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**RESEARCH ARTICLE**

# BOLD-SEEDED AND SEED COAT COLOUR MUTATIONS IN GRASS PEA (*Lathyrus sativus* L.): ORIGIN, MORPHOLOGY, GENETIC CONTROL AND LINKAGE ANALYSIS

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**ABSTRACT**

Three different types of mutations affecting seed size and seed coat colour were detected in gamma ray induced $M_2$ progeny of two grass pea (*Lathyrus sativus* L.) cultivars, 'Nirmal' and 'BioR-231'. Seed size was larger in bold-seeded mutant (BSM) line than cultivars, while normal grey-brown colour of seed coat was modified to black in black seed coat mutant (BSCM) and whitish-yellow in whitish-yellow seed coat mutant (WYSCM). The BSM and BSCM exhibited high grain yield and low seed neurotoxin (ODAP) content, whereas WYSCM showed exuberance in vegetative growth and marginal reduction in seed ODAP content. Among other agronomic desirable traits, BSM manifested erect growth habit and tolerance to seed shattering at maturity. The three mutants were true breeding for their respective traits in $M_3$ generation. Inheritance and linkage analysis revealed digenic non-allelic control of bold seed size, while seed coat colour was governed by monogenic recessive gene with involvement of multiple alleles. Both bold-seeded and seed coat colour muations were linked with each other and also with a flower colour locus, controlling blue colouration in petals of grass pea flower.



## INTRODUCTION

Grass pea (*Lathyrus sativus* L.) is an important protein rich annual pulse crop in India and can grow very well in drought-stricken, rain-fed, saline and even flood prone areas (Talukdar *et al*., 2001a; Biswas, 2007; Talukdar, 2009b). Most of the grass pea genotypes in Indian sub-continent are small-seeded ('*lakhadi*') and genetic variations for seed size and seed coat colour are extremely low in existing germplasms (Mehra *et al*., 1995). The

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small-seeded genotypes exhibit very severe pre-mature shattering problem, resulting in very low harvestable grain yield (Campbell, 1997; Hanbury *et al*., 1999). With increasing importance of grass pea as a dependable crop for sustainable agriculture in ever-increasing threat of drought, salinity and other abiotic stress conditions, a wider genetic base of germplasm is essential for improvement of grass pea (Campbell *et al*., 1994; Vaz Patto *et al*., 2006; Talukdar, 2011a). The potentiality of induced mutagenesis in this context can be an effective source of variation (Waghmare and Mehra, 2000; Rybinski, 2003; Biswas, 2007). In recent times, different morphological mutations showing contrasting variations in stem habit, internode morphology, leaf and stipule morphology, pod indehiscence, flower and seed coat colour, floral axis etc. have been successfully induced through induced mutagenesis in different genotypes of grass pea and their genetic basis with linkage associations were elucidated to develop a comprehensive linkage map in grass pea (Talukdar *et al*., 2001b; Talukdar and Biswas, 2006a,b, 2007b,c; Talukdar, 2009a,b, 2011b). In addition, a robust cytogenetic stock including trisomics, tetrasomics, polyploids, and different translocation lines has recently been developed in grass pea, and can be of great importance in aneuploid-based genetic analysis of a particular trait (Talukdar and Biswas, 2007a, Talukdar, 2008, 2009c, 2010a, b). During ongoing investigations on gamma ray irradiated progenies, the present author isolated a number of morphological mutations in $M_2$ progeny of two grass pea cultivars, BioR-231 and Nirmal, including two variant plants with white flowers and much bolder and larger seeds than the mother variety, three plants with characteristic black seed-coat and one plant with whitish-yellow seed-coat. The literature records induction and isolation of different mutations in other grass pea varieties, but there is scant information on inducing bold-seeded mutants and seed coat colour mutations in grass pea (Waghmare and Mehra, 2000;Talukdar and Biswas, 2005)

Seed coat colour along with flower colour could be of useful genetic markers for identifying lines with low neurotoxin content in grass pea (Quader *et al*., 1988; Das and Kundagrami, 2001). On the other hand, seed size has immense significance in yield and genotype was the most important determinant of seed ODAP content (Hanbury *et al*., 1995,1999). Although a general relationship among seed coat colour, flower colour and seed size was reported in different geographical regions, association of linkage between the genes controlling these three traits was not studied in grass pea. The objectives of the present work were, therefore, to 1) characterise the morphological and yield-related traits of the mutant lines, 2) ascertain the genetic basis of seed-size and coat colour mutations, and 3) elucidate linkage associations between seed size, seed coat colour and flower colour in the present material of grass pea.

## MATERIALS AND METHODS

### Detection, isolation and characterization of the mutants

Fresh and healthy seeds of grass pea cv. 'BioR-231' and 'Nirmal' were irradiated with different doses (50, 100, 150, 200, 250, 300, 350 and 400 Gy) of gamma rays to induce mutations. Treated seeds were sown treatment-wise to raise $M_1$ generation in separate plots with 30 and 50 cm uniform distances between plants and rows, respectively. Seeds of individual $M_1$ plants in all the treatments along with control were separately harvested and sown in different rows in a randomised block design to raise $M_2$ progenies. After careful examination two plants were detected in 300 Gy-irradiated populations of variety 'Nirmal' by their characteristic erect habit and white flowers. These two plants produced much bolder and larger seeds than the mother (control) cultivar at harvest. In addition, three plants were detected by their characteristic black seed coat and one plant was identified by whitish-yellow modification of seed coat at post-harvest stage in $M_2$ progeny raised through 300Gy gamma ray irradiation in variety 'BioR-231'. These six plants were self-pollinated and morphological and yield related traits on 15 randomly selected plants were recorded in detail during $M_3$ generations (Table 1). Seed size was categorised as bold following the criterion of seed weight fixed by earlier workers (Sarwar *et al*.,1997; Sharma *et al*.,1997). The bold-

seed size along with flower colour recorded in the $M_2$ generation bred true in $M_3$ generations, and it was termed as white flowered bold-seeded mutant (BSM). Similarly, seed coat colour also bred true in $M_3$ generation and accordingly, they were designated as black seed coat mutant (BSCM) and whitish-yellow seed coat mutant (WYSCM). The two varieties were treated as control for respective mutant line in similar field condition. The mutant and control were grown in two different agro-climatic zones: Gangetic plain (south Bengal, clay alluvial soil, pH $\geq$ 7.0, mean temperature during winter 23°C and humid) and North Bengal (sandy alluvial soil, acidic, winter mean temperature around 15°C and dry) to test the variation, if any, in seed size, coat colour and seed toxin content. Simple t-tests were performed to determine the significance of differences between the control and mutant line for different yield attributes. Seed ODAP content was estimated using the method of Rao, (1978). Inheritance studies and linkage analysis Inheritance pattern of the mutation was traced in $M_3$ following intercrosses (including reciprocals) between the three mutants and two control cultivars in different combinations (Table 2). The $F_{1s}$ from respective crosses were selfed and backcrossed to mutant parent. Chi-square test was employed to determine the expected genetic ratio.

The BSM line along with BSCM, WYSCM and control varieties was used to study the linkage relationship between seed coat colour and seed size. A flower colour locus, B+ exhibiting multiple allelic control of flower colour in grass pea (Talukdar and Biswas, 2007c) was also included in the present linkage analysis. Intercrosses among the different genotypes ($M_3$) were made in various combinations taking 2 characters at a time to raise $F_1$ progeny (Table 3). The $F_1$ plant was backcrossed to respective recessive parent and was also selfed to grow $F_2$ generation. Test cross data was pooled for same pair of genes in some crosses (Table 3).Chi-square test was employed to determine the goodness of fit in each case. Map distance between concerned genes was estimated in centiMorgans (cM.) from the cross over value (cov%) obtained from test cross segregation using Kosambi's formula (Kosambi, 1944).

# RESULTS AND DISCUSSION

**Bold-seeded mutant (BSM)**

The mutant plants produced white flowers in contrast to blue flowers in the control. There were significant increases in the number of primary branches, pods per plant, grain yield per plant and 100 seed weight over the control. The pods were longer in the mutant, with a comparable number of seeds per pod and showed no tendency to premature pod shattering. The seeds harvested from the mutant were larger and over 2-fold heavier than the control seeds in both agro-climatic zones. Seeds were whitish-grey in colour. Seed ODAP content (0.11–0.14%) had reduced significantly, as compared to the control. No significant variation was observed for seed size, coat colour and toxin content between Gangetic plain and North Bengal condition. Flowering and maturity was earlier by about 12 d and 5 d, respectively (Table 1).

The bold-seeded mutant in the present study had many agronomical desirable characteristics such as erect, compact growth habit, higher number of branches, early flowering and maturity, large seed size, high seed yield and low seed ODAP. Smartt, (1984) considered the development of a more compact growth habit combined with some increase in seed size and elimination of ODAP could transform grass pea into an ideal grain crop. Absence of seed shattering as in the present mutant line is an important criterion for a grain crop. Large-seeded grass pea lines without the shattering habit are common in European lines but lacking in the Indian subcontinent where small-seeded lines are generally grown (Campbell, 1997; Talukdar, 2011b). In this backdrop, isolation of this true breeding mutant line with bold-seed size, easily identifiable flower and seed coat colour and desirable yield components assumes significance.
Reduction in seed ODAP and its stability are two important criteria in grass pea breeding. Large and bold-seeded varieties with low seed ODAP content have been reported in other grass pea varieties (Quader *et al*., 1988; Das and Kundagrami, 2001). Seed ODAP content in a recently released large-seeded and white flowered variety (LUANCO-INIA) in Chile was estimated at 0.175–0.516%

(Mera *et al*., 2003). In the present investigation, there was about 50% reduction in seed ODAP in the bold-seeded mutant of the control and this trend was maintained in $M_3$ generation. Moreover, there was non-significant variation of seed ODAP between two different agro climatic regions, reflecting some stability of the mutant, although further study is needed.

**Black seed coat mutant (BSCM)**

As compared to normal pace of growth and brown seed coat exhibited in control variety, selfed progeny of the suspected three mutant plants ($M_2$) exhibited vigorous growth from very seedling stage and manifested black mosaic modification in seed coat colour in $M_3$ generation. Initiation of branch in the mutant was found to be earlier than in the control (data not in table), and number of primary branches, plant height and yield enhanced significantly ($p$<0.05) in the mutant plants (Table 1). Flowering in these plants initiated in between 29$^{th}$ and 35$^{th}$ d, in contrast to 45$^{th}$ and 52$^{nd}$ d, of germination in control. Maturity occurred at more rapid rate showing 42 d advancement at harvest (Table 1). Seed yield in BSCM was found to be remarkably higher ($p$<0.05) than in the control at $M_3$. Number of pods / plant and seeds / pod increased markedly in the mutant plants. Seed neurotoxin ODAP content has reduced significantly as compared to the control plants (Table 1).

Whitish- yellow seed coat mutant (WYSCM) This mutant line could be distinguished from control by characteristic modification of grey-brown seed coat of control plants into whitish-yellow colouration and as compared to prostrate habit in the control plants, the mutant plants were semi-erect with significantly higher length of indeterminate stem and increased number of branches as well as leaflets/leaf which attributed it as bushy habit. Rate of increase in plant height and primary branch formation was found to be faster and the mutant produced more tertiary and late order branches than control and BSCM line (data not in table). In contrast to normal blue flower, the colour of flower in the mutant was bluish-white. Maturity of the mutant was delayed by 11 d, from the control plant and biological yield increased significantly. Pods in the mutant showed considerable seed shattering at maturity. Compared with control, reduction in seed ODAP content was found to be marginal in the mutant (Table 1).

Variations in seed coat colour (black and whitish-yellow) were prominent and distinctive in two seed coat colour mutant lines. These modifications being specific and stable for each of the mutant in advanced generations could be treated as marker phenotypes. Besides black colouration, BSCM could be distinguished from others as well as from control by its significantly higher grain yield, early maturity and low seed ODAP content. Reduction in the number of late order branches was also a desirable trait to treat

**Table1. Different morphological and yield related traits (± SE) in *BSCM*, *WYSCM, BSM* and two control varieties (Nirmal and BioR-231) in $M_3$ generation of grass pea (*Lathyrus sativus* L.).**

| Traits | BSCM | WYSCM | BSM | Nirmal | BioR-231 |
|---|---|---|---|---|---|
| Plant height (cm.) | 60.51*±0.49 | 100.07*±0.57 | 56.00±0.39 | 64.09±0.48 | 52.53±0.47 |
| Primary Branches/plant | 16.01*±0.32 | 24.35*±0.33 | 20.07*±0.33 | 12.50±0.37 | 12.00±0.32 |
| Leaflets/leaf | 5.00±0.22 | 7.60*±0.47 | 3.40±0.55 | 4.30±0.50 | 4.60±0.46 |
| Days to flowering | 32.50*±0.51 | 52.00±0.43 | 30.01*±0.66 | 42.00±0.65 | 49.87±0.56 |
| Days to maturity | 92.50*±0.52 | 145.00*±0.49 | 128.07±0.43 | 133.00±0.39 | 134.50±0.42 |
| Pods / plant | 90.00±0.21 | 78.00±0.30 | 100.00*±0.37 | 75.00±0.64 | 84.20±0.70 |
| Seeds / pods | 5.67*±0.19 | 3.90±0.20 | 3.80±0.31 | 3.56±0.19 | 3.40±0.32 |
| 100 seed weight(g)- Gangetic plain | 5.90±0.15 | 5.01±0.20 | 11.9*±0.14 | 5.39±0.29 | 5.55±0.08 |
| North Bengal | 5.45±0.22 | 4.86±0.25 | 11.5*±0.19 | 5.40±0.30 | 5.29±0.17 |
| Seed yield/plant | 27.72*±0.64 | 11.29±0.44 | 23.00*±0.43 | 14.82±0.67 | 11.60±0.20 |
| Harvest index % | 60.17*±0.71 | 26.67*±0.66 | 76.67*±0.22 | 41.95±0.45 | 42.98±0.21 |
| Seed ODAP %- Gangetic plain | 0.21*±0.03 | 0.28±0.37 | 0.14*±0.35 | 0.28±0.20 | 0.34±0.21 |
| North Bengal | 0.19*±0.11 | 0.31±0.29 | 0.11*±0.41 | 0.23±0.27 | 0.29±0.11 |

*significant at 5% level from respective control variety; BSCM and WYSCM from 'BioR-231', BSM from 'Nirmal'

Table2.Inheritance of seed size, seed coat colour and flower colour in grass pea (*Lathyrus sativus* L.).

| Cross+ | F1 phenotype | $F_2/BC_1$ generations | | $\chi^2$ | *p* |
|---|---|---|---|---|---|
| **Seed Size** | | **normal** | **bold** | | |
| Control ×BSM | normal-sized seeds | 129 | 09 | 0.01*** | 0.95-090 |
| $F_1$×BSM | - | 46 | 18 | 0.34* | 0.70-0.50 |
| BSCM×BSM | normal-sized seeds | 152 | 10 | 0.001*** | 0.98-0.95 |
| $F_1$×BSM | - | 59 | 17 | 0.14* | 0.80-0.70 |
| WYSCM×BSM | normal-sized seeds | 73 | 05 | 0.006*** | 0.95-0.90 |
| $F_1$×BSM | - | 55 | 16 | 0.23* | 0.70-0.50 |
| Pooled $F_2$ | normal-sized seeds | 354 | 24 | 0.01*** | 0.95-0.90 |
| Pooled $BC_1$ | - | 160 | 51 | 0.06* | 0.90-0.80 |
| **Seed coat colour** | | **grey-brown** | **black/whitish-grey/whitish-yellow** | | |
| Control×BSCM | grey-brown | 98 | 32 | 0.01* | 0.95-0.90 |
| $F_1$×BSCM | - | 42 | 38 | 0.20** | 0.70-0.50 |
| Control×WYSCM | grey-brown | 80 | 24 | 0.21* | 0.70-0.50 |
| $F_1$×WYSCM | - | 13 | 09 | 0.73** | 0.50-0.30 |
| Control×BSM | grey-brown | 100 | 31 | 0.06* | 0.90-0.80 |
| $F_1$×BSM | - | 33 | 29 | 0.26** | 0.70-0.50 |
| | | **black** | **whitish-yellow/whitish-grey** | | |
| BSCM×WYSCMBlack | | 41 | 13 | 0.03* | 0.90-0.80 |
| $F_1$×WYSCM | - | 11 | 08 | 0.47** | 0.50-0.30 |
| BSCM×BSM | black | 59 | 20 | 0.04* | 0.90-0.80 |
| $F_1$×BSM | - | 17 | 13 | 0.53** | 0.50-0.30 |
| | | **whitish-grey** | **whitish-yellow** | | |
| BSM×WYSCM | whitish-grey | 63 | 19 | 0.15* | 0.80-0.70 |
| $F_1$×WYSCM | - | 23 | 18 | 0.61** | 0.50-0.30 |
| **Flower colour** | | **Blue** | **white** | | |
| Control ×BSM | blue | 70 | 24 | 0.01* | 0.95-0.90 |
| $F_1$×BSM | - | 32 | 25 | 0.86** | 0.50-0.30 |
| BSCM×BSM | blue | 56 | 18 | 0.01* | 0.95-0.90 |
| $F_1$×BSM | - | 25 | 22 | 0.19** | 0.70-0.50 |

+BSM-bold-seeded mutant, BSCM-black seed coat mutant, WYSCM-whitish-yellow seed coat mutant.

this mutant as a high grain yielding line. All these favourable traits may be incorporated in plant type exhibiting dwarf, compact and determinate habit in grass pea breeding. On the other hand, WYSCM in addition to whitish-yellow colour of seed coat, was also distinctive by its highest forage yield possibly due to delayed maturity, taller plant height with indeterminate stem, increased number of primary, secondary as well as tertiary branches and number of leaves with leaflets, larger size of leaflets, luxuriant vegetative growth and higher amount of biomass production. This is effective in preventing soil moisture content from drying out and is most desired in ideal forage crop (Campbell, 1997). Pertinently, the observations of Smartt, (1984) would be of adequate significance in this regard. According to him, exuberance of vegetative growth may provide grass pea with the potentialities of forage crop despite lack of improvement in grain yield. Improvement of forage, fodder and straw in grass pea has a large potential in many under-developed regions in India and other places (Biswas, 2007). The WYSCM, in this regard, may be more useful as a forage crop than as a grain crop. Seed coat pigmentation in grass pea is reportedly correlated with flower colour and different antinutritional factors (ANFs) including seed neurotoxin, ODAP content (Deshpande and Campbell, 1992; Campbell, 1997; Das and Kundagrami, 2001). Grass pea types exhibiting white seeds are generally associated with white flowers and low ANFs, and are available in European accessions, whereas dark coloured flowers, deep pigmented seeds and high ANFs are common in Indian subcontinent (Jackson and Yunus, 1984; Campbell,1997). The present study is in partial accordance with this observation as BSCM produced usual blue flower and WYSCM was accompanied with bluish-white flower. However, seed ODAP content recorded inverse relationships; its reduction was significant in BSCM line but marginal in WYSCM. In contrast, the bold-seeded (BSM) line showed white flower colour, whitish-gray seed coat and produced seeds with low ODAP content. Absence of relationship between seed coat or flower colour and seed ODAP content was reported in different germplasms of grass pea, but was useful in some cases (Kaul *et al*.,1986; Pandey *et al*.,1995; Das and Kundagrami, 2001).

Genetics and linkage studies Intercrosses between control cultivar/BSCM/WYSCM producing normal-sized seeds and the BSM gave

rise to $F_1$ plants which produced normal-sized (mean100 seed weight 5.5±0.28) seed (Table 2). In selfed $F_2$ progenies, bold-sized seeds were harvested from a pool of 24 plants whereas the remaining 354 plants produced normal (control)-sized seeds, fitting well to a 1:15 ratio. In the respective testcrosses of $F_1$ × BSM, segregation of mutant and control seed size showed a good fit to a 1:3 ratio, indicating recessive nature of the mutation and involvement of two non-allelic genes (symbols proposed as $S^N$ and $S^B$) to govern seed size in grass pea. Any of the alleles in dominant form produced normal-sized seed while absence of dominant alleles of both the genes produced bold and large seed as in the mutant. The $F_2$ plants with bold and large seed produced similar sized seed in $F_3$ generation also. Digenic control of bold-seeded mutation in grass pea was, however, not in agreement with the inheritance pattern of a gamma-ray-induced bold-seeded mutant in mung bean where it was monogenically controlled (Singh, 1996). Mode of inheritance of normal grey-brown seed coat in control varieties, black seed coat in *BSCM*, whitish-yellow in *WYSCM* and whitish-grey in *BSM* was studied in progenies derived from different intercrosses among these four types of parents. Results in table 2 clearly indicated that normal grey-brown seed coat colour was completely dominant over three mutant seed coat colour. Among the mutant lines, black colouration of seed coat exhibited complete dominance over whitish-grey and whitish-yellow seed, while whitish-yellow in WYSCM was recessive to all.

dominance relationship as $C^{gbr}$ (normal colour)>$c^{bl}$ (black)> $c^{wg}$ (whitish-grey)>$c^{wy}$ (whitish-yellow). This result was in agreement with earlier reports on monogenic recessive nature of seed coat colour and involvement of multiple alleles in controlling this trait in grass pea (Talukdar and Biswas, 2005; Talukdar, 2009b). Segregation of flower colour into blue and white exhibited good fit to 3:1 in F2 and 1:1 in test cross, suggesting recessive monogenic control of white flower in the present BSM line. The result is consistent with earlier reports of inheritance of white flower as a recessive trait in grass pea, where flower colour was digenically controlled (Kumari *et al*., 1993; Mehra *et al*., 1995; Tiwari and Campbell, 1996; Talukdar and Biswas, 2007c). Two different non-allelic genes showing duplicate (15:1) gene interaction were involved in governing seed size in grass pea. However, it is noteworthy that seed coat colour and also, flower colour in BSM plants exhibited monogenic mode of segregation, indicating occurrence of whitish-grey seed colour and white flower colour of the mutant in greater number of $F_2$ progeny plants than that occurred for bold-seed size. This is also indicative that genes controlling bold seed size in BSM have no pleiotropic effect on development of either seed coat colour or flower colour, and the seed size, seed coat colour and flower colour are governed by different genes, as revealed by joint segregation of these traits. Crosses between BSCM as well as control variety and bold-seeded mutants (BSM) in different combinations indicated good fit of flower as well

**Table3. Linkage tests of genes controlling seed coat colour ($C^{gbr}$), flower colour ($B^+$) and boldness of seeds ($S^B$-$S^N$) and estimation of map distances in *Lathyrus sativus* L.**

| Cross | Gene pair (X-Y) | $F_2$/test cross segregation XY | Xy | xX | xy | Total | χ2 at3df* (9:3:3:1 / 45:3:15:1) | χ2 at 3df* (1:1:1:1 / 3:3:1:1) | Map dist (cM) |
|---|---|---|---|---|---|---|---|---|---|
| Control x *BSM* ($C^{gbr}$-$B^+$) | | 100 | 12 | 14 | 25 | 151 | 44.97 | - | - |
| $F_1$ x *BSM* (test cross) | Do | 65 | 12 | 10 | 50 | 137 | - | 66.47 | 16.6 |
| *BSCM* x *BSM* | Do | 82 | 15 | 14 | 20 | 131 | 26.25 | - | - |
| $F_1$ x *BSM* (test cross) | Do | 48 | 08 | 09 | 40 | 105 | - | 49.25 | 16.7 |
| WYSCM x *BSM* | Do | 43 | 06 | 06 | 14 | 69 | 29.64 | - | - |
| $F_1$ x *BSM* | Do | 41 | 08 | 07 | 37 | 93 | - | 43.04 | 16.7 |
| Pooled test cross | Do | 154 | 28 | 26 | 127 | 335 | - | 158.27 | 16.7 |
| *BSCM* x *BSM* | $C^{gbr}$-($S^B$-$S^N$) | 111 | 21 | 55 | 10 | 197 | 37.67 | - | - |
| $F_1$ x *BSM* (test cross) | Do | 160 | 38 | 14 | 18 | 230 | - | 101.64 | 24.2 |
| Control x *BSM* | Do | 58 | 47 | 33 | 10 | 148 | 9.87 | - | - |
| $F_1$ x *BSM* (test cross) | Do | 198 | 40 | 23 | 54 | 315 | - | 117.40 | 21.1 |
| Pooled test cross | Do | 358 | 78 | 37 | 72 | 545 | - | 488.40 | 22.5 |
| Control x *BSM* | $B^+$-($S^B$-$S^N$) | 101 | 08 | 09 | 32 | 150 | 128.20 | - | - |
| $F_1$ x *BSM* (test cross) | Do | 130 | 09 | 11 | 30 | 180 | - | 591.01 | 11.3 |

* deviated significantly at 5% level

The results suggested monogenic control of seed coat colour in the present material and involvement of multiple allelic series in the locus, showing as seed coat colour individually in 3:1 ratio in $F_2$ (Table 2), but their joint segregation deviated significantly ($p$<0.05) from normal dihybrid ratios

both in $F_2$ and test crosses (Table 3). Analysis of joint segregation of seed coat colour (3:1) and seed size (15:1) raised through dihybrid crosses between control variety as well as BSCM and BSM revealed that the $F_2$ and test cross segregations did not fit well to the expected 45:3:15:1 in $F_2$ and 3:3:1:1 in test cross. Similar results were obtained in segregation of flower colour and seed size in control x BSM and in BSCM x BSM. These results suggested that the loci controlling seed coat colour, flower colour and seed size were linked with each other in the present material of grass pea. Cross over values between genes $C^{gbr}$ and $B^{+}$, between $C^{gbr}$ and ($S^{N}$-$S^{B}$) and between $B^{+}$ and ($S^{N}$-$S^{B}$) were calculated as 16.12% (pooled), 21.10% (pooled) and 11.11%, respectively in different test cross segregations and using these values in Kosambi's formula, these 3 loci were tentatively mapped as-

I-------16.72---------I--------11.30--------I
$C^{gbr}$----------$B^{+}$----------($S^{N}$-$S^{B}$)
I--------------------22.51--------------------I

Information about gene mapping based on morphological parameters is not available in grass pea. Recently, however, RAPD, STS and STS/CAPS markers along with isozyme markers have been used to construct linkage maps related to different dwarfing loci, isozyme markers and blight resistance (Chowdhury and Slinkard, 2000; Gutierrez *et al*., 2001;Skiba *et al*., 2003; Talukdar *et al*., 2010c). In the present investigation, isolation and characterization of two high grain yielding mutant lines and one potential forage line, and their linkage association with flower colour and bold seed size strongly favored the idea that induced mutagenesis is highly useful in creation of stable and desirable mutations and construction of classical linkage map in grass pea.

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