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Review

Recent progress on genetic analysis of novel mutants and aneuploid research in grass pea (*Lathyrus sativus* L.)

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A good number of diploid genetic mutants, RILs and different cytogenetic stocks including a complete set of primary trisomics and tetrasomics, some tertiary trisomics, double trisomics and 10 reciprocal translocation lines were developed and being characterized in induced mutant population of seven different grass pea (*Lathyrus sativus* L.) varieties. Genetic basis of mutants has been investigated in intercrossed population and using trisomics some of them were localized on chromosome. Utilization of these novel genetic and cytogenetic stocks in improvement of grass pea with high yield and low seed neurotoxin, ODAP potential has been reviewed in this communication.

Key words: Grass pea (*Lathyrus sativus* L.), recombinant inbred lines (RILs), ODAP.

INTRODUCTION

Grass pea (*Lathyrus sativus* L.) belonging to the family Leguminosae is an annual, herbaceous popular food crop in many Asian and African countries where it is grown for human consumption and as animal feedstock. Its domestication began in Balkan Peninsula in the early Neolithic period and its cultivation has now spread from The Mediterranean basin to the Europe, Australia and South America (Smartt, 1984; Kislev, 1989; McCutchan, 2003). It is the only cultivated grain legume within the genus *Lathyrus* and showed tolerance to drought as well as water logging, resistance to insects and pests, adap-tability to nearly all types of soils and adverse climatic conditions and high seed protein content (Campbell, 1997; Biswas, 2007). A renewed interest about grass pea in Europe is justified by the urgent need to recover marginal lands and to provide an efficient alternative to the lands overexploited by cereal cultivation (Granati et al., 2003). In India this crop has been extensively grown (cultivation area 1.6 m ha) in Eastern and Central part of the country (FAO, 2002) and also in relay cropping system with winter paddy rice (Sharma and Pandey, 2001).

Although cultivated for more than 8000 years, genetic improvement of grass pea has not reached desired scale by conventional breeding methods possibly having narrow range of variations due to self-pollination and inter-specific incompatibility. Moreover, the features like low seed production, prostrate, indeterminate habit, susceptibility to pod shattering, delayed flowering as well as maturity and of course, presence of seed neurotoxin, - N-oxalyl L , -diaminopropionic acid or ODAP serve as limiting factors for broader introduction of grass pea in different geographical areas (Rybinski, 2003; Tavoletti et al., 2005; Talukdar, 2008a). The technique of mutation breeding has, therefore, been adopted as a valuable supplement to conventional breeding to create additional genetic variability and endeavour in this direction has been initiated only in the early 70's (Nerker, 1972, 1976; Chekalin, 1972). Mutation research on grass pea has gained momentum thereafter bringing about arrays of mutagenic changes involving chromosomal anomalies, chlorophyll deficiencies and different types of phenotypic modifications (Prasad and Das, 1980; Waghmare et al., 2001; Talukdar et al., 2001a; Rybinski, 2003) .Among these, morphological mutants are useful as novel genetic variants to elucidate genetic basis of desirable traits in grass pea genetics and breeding. Research is now being conducted to address desirable yield components and reducing seed neurotoxin content. Unlike other closely related grain legumes like pea, lentil and chickpea, genetic control of most of the desirable characters are not studied and thus, no linkage map is available in grass pea. A dense linkage map of agricultural crop species provides valuable information for plant breeding and construction of this type of map with classical as well as

Figure 1. A dwarf mutant line.

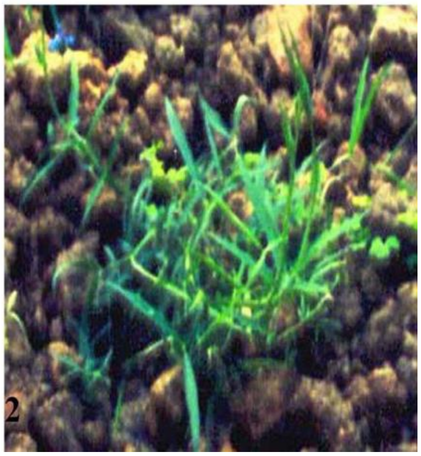

Figure 2. A semi-dwarf mutant.

Figure 3. A high yielding mutant plant.

molecular markers requires wide range of stable mutations, recombinant inbred lines (RILs) and cytogenetic tester stocks. Different trisomics, tetrasomics, double trisomics and translocation stocks have been utilized as tester stocks in linkage mapping (Sybenga, 1996) and in legumes, research on aneuploid based genetic mapping lag far behind such attempt than cereals (Singh et al., 2007). Keeping all these in mind, a detail investigation has been carried out since 1997 - 1998 in large number of induced mutant population of different grass pea varieties to develop genetic and cytogenetic mutant stocks. Some of the significant findings regarding these genetic mutants, their mode of inheritance, linkage relationships and possible chromosomal localization have been reviewed in this communications.

GENETIC MUTANT STOCKS

Crop genetics and breeding benefit from research on genetic mutants that qualitatively and quantitatively influence plant traits. Induction of mutations, as well as their characterization and maintenance are important. During the last 40 years (1970 - 2009) mutation research in grass pea was carried out by the treatment with wide range of physical and chemical mutagens including gamma rays, EMS and NMU or their different combinations and a wide spectrum of viable diploid (2n = 14) mutations affecting plant habit, branching, stem and internodes, leaflets, stipules, flower characteristics, pod characters, seed coat colour and seed size has been detected and characterized in different grass pea varieties (used as control). Characteristic features and genetic basis of the true breeding mutants analysed in present author's lab since 1997 - 1998 are being reviewed.

Mutations affecting vegetative organs (Figures 1 - 5)

Plant habit mutations

As compared to normal tall, spreading, indeterminate and prostrate habit, this type of mutation represents dwarf, semi-dwarf, miniature giant, erect and determinate habit (Nerker, 1976; Prasad and Das, 1980; Talukdar et al., 2001b). In grass pea var.BioR-231 and Hoogly Local three self-fertile, true breeding, recessive dwarf mutants namely *dwf1*, *dwf2* and *dwf3* were isolated and characterized in detail (Talukdar, 2009a). Interestingly, *dwf1* line was identified in colchicine-induced (0.25%, 6 h, 3 days) population but it inherited as a true diploid gene mutation showing 2n = 14 chromosomes and complete absence of any type of chromosomal anomaly. Beside typical dwarf phenotypes in different magnitudes, the three mutant lines exhibited large number of induced mutagenic changes in different organs like leaflet colour and shape, erect and determinate stem, winged modification of internode, stipule morphology, tendril nature, pod cha-

Figure 4. Curly leaflet.

Figure 5. Stipule mutant.

racteristics, seed coat colour and seed neurotoxin content. For *dwf1*, presumably, mutagenic effect of colchicine was not limited to single region of a chromosome, but that mutations might be affected at random in a large number of loci on different chromosomes within one plant whose diploid chromosome number remains unaffected (Talukdar, 2008a) . For *dwf2* and *dwf3*, pleiotropy of dwarfing genes or mutations in several closely linked loci was assumed to be responsible for occurrence of number of contrasting traits within a single line. Till date, two non-allelic genes, *df1/df2* and *df3* were identified in controlling dwarfism in grass pea and their complex interaction resulted in appearance of a miniature mutant plant type showing extreme reduction in plant height and seed yield components in the F_2 progeny (Talukdar, 2009a). This type bred true in advanced generations. With number of desirable morphological traits the three dwarf lines can be used as multiple-marker genetic linkage stocks to associate number of loci in linkage map of grass pea.

Branching mutants

Non-branching and shy-branching mutations were reported in different varieties of grass pea (Nerker, 1976; Prasad and Das, 1980). As compared to normal alternate branching in control varieties, three different types of mutant lines branching mutant 1 (*brm1*), branching mutant 2 (*brm2*) and profusely branched mutant (PBM) were detected by concentric mode of branching, sub- opposite manner of branching and the presence of large number of basal branching in 300Gy gamma ray irradiated and 0.15% EMS (10 h.) treated M_2 progeny of grass pea varieties ‘BioR-231’ and ‘Hooghly Local’, respectively. Preliminary investigation revealed high yielding nature with low seed ODAP content in brm1 and PBM lines (Table 1). The three lines bred true as recessive mutants and more than one gene is suspected to control these mutations in grass pea.

Stem and internode mutants

Mutations affecting these two characters were represented by the presence of non-winged (‘afila’) internode, highly winged internode and fasciated stem in different treatments and varieties of grass pea. In var. BioR-231, non-winged internode mutant (NWIM, 250Gy) was primarily distinguished by its characteristic non-winged roundish circumference of internodes in contrast to angular winged internodal ridges in control variety and also by erect, determinate and semi-dwarf habit, higher number of primary and secondary branches, increased grain yield and low seed ODAP (Talukdar and Biswas, 2006a; Table 1).On the other hand fasciated mutant (250Gy) was recessive and were characterized by broad, strap-like flattened stem, appearance of a cluster of small leaves at top and reduced yield in var. P-27 (Waghmare et al., 2001). All the stem mutants bred true as monogenic recessive mutations in grass pea. A multiple allelic locus, *Wgn* was detected controlling winged modification of stem with dominance relationship as *Wgn* (normal winged stem in control) > *wgn* (highly winged nature) > wgn^o (non-winged) in the present study (Talukdar, 2008a).

Leaflet and tendril mutants

The normal linear-lanceolate shape of leaflets was found modified to ovate-lanceolate, ovate, obovate, short, linear, crinkle, coiled and circinnata in different mutant lines (Nerker, 1976; Talukdar et al., 2001a). Leaflet became dense green and thicker in texture in *dwarf mutant* lines (Talukdar, 2009a) while its number increased in mutants used as forage yielding lines (Talukdar, 2008a). *A coriaceous* and erectoid leaflet mutant (CELM, 300Gy) was isolated with erect leaflets resulting in increased radiation use efficiency (RUE) and higher biomass accumulation in this mutant line (Talukdar et al., 2001a). Wide variation in leaflet morphology was also found in different other accessions of grass pea (Jackson and

Table 1. Agronomic characters (mean±SE) of 15 high grain yielding mutant lines (M_5) in *Lathyrus sativus* L.

Mutant lines*	Plant height (cm)	Number of branches/pl^{+}	Days to flowering	Days to maturity	Length pod	Pods/Plant	Seed yield /Plant(gm)	Seed ODAP%
brm1	49.00 ± 0.18	27.80 ± 0.09	20.00 ± 0.24	104 ± 0.30	3.74 ± 0.32	120.0 ± 0.17	27.90 ± 0.39	0.13 ± 0.30
PBM	52.11 ± 0.20	27.20 ± 0.07	50.20 ± 0.29	138 ± 0.35	3.58 ± 0.25	109.2 ± 0.20	21.72 ± 0.31	0.10 ± 0.16
NWIM	28.09 ± 0.17	20.01 ± 0.05	55.11 ± 0.31	135 ± 0.23	3.89 ± 0.15	99.10 ± 0.26	20.22 ± 0.37	0.19 ± 0.46
PVFM	69.78 ± 0.08	24.70 ± 0.10	31.00 ± 0.21	101.5 ± 0.31	3.67 ± 0.06	102.3 ± 0.16	21.77 ± 0.16	0.17 ± 0.31
CELM	50.50 ± 0.10	17.05 ± 0.11	50.00 ± 0.35	117 ± 0.19	3.54 ± 0.19	90.3 ± 0.20	16.63 ± 0.34	0.20 ± 0.22
WFM	55.43 ± 0.22	20.00 ± 0.14	31.50 ± 0.10	126.2 ± 0.44	3.38 ± 0.27	100.5 ± 0.15	23.09 ± 0.18	0.10 ± 0.15
BSCM	57.47 ± 0.26	18.73 ± 0.20	32.06 ± 0.40	92.50 ± 0.36	3.44 ± 0.07	88.90 ± 0.14	27.46 ± 0.38	0.19 ± 0.17
LGSCM	61.09 ± 0.11	21.50 ± 0.16	40.50 ± 0.21	111.7 ± 0.29	4.57 ± 0.12	105.17 ± 0.10	22.35 ± 0.20	0.14 ± 0.26
WSCM	77.65 ± 0.18	19.50 ± 0.16	52.50 ± 0.22	110.06 ± 0.15	3.65 ± 0.20	107.0 ± 0.07	18.85 ± 0.28	0.09 ± 0.49
DPM	24.65 ± 0.04	19.25 ± 0.09	37.50 ± 0.19	95.02 ± 0.30	3.50 ± 0.11	92.10 ± 0.23	29.89 ± 0.34	0.13 ± 0.32
MPM	26.05 ± 0.06	21.50 ± 0.21	40.00 ± 0.23	101.2 ± 0.26	3.39 ± 0.05	143.3 ± 0.35	33.12 ± 0.30	0.09 ± 0.16
LPM	63.57 ± 0.21	13.43 ± 0.23	35.20 ± 0.42	114.2 ± 0.16	3.09 ± 0.07	100.75 ± 0.20	22.54 ± 0.23	0.13 ± 0.18
BSM	56.00 ± 0.14	17.10 ± 0.43	29.70 ± 0.41	130.0 ± 0.23	4.05 ± 0.10	99.75 ± 0.16	23.00 ± 0.39	0.14 ± 0.16
HYM-I	40.76 ± 0.07	19.06 ± 0.25	37.06 ± 0.32	134.4 ± 0.46	4.33 ± 0.24	103.3 ± 0.16	20.08 ± 0.33	0.29 ± 0.67
HYM-II	55.52 ± 0.10	17.70 ± 0.16	50.20 ± 0.26	130.5 ± 0.37	3.38 ± 0.19	89.50 ± 0.20	19.50 ± 0.24	0.20 ± 0.37
HYM-III	49.11 ± 0.32	16.67 ± 0.12	44.55 ± 0.19	134.4 ± 0.55	3.40 ± 0.28	89.00 ± 0.18	19.57 ± 0.17	0.17 ± 0.44
Control**	55.55 ± 0.63	13.40 ± 0.41	48.00 ± 0.50	121.3 ± 0.40	3.50 ± 0.36	84.80 ± 0.33	12.60 ± 0.60	0.33 ± 0.40

* see text for full names of the mutants, trialed in two different agro-climatic regions-Eastern Himalayan plains and Indo-Gangetic plains for above yield attributes** average values of different control varieties; + only primary and secondary branches.

Yunus, 1984). Linear -lanceolate leaflet showed complete dominance to ovate-lanceolate and monogenically controlled by *Lfts/lfts* locus in grass pea. Identifications of other loci are in progress.

Usual presence of long terminally coiled tendril in compound leaf of grass pea varieties was modified to short curly, straight, unbranched, abnormal branching, hooked, highly coiled tips and even complete absence in different mutant lines (Talukdar, 2008a, 2009a, unpublished observation). The tendril-less mutant produced a tuft of small leaflets in place of terminal tendril in compound leaf of four different mutant lines and may provide a valuable clue to the developmental genetics of compound leaf in grass pea.

Stipule mutants

Phenotype of usual large foliaceous free-lateral stipules has been modified to short, ovate lanceolate, round-ovate, acicular-linear or complete absence in different mutant lines (Talukdar et al., 2001a; Talukdar D, Biswas AK (2006b) Talukdar 2009a). Its normal green colour and parallel orientation against stem axis was found mutated to blackish-purple colour with perpendicular orientation in a mutant designated as blackish-purple stipule mutant (250Gy) which showed high forage yielding capacity (Talukdar, 2008a). Involvement of multiple alleles showing dominance relationship as St^{n} (normal stipule) > St^{ir} (ovate-round) > St^{al} (acicular-linear) > *st* (estipulate) and *Par/par* (Parallel/perpendicular) with monogenic inheri-tance were identified in controlling stipule shape and orientation, respectively (Talukdar and Biswas, 2005a; Talukdar and Biswas, 2007c). Two different non- allelic genes, St^{G} and St^{Bp} were however detected complementing (9:7) with each other to produce normal green stipule in control plants. Absence of dominant alleles in any one of the genes resulted in blackish-purple color in sti-

Figure 6. Double flower formation with distichous pedicel.

Figure 7. A pale-violet flower colour mutation.

Figure 8. Field view of reddish-purple flower mutation.

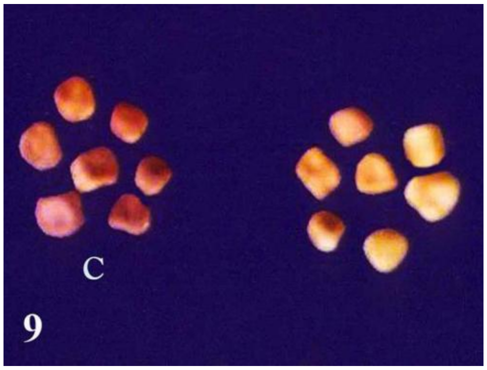

Figure 9. Normal brown (c) and yellow.

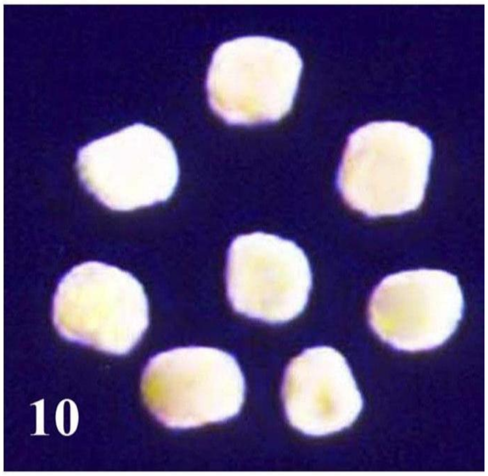

Figure 10. White.

pules of mutant plants (Talukdar and Biswas, 2007c).

Mutations affecting reproductive organs (Figures 6 - 12)

Flower colour polymorphisms

Wide variations in flower (corolla) colour from normal blue were exhibited as pale- violet, reddish- purple, blue-patched white and white in different mutant lines of grass pea var. 'BioR-231', 'BioL-203' and 'Nirmal'(Talukdar et al., 2002). Genetic analysis revealed presence of two different non- allelic genes; multiple allelic *B+* locus (blue) with different degree of dominance on other colours and *Pv/pv* locus controlling pale-violet colour in grass pea. Interaction between these two loci gave rise to light-blue and pinkish-purple corolla phenotypes in hybrid plants while white is recessive to all (Talukdar and Biswas, 2007c). Mutant with pale-violet (PVFM) and white flower (WFM) exhibited high grain yielding potentialities (Table 1). Induced mutation in flower colour was also reported in different other varieties of grass pea (Nerker, 1976; Prasad

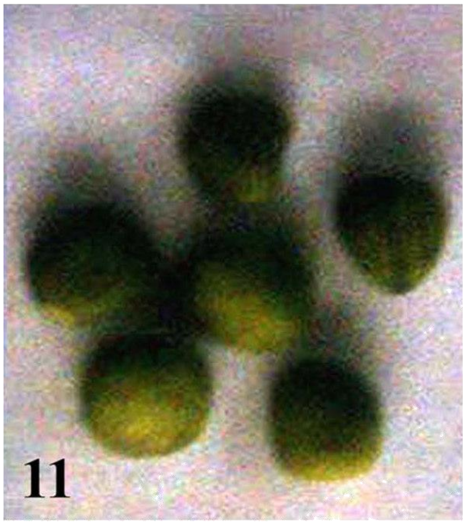
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Figure 11. Light-green.

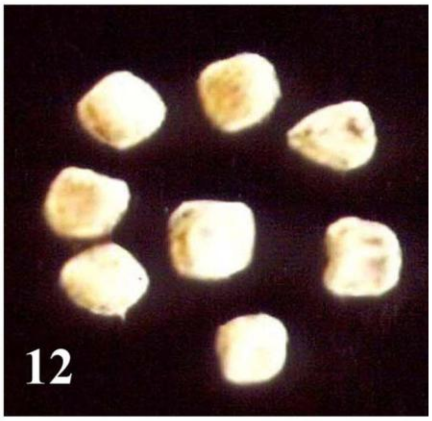
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Figure 12. Yellowish-white seed coat.

and Das, 1980; Rybinski, 2003). More than one non-allelic locus in flower colour polymorphism was also detected in different other inter -varietal crosses of grass pea (Mehra et al., 1995; Tiwari and Campbell, 1996; Das and Kundagrami, 1999).

Seed coat color mutants

Like flower colour, normal brown and grey-brown speckled seed have also been modified to black-mosaic, light green, yellow but non-mosaic, yellowish white, grey-white, creamy white non-mosaic and complete white non-mosaic seed coat in different induced mutant lines (Prasad and Das, 1980;Talukdar, 2009a; Talukdar and Biswas, 2005b). In var. BioR-231, mutants with black-mosaic seed coat (BSCM) and white non-mosaic seed coat (WSCM) showed significantly high grain yield and very low seed ODAP content (Table 1). At least three different loci were initially detected in controlling seed coat colour in grass pea of which one was multiple allelic exhibiting dominance as *cbr* (brown) > *cbl* (black) > *cy* (yellow) > *cw* (white) in different mutant lines (Talukdar and Biswas, 2005b). White seed coat was assumed to be controlled by another locus, tentatively designated *cw1* with three alleles participated in the order of dominance as *cgw* (grey-white) > *cyw* (yellowish-white) > *cw1* (white). A completely different locus, however, was suspected to be behind retention of light green colour in mature seed of another mutant line (LGSCM) which is high grain-yielding with desirable yield components (Table 1).

In general, the white-flowered lines produced white seed coat in different intensity with other light colour and showed low seed ODAP content in the present material than coloured-flowered, coloured-seeded lines (Table 1) which generally predominates in many western countries (Jackson and Yunus, 1984). In this context, isolation of different white-flowered, white-seeded true breeding mutant lines with high grain yield potential in Indian varieties assume significance.

Malformed flower mutant

In contrast to typical pentamerous and papilionaceous corolla, half-split keel, extra whorl of corolla, epicalyx and exposed stigma characterized these types of mutations (Nerker, 1976; Talukdar and Biswas, 2002). In var.BioR-231, the malformed flower mutation, *mfl* showed recessive gene action and probably responsible for high pollinator activity on exposed and receptive stigma leading to high degree of out-crossing (26 - 32% compared to 1 - 1.9% in variety BioR-231) in this mutant line.

Distichous to polystichous pedicel mutants

Occurrence of solitary floral pedicel (monostichous) with single flower on racemal pedicel (peduncle) is the normal feature in grass pea. Conspicuous modification of monostichous pedicel in different degrees to distichous (two floral pedicels/peduncle), tristichous (three pedicels/ peduncle), tetrastichous (four pedicels/peduncle) and polystichous (> four pedicels/ peduncle) was manifested in six different induced mutant lines including one *dwarf mutant* line in grass pea var. Biol- 203, ‘Nirmal’, ‘LSD-3’, ‘P-24/3’, ‘BioR -231’, ‘Hoogly Local’ and in two local varieties cultivated in hilly tracks of the eastern Himalayas (Darjeeling District, West Bengal, India 27°03 N/ 88°18 E). The hill genotypes locally adapted to humus and acidic soil, high annual rainfall (3000 mm) and very low winter temperature (1 - 4°C) exhibiting properties of both desirable and undesirable yield components comparable to those studied in mid-western Himalayan region of India (Kumari and Prasad, 2005).

Increase in the number of floral pedicels lead to enhancement of pod production in double (DPM) triple (TPM), multiple podding (MPM) mutants which is a desirable yield component in legumes including grass pea (Campbell, 1997; Rubio et al., 1998; Srinivasan et al., 2006; Talukdar 2009b; Table 1). Double podding inherited as a recessive trait in dwarf mutant line, *dwf2* in grass pea (Talukdar, 2009a) although it is not known if the trait is the result of pleiotropic action of dwarfing gene, *df1/df2* or controlled by different gene/s in different mutant lines. A breeding strategy comprising of increased pod number/ peduncle, increased flowering nodes with peduncle, increase in seed number or seed size/pod with erect, dwarf habit, early flowering and low ODAP content would be beneficial for grass pea.

Long pedicelled mutant (LPM)

Abnormal elongation of racemal pedicel or peduncle was manifested in this mutant line. The mutant line was high-yielding with low seed ODAP content and the pedicel trait was believed to be controlled by several genes with cumulative effect (Polygenic) in grass pea (Talukdar and Biswas, 2007b).

Bold-seeded mutant (BSM)

Isolated in 300Gy treated M_2 progeny of var. BioR-231this true breeding mutant line showed large and bold seed, white flower, erect and dwarf habit, higher branches, high grain yield and low seed ODAP (Table 1). The mutant trait was controlled by two non-allelic genes, S^B-S^N in duplicate (15:1) interaction and resulted in presence of recessive alleles of both the genes (Talukdar and Biswas, 2008c).

Genetic variability and interrelationships of quantitative traits

Range of variations and mean values of plant height, number of primary branches, days to flowering, days to maturity, pods per plant, pod length (cm), seeds per pod, seed yield per plant, 100 seed weight, biological yield per plant and harvest index (HI%) were taken into account and mean values shifted positively in fifteen high-yielding mutant lines (Table 1). Although no distinct macro-morphological feature was present, three different high-yielding mutants (HYM-I, II and III) were isolated and characterized in var. 'BioL-203', 'Nirmal' and 'P-24/3'. On the other hand significant increase in biomass production provided six induced mutant lines with fodder yield capabilities.

Analysis of variance revealed that pooled data showing variation in each of the yield influencing traits in different mutant lines were significantly different from one another as well as from control but the variations observed in different generations were not significant. Barring days to flower, heritability (broad sense) was very high and magnitudinal differences between genotypic coefficient of variations (GCV) and phenotypic coefficient of variations (PCV) were very low in all other traits indicating negligible role of environment in expression of these traits in mutant lines. Higher value of heritability coupled with high genetic advance (G. A.) was recorded in all important yield components except days to maturity only (Talukdar and Biswas, 2008a). While additive gene effect was attributed for high heritability with high G. A. and improvement of the concerned trait/s was suggested through individual plant selection, high heritability but low to moderate G.A on the other hand was ascribed for non-additive gene action, in which selection would rather be ineffective. However, performance can be improved by intermating with superior genotypes through accumulation of desirable genes in the selected mutant lines (Talukdar, 2008a).

Correlation of seed yield with plant height, number of primary branches per plant, pods per plant and 100 seed weight was found to be positive and significant; of which maximum contribution was recorded due to pods per plant (Talukdar, 2009b). Relationship of seed yield with days to maturity and seeds per pod was positive but not significant. Significantly positive correlation was also noticed between plant height and pods per plant as well as between number of primary branches and pods per plant. Days to flower had shown significantly negative correlation with seed yield, pods per plant and plant height. The results suggested favorable influence of earliness towards pod setting and seed yield per plant and number of pods, therefore, appeared to be most important yield contributing factors. Seed size could also contribute positively towards yield (Hanbury et al., 2000). Association of seed ODAP content with number of pods and 100 seed weight was negatively significant but its relationship was positive and significant with days to flower suggesting that early flowering and bold seed size might have contributed towards reduced ODAP content in seeds (Talukdar, 2008a, 2009b).

Seed protein variation and isozyme polymorphism in induced mutant lines

Analysis of SDS-PAGE banding profiles of total seed protein in 31 elite induced mutant lines (15 high seed yielding, 10 moderate yielder but with desirable morphological traits and six high forage yielding) and six grass pea varieties revealed as many as 41 different bands with variation in width and intensity. Out of 31, 19 mutant lines (11 of them high yielding) possessed one or more than one unique band/s. The six varieties also differed from each other but the magnitude of differences was far less in control varieties than mutant lines (Talukdar, 2008a; Talukdar, unpublished observation). Presence of unique band/s can be used as biochemical marker for identifica-

Table 2. Allelic constituents of 13 important polymorphic isozyme loci detected in 15 high yielding induced mutant lines of grass pea (*Lathyrus sativus* L.)

Mutant lines*	**Polymorphic isozyme loci****												
	Acp-1	**Acp-2**	**Aco-1**	**Aco-2**	**Est-1**	**Est-3**	**Prx-1**	**Prx-3**	**Lap-1**	**Lap-2**	**Sod-1**	**Pgm-2**	**Aat-c**
brm1	aa	aa	cc	bb	bb	bb	aa	cc	cc	aa	-	aa	-
PBM	bb	aa	aa	bb	bb	aa	cc	cc	aa	aa	aa	-	-
NWIM	null	aa	aa	bb	cc	aa	-	-	cc	aa	aa	aa	bb
CELM	aa	cc	bb	cc	aa	bb	bb	aa	aa	cc	bb	aa	cc
PVFM	aa	bb	aa	bb	null	cc	aa	cc	aa	bb	aa	cc	-
WFM	cc	bb	cc	aa	aa	aa	cc	bb	aa	bb	-	aa	cc
BSCM	aa	cc	aa	bb	bb	bb	aa	cc	bb	aa	aa	bb	cc
LGSCM	aa	cc	aa	cc	bb	null	bb	cc	bb	aa	cc	bb	cc
WSCM	aa	cc	bb	cc	cc	cc	aa	bb	cc	bb	cc	-	-
DPM	cc	cc	cc	aa	bb	aa	aa	bb	cc	aa	-	aa	bb
MPM	cc	cc	cc	bb	aa	aa	bb	bb	cc	aa	aa	aa	cc
LPM	aa	bb	aa	bb	aa	bb	aa	cc	bb	bb	cc	-	aa
BSM	cc	bb	aa	cc	bb	cc	aa	null	aa	aa	aa	aa	-
HYM-I	aa	bb	bb	bb	aa	aa	cc	bb	bb	aa	bb	bb	cc
HYM-II	bb	aa	cc	bb	aa	aa	cc	aa	bb	bb	cc	cc	aa
HYM-III	aa	null	aa	bb	aa	bb	bb	aa	bb	cc	cc	aa	aa

*See text for full names of mutant lines, ** Acp-acid phosphatase, Aco-aconitase, Est-esterase, Prx-peroxidase, Lap-leucine aminopeptidase, Sod-super oxide dismutase, Pgm-phosphogluco mutase, Aat-c-aspartate amino transferase (cytosolic), nomenclature for isozyme loci according to Weeden (1988). '-' not studied.

tion as well as maintenance of genetic purity of mutant lines and their hybrids in grain legume crops including grass pea (Ladizinsky and Adler, 1975; Naik and Kole, 2002; Roy et al., 2004).

Polymorphism in different isozyme loci including acid phosphatase, aconitase, esterase, peroxidase, superoxide dismutase, aspartate amino transferase, leucine amino peptidase and phosphoglucomutase was detected by the presence of more than one allele, differences in banding intensity, presence of unique allele and also null mutation in the present high yielding mutant lines in different buffers (Table 2). The varieties, however, were more uniform in isozyme diversity like an earlier study (Roy et al., 2004) on leaf esterase and peroxidase banding in some local grass pea cultivars. Studies on genetic basis of isozyme in the present mutant lines are in progress.

RILs, cross-over segregants and reciprocal translocation stocks

As many as 210 recombinant inbred lines (RILs) were developed by random selection of plants in F_2 generation of distant parental crosses and by selfing them up to F_9 generation (2007 - 2008) for nine quantitative traits *viz*., days to flowering, days to maturity, number of branches per plant, pods per plant, pod length (cm), seeds per pod, 100 seed weight, seed ODAP content and per plant seed yield (gm). About 172 RILs showed higher mean value for a specific trait over respective control value and were selected as promising lines. However, undesirable features like pod shattering, prostrate and indeterminate habit, hard seed coat, small seed size and high seed ODAP were also recorded in some other RILs. Superior lines isolated through selection in recombinant inbred population having high yielding traits may be used as donors as grass pea improvement programmes. The results indicated successful use of present mutant lines with contrasting traits in wide hybridization.

Among the 78 cross-over segregants in F_2 progeny derived from crosses between different control varieties and mutant lines 49 types were selected as promising for combination of different qualitative traits viz., plant habit, flower as well as seed coat color, floral architecture, pedicel morphology, seed size etc.

So far, 10 reciprocal translocation (RT) or chromosomal interchanges have been identified through gamma ray induced (250Gy, 300Gy, 350Gy, 400Gy) progeny of grass pea var.BioK- 231, BioR-231, P-24/3 and LSD-3 (Biswas and Biswas, 1997; Talukdar, 2008a, 2009c). The RT stocks involve both RT homozygotes and RT heterozygotes. While the former one possessed presence of 7II (2n = 14) at meiosis-I, low level of pollen sterility (2.1 - 3.45%) and some good agronomic traits the latter showed semi-sterility, association of one ring quadrivalent (1IV + 5II, 2n = 14) and heterozygous superiority over other progeny plants in some instances. RT homozygotes are important source of RT heterozygotes which produce tertiary trisomics in selfed and inter-crossed progenies.

PROGRESS OF ANEUPLOID RESEARCH AND LINKAGE MAPPING

The value of diploid genetic mutants is enhanced manifold once their locations are identified in the genome of a crop plant and different aneuploid materials can be used successfully in this mapping. Since 2000, efforts are being made to develop cytogenetic stocks in grass pea and number of aneuploid types has been identified, characterized and maintained in population.

Trisomics

A complete set of seven different primary trisomics were characterized in genetic background of diploid grass pea var. 'BioR-231' (2n = 14) by the presence of an extra chromosome (2n + 1; 2n = 15) in their genetic complement (Talukdar et al., 2001a; Talukdar and Biswas, 2007a). The trisomics could also be distinguished phenotypically from each other and from diploid plants even at early seedling stage by specific modifications in leaflet and stipule morphology and also by variation in band width, mobility, number and intensity of leaf esterase and peroxidase isozyme banding profile (Table 3). Three separate zone of enzyme activity in esterase and two zones of peroxidase activity along with some trisomic specific allozyme were detected in the zymogram phenotype of the seven grass pea trisomics (Talukdar, 2008a). Trisomics were self-fertile and showed average transmission rate of 23% in selfed and inter-crossed population mainly as female parent through small sized seeds (Talukdar and Biswas, 2008b) . Consistent presence of extra chromosome in the form of trivalent (chain, frying pan or Y- shaped) or as a univalent was ascribed for phenomenon of primary trisomy in population.

Preliminary analysis in selfed and inter-crossed progenies of different translocation lines (RT1-RT10) revealed occurrence of interchange and tertiary trisomics in grass pea with extra chromosome containing translocated part from other non-homologous chromosome. In general, the trisomic plants manifested weak growth habit, slender stem, higher pollen sterility and variation in other morphological characters than their diploid counterpart but they were more or less self-fertile and an important source of primary trisomics (D. Talukdar, unpublished observation).

Primary tetrasomics

Seven different primary tetrasomics (2n + 2, 2n = 16) showing regular chromosomal association of 1IV + 6II, 1III + 6II + 1I at meiosis -I was identified in advanced selfed generations of different trisomic types (Talukdar, 2008b). Occurrence of linear or 'Y'-shaped trivalent indicated unmodified (primary) nature of present tetrasomics exhibiting influence of two extra homologous chromosomes in more exaggerated way. The tetrasomic types were easily distinguishable, self-fertile but its pollen sterility increased over diploid and trisomic plants.

Double-trisomics

Five double-trisomic plants (2n + 1 + 1; 2n = 16) with two different non-homologous extra chromosomes were isolated in gamma radiation (200Gy, 350Gy and 400Gy) induced M_2 progeny of 'BioR -231', 'Midnapore Local' and 'BioL-203'. Interestingly, the double trisomic plants often exhibited combinations of two different trisomic phenotypes and produced primary trisomics in selfed progeny. Pollen sterility varied greatly (9.37 - 33.46%) in these double-trisomic plants. Zymogram phenotypes also contained combination of respective trisomic specific isozyme banding pattern in gel.

Genetic linkage and chromosomal localization of mutant phenotypes

Through linkage analysis and with the help of modified trisomic segregation ratio in diploid portion of F_2 progeny of trisomic parent (seed parent) × diploid recessive mutant (as pollen parent), *df1/df2* gene (dwarfism) was mapped 24.80cM apart with *lfc* gene (leaflet colour) and loosely linked with *wgn* gene (winged internode) on extra chromosome of trisomic I; *lfc* and *wgn* was rather tightly linked (9.75cm) on the same chromosome while *df3* (dwarfism) was mapped with *cbl* (seed coat colour) in 11.79 cm. map distance on the extra chromosome of trisomic-III (Talukdar, 2009a). It has also been noted that erectness and determinate growth habit in grass pea is not the result of pleiotropy of dwarfinggenes, rather these two traits are controlled by two different genes which are supposed to be linked with dwarfing gene *df1/df2* on the same chromosome but assorted independently with another dwarfing gene, *df3* (D. Talukdar, unpublished data). Similarly one of the flower colour locus *B*+, seed coat colour locus *cbr* and bold-seed size exhibited linkage

Table 3. Variation in leaf isoesterae and peroxidase isjozyme banding profiles among 7 trisomics (Tr) and diploid control variety BioR-231 in grass pea (Lathyrus sativus L.)

Band number	Rm value	Tr-I	Tr-II	Tr-III	Tr-IV	Tr-V	Tr-VI	Tr-VII	Diploid cultivar
Leaf iso-esterase banding									
1	0.063	+	+	+		+	+	+	+
2	0.105				(+)*				
3	0.126						(+)*		
4	0.189		(+)*						
5	0.274				+	+	+	+	
6	0.305	+	+	+					
7	0.358							(+)*	
8	0.379	+	+	+	+	+	+		+
9	0.395			(+)*					
Leaf peroxidase banding									
1	0.074	+	+	+	+	+		+	+
2	0.100						(+)*		
3	0.110	+	+	+	+	+	+	+	
4	0.260	+		+		+	+	+	+
5	0.290				(+)*				

(+)*- trisomic specific novel band.

with each other. Close linkage (9.15 cM) between *lfts* (leaflet shape) and *St*^{n} locus (stipule morphology), between (4.33 cm) *crl1* (a type of corolla phenotype) and *tnd* (tendril morphology), between (10.64 cM) *lfts* and *crl1* and weak linkage (30.49 cM) between *lfts* and *tnd* was estimated on the same linkage group (Talukdar, 2008a). Pleiotropy or close linkage, however, was present between presence of additional petals (*crl 2*) and estipulate (*St*^{n}) mutation in grass pea (Talukdar and Biswas, 2002). Linkage relationships between different polymorphic isozyme loci and genetic mutants are also being carried out by means of RT lines and modified aneuploid segregation ratios in different mutant lines. This will certainly be a great useful in construction of classical and molecular linkage map in grass pea in near future.

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