2001a; Talukdar and Biswas 2007b). The seven primary trisomics were characterized as '*bicular*' (type I), '*bifid*' (type II), '*ternate-fasciculate*' (type III), '*ternate-verticillate*' (type IV), '*alternate*' (type V), '*revolute*' (type VI), and '*revolute-lanceolate*' (type VII). The trisomic plants were used as female parent in crosses with homozygous diploid dwarf plants. On the basis of typical leaflet and stipule characters, different trisomic phenotypes in segregating populations could be readily identified at seedling stages. Each F_1 plant was harvested individually and the trisomic F_1 plants were selfed to obtain F_2 seeds and also backcrossed with dwarf types to raise BC_1 population. Chromosome of F_1 plants, F_2 recessive homozygotes and BC_1 plants obtained from different crosses were analysed at meiosis-I following the procedure of Biswas (1998). In the segregating F_2 and BC_1 progenies, diploid and trisomic plants in different crosses were classified as dominant normal plant type and recessive dwarf mutant type. Segregation data for normal and mutant phenotypes in diploid portion were tested by means of χ^2 test for normal disomic segregation ratio of 3:1 in F_2 and 1:1 for test cross. Significant deviation from this ratio was again tested for 8:1 in F_2 and 2:1 in test cross to locate possible trisomic chromosome/s bearing gene/s for dwarf mutations.

Linkage test: dwarf mutants with other phenotypic markers

Two prominent and phenotypically easily recognizable true breeding mutant lines namely, *pale violet flower mutant (Pv)* and *black seed coat mutant (Cbl)* were earlier characterized in grass pea (Talukdar *et al*. 2002; Talukdar and Biswas 2005a). Inheritance pattern of stipule character was also studied and accordingly, gene symbol St^n for the presence of normal foliaceous stipules/St^e for absence of stipules in grass pea was assigned (Talukdar and Biswas 2005b). For leaflet colour and winged modification of internode gene symbols, *Lfc* (normal green)/*lfc* (dark green) and *Wgn* (normal winged)/ *wgn* (highly winged), respectively, were proposed for the present study. These marker characters have been included in the present investigation to study the linkage relationship with three dwarf mutant lines (table 4). Since 2003–2004, investigations were carried out to trace the linked association between genes controlling dwarfism

and these five genetic markers through intercrossing between dwarf-mutant lines and flower as well as seed coat colour mutants along with two control cultivars and, subsequently, F_1 hybrid in each cross was raised. F_1 plant in each cross was backcrossed to respective recessive parent and also selfed to produce F_2 progeny. Recessive F_2 progenies were also advanced to F_3 generation. Chi-square test was employed to test for single-locus goodness-of-fit between the observed and expected ratios for individual traits and to test joint segregation ratios for both loci in F_2. Cross over value in percentage (cov%) was calculated from test cross data and incroporating this value in Kosambi's formula (Kosambi 1944), map distance between the linked genes was estimated.

Results and discussion

Characteristics of three dwarf mutants

Characteristic features of three dwarf mutants; namely, *dwf1, dwf2* and *dwf3* and two parental accessions; namely 'BioR-231' and 'HL' used as control in the present study have been compared in table 1. In comparison to control plants, plant height was reduced to different magnitude in three dwarf mutants and the shortest height was recorded in *dwf1*, followed by *dwf3* and *dwf2*. This reduction in plant height was mainly due to short internodal distances manifested in three dwarf mutants, as also seen earlier in barley dwarf mutant (Sethi 1974), grass pea internode mutant (Talukdar and Biswas 2006) and chickpea mutant (Lather 2009; Gaur *et al.* 2008). Usual prostrate, spreading and indeterminate stem of control plants in the present material were found to be modified to erect habit accompanied with determinate stem in all the three dwarf-mutant lines (figures 1a–c and 2, a&b). The orientation of early formed branches were very close and inclined with each other to form a compact and bushy plant type which was very distinct particularly in *dwf2*. Number of primary and secondary branches in *dwf2* and *dwf3* reached very close to that of control variety but tertiary and later order branches reduced considerably in all the three mutants. Internodes in *dwf1* and *dwf2* were prominently winged which might be attributed to their rigid habit of stem. Normal winged nature was however manifested in *dwf3* and its stem was tender and soft but formation of its branches in close vicinity and their low angle of divergence with main axis gave this mutant a characteristic bushy habit. Leaflets and stipules in *dwf1* and *dwf2* were shorter in size, ovate–lanceolate in shape and dark green in colour. In *dwf3*, leaflets were linear–lanceolate and normal green, like control plants, and the stipules did not develop at all. As compared to control variety, inter-leaflet distances decreased in all the three dwarf types, and leaflets were thicker and more upright in *dwf1* and *dwf2* but similar to controls in *dwf3*. Weigle and Butler (1983) also reported thicker, shorter and darker green leaves in EMS induced dwarf mutant showing complete absence of internode in *Impatiens platypetala*. Modification of long terminally coiled tendrils in the present normal type was exhibited as very short, curled and intermingled with each other in *dwf2*. Terminally coiled short tendril was however developed in the other two mutant lines. Usually, flower was solitary axillary on peduncle with a height between 3.15 and 3.28 cm in the control plant but peduncle length increased in *dwf3* and decreased markedly in *dwf1*. It however, attained normal length in *dwf2* type. Talukdar and Biswas (2007a) explained the involvement of more than one gene showing cumulative effect in controlling peduncle length in grass pea. In an induced barley dwarf mutant, Sethi (1974) discussed the presence of longer peduncle and explored its significance in genetic and breeding research. Kolb and Marshall (1984) attributed the reduction in peduncle length in dwarf oat to shorter parenchyma cells and lower percentage of dividing cells in the meristem. In grass pea, usually solitary pod developed, but in *dwf2*, clustered pods (known as 'double poddedness') were manifested on a single peduncle. All the three mutants showed tendency to premature pod shattering and matured earlier than control varieties in all the generations studied.

Post harvest identification of three dwarf mutants was also possible by conspicuous modifications in their seed coat colour. Like parental variety 'BioR-231', was brown in *dwf1* was modified to milky white in *dwf2* (figures 3,a&b). On the other hand, brownish grey colouration in seed coat of variety 'HL' was modified to yellow tinged white in *dwf3* line (figure 3c&d). As compared to parental varieties (0.33% in BoR-231 and 0.28% in HL), mean seed ODAP content decreased to 0.11% in *dwf2* and 0.14% in *dwf1* but increased to 0.39 ± 0.49 in *dwf3* which showed significance difference (at 0.05 level) with that of control values.

Meiotic analyses revealed regular occurrence of 7II at metaphase-I and 7–7 separations at anaphase-I in microsporocytes of all three dwarf mutant lines and no chromosomal abnormalities were observed. It seems that the phenotypic modifications exhibited in these mutant lines have arisen through gene mutation but not through any type of structural or numerical aberration in chromosomes. This carries special significance in case of colchicine-induced *dwf1* as colchicine is generally used to induce polyploidy in plants. Foster *et al*. (1961) postulated that colchicine caused a somatic reduction of the chromosomes after or, perhaps, concurrent with mutagenic effects, and that subsequently the diploid number was restored. According to him, mutagenic action of colchicine was not limited to a single locus of a chromosome but might affect mutations at random involving many genes probably on different chromosomes within that one plant resulting in large number of mutant phenotypes in a single plant as also exhibited in the present *dwf1* in grass pea. Colchicine-induced mutants or variants were also recovered earlier in flax (Dirks *et al*. 1956) and soybean (Downes and Marshall 1983).

Inheritance and allelic relationship of gene(s) controlling dwarfism

Reciprocal crosses among *dwf1* as well as *dwf2* and

Table 1. Morphological features of *dwf1*, *dwf2* and *dwf3* and the two parental varieties in *Lathyrus sativus* L.

Character	*dwf1** (Mean ± s.e.)	*dwf2** (Mean ± s.e.)	*dwf3** (Mean ± s.e.)	BioR-231 (Mean ± s.e.)	Hooghly Local (HL) (Mean ± s.e.)
Plant height (cm)	16.37 ± 0.05	25.1 ± 0.19	20.11 ± 0.12	52.51 ± 0.29	64.09 ± 0.48
Length of internodes (cm)	1.63 ± 0.17	1.72 ± 0.11	2.09 ± 0.18	3.30 ± 0.37	3.51 ± 0.49
Number of primary branches/plant	6.8 ± 0.24	11.2 ± 0.34	9.0 ± 0.31	13.2 ± 0.21	11.90 ± 0.19
Leaflet length (cm)	3.0 ± 0.19	3.1 ± 0.15	3.17 ± 0.20	6.1 ± 0.04	5.7 ± 0.11
Leaflet breadth (cm)	0.49 ± 0.07	0.64 ± 0.0	0.53 ± 0.13	0.50 ± 0.08	0.47 ± 0.09
Length of stipules	0.50 ± 0.09	0.70 ± 0.1	-	1.79 ± 039	1.61 ± 0.22
Stem habit	Erect and determinate	Semi-erect and determinate	Erect and determinate	Spreading and indeterminate	Semi-spreading and indeterminate
Internodes	Highly winged	Highly winged	Normal winged	Normal winged	Normal winged
Length of Peduncle (cm)	1.20 ± 0.27	3.9 ± 0.42	7.22 ± 0.29	3.25 ± 0.51	3.16 ± 0.42
Days to flowering	63.0 ± 0.31	80.4 ± 0.56	37.8 ± 0.35	48.8 ± 0.67	40.0 ± 0.57
Days to maturity	106.1 ± 0.48	123.0 ± 0.33	90.2 ± 0.3	134.9 ± 0.55	117.0 ± 0.37
Pods/peduncle	01	02	01	01	01
Pods/plant	16.9 ± 0.07	75.4 ± 0.47	51.5 ± 0.32	8.1 ± 0.56	73.17 ± 0.69
Leaflet thickness	Thick, dark	Thick, dark	Normal thin	Normal thin	Normal thin
and colour	green	green	and green	and green	and green
Pod shattering habit	Absent	Absent	Absent	Present	Present
Seed yield/plant (gm)	2.2 ± 0.69	12.3 ± 0.66	11.6 ± 0.79	1.67 ± 0	15.09 ± 0.19
Seed coat colour	Brown	White	Yellow tinged white	Brow	Gray–brown
Seed ODAP%	0.14 ± 0.33**	0.11 ± 0.37**	0.39 ± 0.49**	0.33 ± 0.43	0.28 ± 0.56

**dwf1* and *dwf2* derived from variety 'BioR-231', while *dwf3* is derived from 'Hooghly Local'. **Significant at 0.05 leve*

Figure 1. Plant types of various genotypes of grass pea (*Lathyrus sativus* L.): (a) control variety, BioR-231, (b) dwarf mutant 1, *dwf1*, (c) dwarf mutant 2, dwf2, and (d) [illegible]ture plant type.

Figure 2. Plant habits of various genotypes of grass pea (*Lathyrus sativus* L.): (a) control variety, Hooghly Local, (b) dwarf mutant 3, *dwf3*, and (c) miniature plant type.

Figure 3. Seed coat colour of different mutant lines and control varieties: (a) brown seed coat of variety BR-231 and *dwf1*, (b) white seed coat of *dwf2*, (c) grey-brown seed of variety Hooghly Local, (d) yellow tinged white seed coat of *dwf3*, and (e) black seed coat of *BSCM* line.

control variety yielded F_1 plants with normal phenotypes-like control indicating dominance of normal type over dwarf in each case. A good fit of normal and dwarf plant type to the ratio of 3:1 in the F_2 generation and test cross suggested involvement of recessive gene in controlling the dwarf trait and no cytoplasmic factor was involved. Similar results were obtained in F_1 and F_2 generations of the cross between *dwf3* and control plant (table 2). The three recessive dwarf types recovered in F_2 generation were selfed and in F_3 all the 145 plants derived from *dwf1* were true breeding. Similarly, no segregation of *dwf2* type and *dwf3* type was noticed in F_3 progeny of all the 201 plants *(dwf2)* and of 131 plants *(dwf3)*, respectively (data not shown). Although dwarf mutations generally originated through recessive mutation in most of the plants, Qin *et al*. (2008) recently reported a novel dominant dwarf mutation, controlled by a single dominant gene in rice.

Inter-mutant crosses were also performed to study the allelic relationships of genes governing dwarf nature in grass pea. For this purpose, *dwf1* and *dwf2* were crossed and in the F_1 progeny characteristic phenotype of *dwf1* appeared in all plants. In subsequent F_2 generation, phenotypes characteristics of *dwf1* and *dwf2* segregated and fit well to 3:1 ratio indicating monogenic complete dominance of *dwf1* type over *dwf2*. A 1:1 segregation of these two types in corresponding backcross confirmed the result (table 2). Monogenic complete dominance of normal type over *dwf1* and *dwf2* and again *dwf1* over *dwf2* suggested that the locus is multiple allelic showing an order of dominance as normal type > *dwf1* > *dwf2*. The gene symbols for these three types were proposed as *Df, df1* and *df2*, respectively.

A completely different result however was obtained in the crosses between *dwf1/dwf2* and *dwf3*. All the F_1 plants derived from the crosses between *dwf1* and *dwf3* as well as between *dwf2* and *dwf3* were of normal types. In F_2, four types of plants—normal type, *dwf1, dwf3* and a variant type in case of first cross, and normal type, *dwf2, dwf3* type and again a variant type in second cross appeared in the progeny showing good fit to 9:3:3:1 ratio in both cases. This indicated involvement of two non-allelic loci *Df/df1/df2* and *Df3/df3* (designated for *dwf3* type) in controlling dwarfism independently in grass pea and both the genes (*Df* and *Df3*) exhibited dominance over their respective recessive alleles (*df1/df2* and *df3*). In the presence of both the genes in dominant form (*Df-Df3*) normal phenotype appeared whereas presence of *df3* gene in double recessive form (*df3df3 Df-*) produced phenotypes characteristic of *dwf3* type. On the other hand, *dwf1* or *dwf2* type occurred in the presence of double recessive nature of *df1/df2* gene (*Df3-df1 df1* or *Df3-df2df2*). In homozygous recessive condition of both the genes (*df1df1df3df3* or

Table 2. Inheritance of dwarf mutations in F_1 and F_2 generations of different crosses in *Lathyrus sativus* L.

Cross	F_1 phenotype	*Observed frequencies in F_2 and backcross progeny					Expected genetic ratio
		Normal	*dwf1*	*dwf2*	*dwf3*	Miniature	
BioR-231 × *dwf1*	Normal type	113	37	–	–	–	3:1
F_1 × *dwf1*	–	30	23	–	–	–	1:1
BioR-231 × *dwf2*	Normal type	290	–	95	–	–	3:1
F_1 × *dwf2*	–	81	–	77	–	–	1:1
HL × *dwf3*	Normal type	153	–	–	48	–	3:1
F_1 × *dwf3*	–	79	–	–	72	–	1:1
dwf1 × *dwf2*	*dwf1* type	–	67	24	–	–	3:1
F_1 × *dwf 2*	–	–	29	26	–	–	1:1
dwf2 x *dwf3*	Normal type	159	–	49	42	1	9:3:3:1
Dwf1 x *dwf3*	Normal type	112	39	–	37	10	9:3:3:1

*Observed values are consistent with the expected genetic ratios at 0.05 significance level.

df2df2df3df3) a variant plant type showing extreme reduction in height, deformed leaves and swollen stem resulted in the F_2 progeny. This type is bred true in advanced generations and tentatively designated as 'miniature type' in grass pea (figures 1,d and 2,c).

Genetic segregation and linkage of dwarfing genes in primary trisomics

In the last couple of years, linkage tests were conducted to study the association of dwarfing genes with the extra chromosomes of the seven different primary trisomics isolated in grass pea. The results given in table 3 indicated that the segregation of normal and recessive dwarf types in the diploid portion of F_2 progenies obtained from the crosses namely *dwf1* × '*acicular*', *dwf2* × '*acicular*' and *dwf3* × '*ternate-fascicled*' showed significant deviation from the expected normal Mendelian disomic ratio of 3:1 but exhibited good fit to the expected trisomic ratio of 8:1. The segregation ratio in BC_1 population in all the three crosses also manifested deviation from the expected disomic ratio of 1:1, instead fitted well to 2:1. The deviations from the normal segregation ratio are ascribed to the phenomenon of primary trisomy. No recessive homozygote plant in trisomic portion was traced in these three crosses and all the recessive homozygotes, 15 from the first cross, 24 from the second cross and 17 plants in the third cross recovered were cytologically analysed and found to be diploids possessing $2n = 14$ chromosomes. On the other hand, segregation of normal and dwarf type in rest of the crosses showed significant deviation from the expected 8:1 ratio but fit well with disomic 3:1 ratio in F_2 and normal 1:1 ratio in corresponding test crosses in diploid population. Also an appreciable number of recessive homozygotes in $2n + 1$ portion of these crosses were trisomic plants carrying one extra chromosome ($2n + 1 = 15$) in their gametic complement.

The observed differences in the results obtained from the crosses between *dwf2* and trisomic type III and between *dwf3* and type III justified independent nature of *df1/df2* locus with *df3* and the two genes were located on two different chromosomes in grass pea. Presumably, the gene *df1/df2* controlling phenotypes of *dwf1/dwf2* type was located on the extra chromosome of trisomic I, whereas the second locus *df3* was most likely present on the extra chromosome of trisomic III in grass pea. In tomato, Lesley (1926) studied the trisomic inheritance of a recessive dwarf character. Khush *et al*. (1984) discussed the good fit of segregation in the diploid portion of trisomic F_2 population in 8:1 instead of 3:1 for recessive nature of concerned genes irrespective of rates of transmission of the extra chromosome or the distance of the marker gene from the centromere. Tsuchiya and Haines (1975) observed similar results to locate several recessive mutations on barley chromosome by means of trisomic analyses. Honeycutt *et al*. (1989) recorded strong deviation of segregation of normal variegated leaf mutation from the theoretical disomic ratio of 3:1 and held the view that gene controlling variegated leaf mutation in soybean was present on the extra chromosome of trisomic A.

Linkage test of df1, df2 and df3 genes with other phenotypic markers

Linked associations of *df1/df2* and *df3* with seed coat colour, leaflet colour, nature of stipules, winged modification of internode and flower colour were also investigated in different cross combinations (table 5). Two true breeding mutant lines, *BSCM* producing black mosaic seed coat (figure 3,e) and *PVFM* showing characteristic pale violet colour instead of normal blue flower were utilized. Results in table 5 showed a significant and large χ^2 value ($\chi^2 = 76.44$ at 1 df) for joint segregation of genes *cbl* controlling seed coat colour with *df3* but manifested a non-significant value with genes *df2* although in both cases single locus segregation of individual gene was not significant, fitting well with normal 3:1 ratio in F_2. Segregations of four phenotypes in corresponding test cross involving *df3* and *cbl* deviated strongly ($\chi^2 = 60.25$ at 3 df) from the expected 1:1:1:1 but showed good fit in case

Table 3. Segregation of normal and dwarf plants in F_2 and BC_1 generations of crosses between *dwf1/dwf2* and different primary trisomics (types I–III, V and VII) and between *dwf3* and trisomic types I–V of grass pea (*Lathyrus sativus* L.).

	F_2 and BC_1 phenotype									
	$2n$			χ^2				$2n+1$		
Trisomic F_1	Normal	Dwarf type	Total	(1:1)	(2:1)	(3:1)	(8:1)	Normal type	Dwarf type	Total
(*dwf1* × type I) selfed	118	15	133	–	–	13.36	0.0037*	37	0	37
(*dwf1* × type I) × *dwf1*	49	21	70	11.7	0.35	–	–	08	0	08
(*dwf2* × type I) selfed	209	24	233	–	–	26.85	3.37*	29	0	29
(*dwf2* × type I) × *dwf2*	65	29	94	3.79	0.26	–	–	09	0	09
(*dwf2* × type II) selfed	80	23	103	–	–	9.39**	13.14	65	16	81
(*dwf2* × type II) × *dwf2*	27	18	45	1.80	–	–	–	11	07	18
(*dwf2* × type III) selfed	111	25	136	–	–	3.17**	7.82	70	20	90
(*dwf2* × type III) × *dwf2*	18	11	29	1.69	–	–	–	12	9	21
(*dwf2* × type V) selfed	32	09	41	–	–	0.20**	4.86	29	06	35
(*dwf2* × type VII) selfed	41	11	52	–	–	0.41**	5.39	18	04	22
(*dwf3* × type I) selfed	191	56	247	–	–	0.71**	33.16	37	10	47
(*dwf3* × type I) × dwf3	51	40	91	1.33	–	–	–	13	10	23
(*dwf3* × type II) selfed	70	21	91	–	–	0.17**	17.20	23	07	30
(*dwf3* × type II) × *dwf3*	16	09	25	1.96	–	–	–	09	06	15
(*dwf3* × type III) selfed	145	17	162	–	–	18.19	0.063*	15	0	15
(*dwf3* × type III)× *dwf3*	55	24	79	12.16	0.31	–	–	07	0	07
(*dwf3* × type IV) selfed	100	31	131	–	–	0.12**	20.88	21	06	27
(*dwf3* × type V) selfed	55	16	71	–	–	0.23**	9.381	17	04	21
(*dwf3* × type V) × *dwf3*	23	17	40	0.90	–	–	–	15	11	26

*Consistent with 8:1 ratio and **consistent with 3:1 ratio at 0.05 level of significance.

Table 4. Morphological characters of different parents used in linkage study in grass pea (*Lathyrus sativus* L.).

Character	*Dwarf mutant 1*	*Dwarf mutant 2*	*Dwarf mutant 3*	*PVFM*	*BSCM*	BioR-231	HL
Seed coat colour	Brown	White	Yellow tinged white	Brown	Black	Brown	Grey–brown
Leaflet colour	Dark green	Dark green	Normal green	Normal green	Normal green	Normal green	Normal green
Winged nature of internodes	Highly winged	Highly winged	Normal (moderate winged)	Normal winged	Normal winged	Normal winged	Normal winged

of genes *df2* and *cbl*. Thus, it was evident that gene *cbl* inherited independently with gene *df2* but was linked to gene *df3* with an estimated map distance of 11.79 cM, calculated from cross over value of corresponding test cross of (*dwf3* × *BSCM*) F_1 × *dwf3*. Similarly, for genes *lfc-df2*, *wgn-df2* as well as *lfc-wgn* single locus segregation was not significant in each case but their joint segregation in F_2 exhibiting a large χ^2 value (45.59 for *df2-lfc*, 66.0 for *df2-wgn* and 145.2 for *lfc-wgn*) indicated linked association of both the genes *lfc* and *wgn* with *df2* locus and also between *lfc* and *wgn*. The respective test cross segregations also deviated significantly from the expected 1:1:1:1 ratio. Cov%, calculated from test cross data, was incorporated into Kosambi's (1944) formula and map distances of genes *lfc* and *wgn* from *df2* and between *lfc* and *wgn* were ascertainexd as 24.80 cM, 30.34 cM and 9.75 cM, respectively. Regarding stipule character, Talukdar and Biswas (2005b) earlier pointed out that the trait in grass pea was monogenically inherited and the locus was multiple allelic. In the present investigation, segregation of gene *St*^{n} governing stipule character with *df1/df2* and *df3* showed non-significant χ^2 value for both single locus and for joint segregation (0.017 with *df2* and 0.085 with *df3*) in F_2 and exhibited good fit to the expected 1:1:1:1 ratio (0.93 with *df2* and 0.27 with *df3*) in test cross (data not shown). This suggested independent inheritance of gene *df1/df2* as well as *df3* with St^n. Linked association of dwarf habit with flower colour was tested in two different cross combinations involving a mutant line *PVFM* in each case with *dwf2* and *dwf3*, separately. Mehra *et al*. (1995), Tiwari and Campbell (1996), Das and Kundagrami (1999) and recently, Talukdar and Biswas (2007c) established digenic mode of inheritance involving various types of non-allelic interaction in genetics of flower colour production in grass pea. In the present material, preliminary investigation on the segregating F_2 progeny indicated a hybrid pattern of inheritance of flower colour with a definite ratio (data not shown). This result apparently indicated absence of linkage between dwarf habit and flower colour in grass pea. Further investigation in advanced generations is however, necessary.

Information about gene mapping based on morphological parameters in grass pea are not available in review of literature. Recently, however, molecular markers have been used to construct genetic linkage map. Using RAPD, isozyme and one morphological marker in F_2 segregating individuals of *L. sativus*, Chowdhury and Slinkard (2000) constructed a genetic linkage map and subsequently detected linkage between two isozyme loci *Acp-2* and *Pgm-1*. Gutierrez *et al*. (2001) mapped Six other isozyme loci in three linkage groups of grass pea, whereas Ikiba *et al*. (2004) detected two *QTLs*, namely *QTL1* and *QTL2* associated with blight resistance in linkage group one and two, respectively.

In the present investigation on the basis of inheritance and linkage data maps of three different marker genes with *df1/df2* and *df3* can be tentatively constructed in cM as:

df1/df2-----24.80----------*lfc*-----9.75---*wgn*; *df3*——11.79——*cbl*

I----------30.34----------I

Linkage studies of two loci, namely *df1/df2* and *df3* controlling dwarf mutations in grass pea by means of primary trisomic analyses and with five other markers genes pointed out the fact that in grass pea genes controlling leaflet colour (*lfc*) and winged nature of internode (*wgn*) were located on extra chromosome of trisomic I along with *df1/df2* locus while gene *cbl* governing seed coat colour was most likely present with *df3* locus on the extra chromosome of trisomic III. It was also clear that *lfc* and *wgn* were much more closely linked on this chromosome than either was to *df1/df2* whereas the locus controlling stipule characters and presumably, flower colour were either not carried by any one of these two chromosomes or they were distantly located on the same chromosome in grass pea. Precise cytological identification of extra chromosome in seven primary trisomics involved in the present investigation will certainly give more specific information regarding the chromosomal location of dwarf and other mutations in grass pea in near future.

Dwarf mutations and grass pea breeding

Isolation and characterization of three true-breeding dwarf mutant lines and studies on their pattern of inheritance, chromosomal location and linkage with five other stable genetic markers revealed various aspects in grass pea genetics which can be used to improve this crop. Rybinski (2003) pointed out that prostrate habit, indeterminate growth, late in maturity, pod shattering and of course, seed neurotoxin served as limiting factors for broader introduction of grass pea in different environmental conditions. According to Khanna-Chopra and Sinha (1990), indeterminate stem promotes excessive vegetative growth, which acts as competitive sink for developing pods and seeds. Jain (1975) suggested that improvement in grain yield in legume crops could be achieved through restructuring of plant type to determinate, erect and

Table 5. Linkage study of genus controlling dwarfism (*df1/df2* and *df3*) with seed coat colour (*cbl*), leaflet colour (*lfc*), presence of stipules (St^n/St^e) and winged nature of internode (*wgn*) in grass pea (*Lathyrus sativus* L.).

Cross	Gene pair (X/x-Y/y)	F_1 and test cross segregations					χ^2 (1 df)			χ^2 at 3 df (1:1:1:1)	Cov%	Map distance (cM)
		XY	Xy	xY	xy	Total	Locus X (3:1)	Locus Y (3:1)	Joint			
BSCM × *dwf2*	*Df/df2-Cbl/φbl*	55	25	19	07	106	0.013	1.522	0.21	–	–	–
F_1 × *dwf2*	Do	18	1	17	13	62	–	–	–	1.11	–	–
BSCM × *dwf3*	*Df3/df3-Cbl/φbl*	115	11	11	33	171	0.05	0.16	76.44	–	–	–
F_1 × *dwf3*	Do	49	5	5	35	95	–	–	–	60.25	11.58	11.79
Bio R-231 × *dwf2*	*Df/df2-Lfc/lfc*	112	19	18	30	179	0.31	0.54	45.59	–	–	–
F_1 × *dwf2*	Do	50	15	13	44	122	–	–	–	36.48	22.95	24.80
Bio R-231 × *dwf1*	*Lfc/lfc-Wgn/wgn*	122	10	09	48	189	2.68	3.26	145.2	–	–	–
F_1 × *dwf1*	Do	58	07	06	64	135	–	–	–	89.90	9.63	9.75
BioR-231 × *dwf2*	*Df/df2-Wgn/wgn*	107	17	12	33	169	0.24	1.90	66.07	–	–	–
F_1 × *dwf2*	Do	69	17	25	44	155	–	–	–	41.41	27.10	30.34

compact growth habit. Smartt (1984) considered development of more compact growth habit combined with some increase in seed size and elimination of neurotoxin could transform this neglected crop into one of great value. Presence of dwarf stature along with compact and determinate growth habit reduced the formation of tertiary and late order branches and thereby prevented the loss of nutrients channeled to these undesirable sinks. At the same time, dwarf and erect habit could accommodate more plants per unit area and might compensate the loss of harvestable grain incurred due to spreading stem and close vicinity of pods to the soil moisture in conventional varieties. Moreover, non-shattering nature of pods and double pod formation in *dwf2* line could enhance seed yield. These desirable traits along with low-seed ODAP content as recorded in *dwf1* and *dwf2* lines might be introduced into various high yielding lines in grass pea. Isolation of colchicine-induced *dwf1* and appearance of number of contrasting morphological features unaltered in the three dwarf types in successive-selfed generations suggested true-breeding nature of mutations and could be developed into a valuable multiple marker stock in genetics and breeding research in grass pea. Downes and Marshall (1983) described colchicine as a powerful mutagen in at least some genotypes of several crops and as colchicine-induced variant developed from a mass of undifferentiated tissue; it might be a source of novel genotypes for plant breeding programmes. After studying the effect of dwarfing genes on various morphological parameters in pearl millet, Rai and Hanna (1990) opined that dwarfing gene(s) might become active during the early stages of plant development and could affect numerous other characters either pleiotropically or through linkage. In the present material, detection of chromosomal location of two dwarfing genes and their linked association with leaflet colour (*lfc*), winged internode (*wgn*) and seed coat colour (*cbl*) suggested absence of pleiotropic effect of the two dwarfing loci on these associated characters and can be used to identify linkage groups in grass pea. The genetic differences manifested in the dwarf types due to presence of two different dwarfing genes could be exploited as base material in genetic research and different breeding programmes of grass pea.

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