Endeavour to identify different types of mutant lines by screening out conspicuous heritable modifications in the post irradiated M2 and subsequent generations resulted in isolation of 14 distinct mutant lines and 7 types of trisomics in grass pea cultivar BioR-231. A colchicine induced mutant detected in C2 was also studied in the advanced generations. Among the employed doses of gamma rays, 350Gy was most effective inducing wider spectrum of mutations than all other doses. Biswas (1998) also obtained maximum number of chlorophyll and other mutations following 35 Kr (350 Gy) irradiation. The mutants and trisomic types were characterized with their respective specific marker phenotypes. Accordingly, five different flower colour mutants (pale-violet, reddish purple, blue-patched-white, white flower-I and II), four seed coat colour (black, light green, yellow and white), three growth habit (non-winged internode mutant, profusely branched mutant and dwarf mutant), four stipule characters (exstipulate, acicular-linear, ovate-round, blackish purple), a long pedunculate mutant and seven different types of trisomics were characterized. Among these, characteristic features of dwarf mutant (DM), pale violet flower mutant (PVFM), blue-patched-white flower mutant (BPWFM), different seed coat colour mutants, exstipulate mutant, long pedunculate mutant (LPM) and trisomic types have already been elaborated concisely (Talukdar et al. 2001a, 2001b, 2002; Talukdar and Biswas 2005, 2006, 2007). All of the 15 mutant lines identified by their marker phenotypic manifestations were stable in advanced generations (M3, M4 and M5). In contrast to the usual blue colour of petals in the mother cultivar BioR-231, flower colour has modified to pale violet, reddish purple, blue-patched-white and white in different mutant lines. These modifications in flower colour being highly contrasting were used as marker characters for PVFM, RPFM, BPWFM, WFM-1 and II.
The flower colour mutants were otherwise phenotypically normal like control except reduced yield in BPWFM and enhanced yield in PVFM and WFM-II. Seed ODAP content reduced significantly in PVFM and WFM-II but marginally in RPFM, BPWFM and WFM-I lines. Frequent occurrence of multivalent association and subsequent anaphasic disturbances indicated possibility of reciprocal translocation, induced by gamma ray irradiation in BPWFM. Meiotic abnormalities resulting preponderance of pollen sterility might be ascribed for reduced fertility and seed yield in this mutant. Increase in pollen sterility was encountered in induced M₁ and M₂ progenies of 6 grass pea varieties studied by Prasad and Das (1980). Shaikh and Godward (1972) observed multivalent association and formation of anaphase bridge and laggard along with other meiotic abnormalities in post-irradiated grass pea.

In the present investigation higher yield of grain in PVFM might be attributed to its normal fertility level and traits contributing favourably towards its yield. Besides white colouration in petals, the uniqueness of white flower mutant-II (WFM-II) was also due to its bold size of seeds and non-shattering pods which provided it with two times increase in 100 seed weight as well as per plant seed yield. Superiority of this mutant was also due to earliness in maturity and significantly low seed ODAP content. Indian grass pea genotypes are usually small seeded, and pod shattering is a normal phenomenon; whereas large seeded types without shattering habit occur predominantly in Mediterranean region and Europe (Hammer et al. 1989; Campbell et al. 1994). A few number of bold seeded pure lines have been reported in Chile (Mera et al. 2003) and in India (Kumari et al. 1996, Pandey et al. 1997). The present author is pioneer in isolating this non-shattering, bold seeded and high yielding mutant through gamma ray irradiation in grass pea cv. BioR-231. The seeds of this mutant line were bolder and heavier (100 seed weight =
12.88 gm.) than the bold seeds (100 seed weight = > 5.1 gms.) of different accessions maintained by Sarwar et al. (1997) and was comparable to the bold seeds (100 seed weight = >12 gms.) reported by Pandey et al.(1997). Marginal increase in the average value of 100 seed weight over control was also recorded earlier in different grass pea cultivars (Chekalin1971, Singh and Chaturvedi1990).

Occurrence of induced variations including flower colour modifications like 'albus'(white), 'roseus'(crimson) and 'cyaneus'(light blue) in $M_2$ generation of different grass pea varieties 'Rewa-1', 'Rewa-2', 'T-2-12', 'L-C-76' and 'Tabriz' was reported by Nerker (1972,1976). Prasad and Das (1980) also detected 'albus' type of flower colour modification in gamma ray induced $M_2$ generation of cultivar 'P10'. A lot of flower colour mutants showing white, pink, red, light violate, reddish-blue, pinkish-blue and light blue modifications in colourations of petals were identified by Sarwar et al.(1993,1995). Induced mutations in flower colour were also mentioned in 2 polish grass pea cultivars, 'Derek' and 'Krab' (Rybinski 2003).

Variations in seed coat colour (black, light green, yellow and white) were prominent and distinctive in four seed coat colour mutant lines. These modifications being specific for each of the mutant was treated as marker phenotype and accordingly the mutants were designated as black seed coat mutant (BSCM), light green seed coat mutant (LGSCM) yellow seed coat mutant (YSCM) and white seed coat mutant (WSCM). Among these, black and white seed coat exhibited mosaicism with gray spots. Besides black colouration with gray mosaic, BSCM could be distinguished from others as well as from control by its remarkably higher grain yield, early maturity and low seed ODAP content in all the generations studied. In addition to white colour of seed coat with gray mosaic, WSCM showing comparable grain yield was also distinguished
from others by its highest forage yield possibly due to delayed maturity, taller plant height, increased number of primary branches and leaves with leaflets, larger size of leaflets, luxuriant vegetative growth and higher amount of biomass production, which is effective in preventing soil moisture content from drying out and most desired in ideal forage crop as explained earlier by Campbell et al. (1994). This white seeded mutant line could be more potential as a fodder crop than as a grain crop. Pertinently, the observations of Smartt (1981, 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. The WSCM, thus, may be more useful as a forage crop than as a grain crop. Modifications of seed coat colour through induced mutation in M\textsubscript{2} generation of different varieties of grass pea were mentioned by Nerker (1976) and Rybinski (2003). Prasad and Das (1980) isolated 3 induced mutants showing black, shining red and white colouration of seed coat in M\textsubscript{2} generation of grass pea variety 'P10'.

Among the three growth habit mutants isolated in the present study \textit{profusely branched} mutant (\textit{PBM}) manifested prostrate and indeterminate stem habit like control, whereas \textit{non-winged internode} mutant (\textit{NWIM}) and \textit{dwarf} mutant (\textit{DM}) were erect and determinate in habit. Instead of usual alternate orientation of branches in grass pea, numerous branches originating in concentric manner provided the \textit{PBM} with bushy appearance and significantly high fodder as well as grain yield. It was followed by \textit{NWIM} in number of branches, seed yield and ODAP content while yield was very poor in \textit{DM} line. Nerker (1976) reported occurrence of 'shy-branching' and 'non-branching' type of mutants in grass pea. Prasad and Das (1980), however, identified an unbranched stem mutation which was completely sterile. Occurrence of non-winged (‘afila’) stem mutation and miniature mutant in M\textsubscript{2} progeny
of grass pea was also reported (Nerker 1976, Prasad and Das 1980). Various types of stem habit mutations were detected in post irradiated $M_2$ progeny of different cultivars of grass pea (Waghmare et al. 2001, Kumar and Dubey 2003, Rybinski 2003). Further, according to Rybinski (2003) prostrate stem with indeterminate growth habit is highly undesirable for introduction of grass pea as a grain crop in different environmental conditions. In this backdrop, erect habit with determinate growth of stem in NWIM and DM and also more compact growth habit in DM may be utilized in the breeding of grass pea as earlier suggested by Smartt (1984) and Arora et al. (1996). In addition to its growth habit and tremendous reduction in height the dwarf mutant could be distinguished from the control mother plants by its ovate-lanceolate shape of leaflets, ovate-round stipules, short tendril and markedly winged internode modifications as usually manifested in tetraploid plants. Cytologically it was diploid ($2n = 14$) in nature showing normal meiosis and bred true in subsequent advanced generations. Such phenotypic modifications in the colchicine treated plants comparable with stable changes induced by mutagenic agents following expected Mendelian inheritance without showing any evidence of chromosomal anomalies may be treated as manifestations of gene mutations. Colchicine induced gene mutations has also been reported in sorghum (Franzke and Ross 1952, 1957, Foster et al. 1955, 1961, Ross et al. 1954, 1961, Chen and Ross 1961), flax (Dirks et al. 1956) and soybeans (Porter and Weiss 1948). It has been opined that the mutagenic effect of colchicine is not limited to single region or locus of a chromosome, but that mutations may be effected at random at a large number of loci on different chromosomes within one plant whose diploid chromosome number remains unaffected (Harpstead et al. 1954, Foster et al. 1961).

Four different mutations for stipule character recognizable at seedling stage as exstipulate, acicular-linear shaped stipule (highlighted
in detail as BPWFM, ovate-round stipule (highlighted in detail as dwarf mutant) and blackish purple stipule mutant (BPSM) were poor grain yielder. These marker characters could be of adequate significance in genetic studies. BPSM having lower seed ODAP content vis-à-vis higher forage yield was potential enough to be treated as a good fodder yielding plant. Modification in stipule shape (serrated stipule) through mutagenic treatment has also been reported in grass pea (Nerker1976).

The LPM showing desired characteristics like enhanced (2-fold) yield of seed with significantly low ODAP content and earlier maturity by 17-20 days than control has the potentiality to be a promising improved line. Its stable unusually elongated peduncle length (3-fold increase) provided it with a novel marker phenotype.

Among the fifteen different mutants isolated in the present investigation, six possessed high grain yield potentialities and low seed neurotoxin (ODAP) content as compared to control and other mutant lines. Black seed coat mutant (BSCM) manifesting more than 2.5 times increase in seed yield and 52 days earlier in maturity than control with low level of seed ODAP in all the generations studied was superior to all. It was followed by white flowered (bold seeded) mutant-II (WFM-II), LPM, NWIM, PBM and PVFM in yield potential. Among these high yielders, LPM was earlier in maturity by 17-20 days and was followed by WFM-II and PVFM. On the contrary, PBM and NWIM were relatively late. As compared to control (0.35%), seed ODAP content reduced significantly in all the six high yielders. The mutant lines namely, BPSM and WSCM were distinguished from others by significant increase in forage yield and low seed ODAP content. In different agro-climatic conditions seed neurotoxin ODAP content remained unchanged in all but WSCM. Using single and recurrent mutagenic treatment endeavour was initiated to isolate high
yielding and low seed ODAP lines in different varieties of grass pea (Swaminathan et al. 1971). Mean value of seed ODAP level shifted in ‘+’ (higher) as well as in ‘-’ (lower) direction showing wide range of variations in different induced mutants of grass pea (Nerker 1972). Induced variability in seed ODAP content was also reported subsequently by different authors (Prasad and Das 1980, Singh and Chaturvedi 1990, Das and Kundagrami 1999). Analysing stability parameters and field performances at different locations, some stable varieties for low ODAP content were recommended by Ramanujam et al. (1980) and Sharma et al. (1997).

Study of mode of inheritance in flower colour, seed coat colour, growth habit and stipule character in F2 generation raised separately by intercrossing control with individual mutant lines and in backcross progenies revealed monogenic segregation in most of the cases. Involvement of a single pair of alleles was thus evidenced and the genotypes B'B', BbpBbp, B'rB'r and BwBw were proposed for blue colour of flower in control, true breeding blue-patched-white colour in BPWFM, reddish purple flower colour in RPFM and white colour in WFM-I and II respectively. Following intercrosses in all possible combinations transmission of flower colour in F1, F2 and backcross progenies manifested complete dominance of blue colour over blue-patched-white and white. Occurrence of blue-patched-reddish purple colour of flower in F1 hybrid of control x RPFM, presence of both blue colour of control and reddish purple colour of mutant line, and segregation of flower colour into 1 blue : 2 blue-patched-reddish purple : 1 reddish purple in F2 suggested co-dominance of the alleles governing blue (B') and reddish-purple (B'r) colour of flower. On the other hand, pink (B'rB'w) or bluish pink (B'rB'bp) i.e intermediate colour of flower in F1 generation of RPFM x WFM-I or RPFM x BPWFM respectively and 1: 2: 1 segregation of reddish-
purple: pink/ bluish pink: white /blue-patched-white respectively in the corresponding F₂ progeny indicated incomplete dominance of reddish-purple to white and blue-patched-white. When crosses were made between control (B⁺B⁺) and pink flowered hybrid (BᵖBʷ) equal number of blue-patched-reddish purple (B⁺Bᵖ) and blue (B⁺Bʷ) flowered plants appeared. In the selfed progeny of the former (B⁺Bᵖ) blue flowered, blue-patched-reddish purple plants and reddish purple plants occurred in 1:2:1 proportion, while selfing of the latter (B⁺Bʷ) yielded blue and white flowered plants in 3:1 ratio. Flower colour in the F₁ plants of control x BPWFM and control x WFM-I and II were blue; and monogenic segregation in the corresponding F₂ and backcross progenies suggested complete dominance of blue over blue-patched-white and white. It is, thus, evident from the present investigation that different modifications of flower colour occurred through monogenic segregation involving a series of multiple alleles B⁺, Bᵖ, Bᵇp and Bʷ for blue, reddish purple, blue-patched-white and white colour of flower respectively. B⁺ was completely dominant over Bᵇp and Bʷ but co-dominant to Bᵖ which showed incomplete dominance over both Bᵇp and Bʷ. Bᵇp, on the other hand was completely dominant over Bʷ. Complete dominance of coloured petals over white or colourless petals was reported in different intervarietal crosses of grass pea (Kumari et al. 1993, Sarwar et al. 1993), while inhibitory (13blue: 3white) and supplementary (9blue: 3pink: 4white) type of gene interactions were suggested by Tiwari and Campbell (1996) and Das and Kundagrami (1999) respectively. Complementary (9blue: 7 pink) type of interaction has also been reported to be involved in flower colour modifications in the segregating F₂ progenies of blue and pink flowered varieties (Niral et al. 1991, Mehra et al. 1995, Das and Kundagrami 1999).

Transmission of flower colour in the F₂ progenies of the intercrosses blue x pale violet and pale violet x reddish purple showed
normal (9 light blue: 3 pale violet: 3 blue: 1 white) and modified (9 pinkish purple: 1 reddish purple: 2 pink: 3 pale violate: 1 white) dihybrid ratios respectively. Two pairs of genes being involved in both the intercrosses acted for the same character i.e. flower colour and modifications of colour occurred through recombination of the two pairs of genes showing complete dominance in the former and one pair showing incomplete dominance in the latter. The genes $B^+$ and $Pv$ governed blue and pale violet colour of flower respectively showing complete dominance. In case of former, following dihybrid cross $B^+$ and $Pv$ together ($B^+-Pv-$) produced only light blue flowers in most of the plants bringing about a new modification in pigmentation while flower colour was blue and pale violet in $B^+-PvPv$ and $Bw Bw Pv$-respectively as in the parents but absence of dominant genes resulted in striking modifications in pigmentation in a few white flowered ($BwBw pv pv$) plants. Mode of segregation has been modified in the latter due to incomplete dominance of $B^p$ over $Bw$. The genes $Pv$ and $B^p$ together resulted in spectacular colour modification and manifested a completely new flower colour (pinkish purple) in most of the $F_2$ plants having the genotypes $Pv- B^pB^p$ while reddish purple flowers was produced only in a few plants possessing the genotypes $B^p B^p pv pv$, but due to incomplete dominance pink colour was manifested in the flower of $B^p B^p pv pv$ plants. Like the earlier intercross absence of dominant genes ($BwBw pv pv$) produced another new flower colour, white. This explanation is in agreement with earlier reports of flower colour segregation in lentil in which beside parental colour of pink and white two new flower colours violet and rose were manifested (Lal and Srivastava 1975).

Genetic basis of colour variation and mosaic pattern in the seed coat of different mutant lines was studied by tracing mode of inheritance separately in the progenies of different intercrosses between the individual seed coat colour mutants and the parent variety BioR-231.
Brown colour of seed coat was monogenic dominant over all other modifications, black colour to light green, yellow and white, light green to yellow and white, and yellow to white colour of seed coat only. A series of multiple alleles showing dominance in the order of $C_{Br} > C_{Bl} > C_{Lg} > C_{Y} > C_{w}$ has been suggested for such variations in seed coat colour. Monogenic complete dominance of normal brown to black colour of seed coat in the present investigation was in agreement with the earlier findings of Prasad and Das (1980) in grass pea. Mode of inheritance of seed coat colour has been investigated in different varieties and induced mutant lines of different other leguminous crops (Phadnis 1978, Chaudhari and Thombre 1983, Ahmad et al. 1983, Ghatge et al. 1985, Henry 2001, Meena et al. 2004).

Among the different growth habit mutations in the present material, inheritance of plant height, stem habit, winged modification of internode, number of nodes, shape of leaflets and length of tendril was simple monogenic, but prostrate habit of stem showed incomplete dominance over erect habit. A series of multiple alleles were assumed to be involved in regulating variations in plant height and winged nature of internode. Normal height in control cultivar BioR-231 was completely dominant over semi-dwarf and dwarf, and semi-dwarf has also shown dominance over dwarf indicating order of dominance $P_{hn} > P_{hsd} > P_{hd}$. Dominance of usual moderately winged internode in control ($W_c$) over markedly winged ($W_m$) in dwarf mutant and non-winged ($W^n$) nature in another mutant was exhibited following the order $W_c > W_m > W^n$ in winged modifications of internode. Monogenic control of plant height, stem habit and number of nodes was reported earlier in several other legumes (Wellensiek 1965, Sandhu and Khehra 1980, Raje et al. 2001, Talukdar and Talukdar 2003, Kumar et al. 2004).
Transmission of existence vs. non-existence as well as variations in shape and orientation of stipules followed simple monogenic segregation in the intercrossed progenies of control and the mutant lines. The order of dominance of the alleles St$n > St^{or} > St^{al} > St^{e}$ involving in control of shape and existence suggested multiple allelic control of the trait.

Digenic mode of inheritance was exhibited in branching pattern, stipule colour and shape and size of seed. Segregation of alternate, sub-opposite and concentric branching patterns in the F$_2$ (12:3:1) and backcross (2:1:1) progenies of control (alternate branching) x mutant (recessive) showing concentric branching suggested involvement of 2 pairs of non-allelic genes assumed to be Al Al Op Op controlling alternate and sub-opposite (al al Op-) orientation of branching of which gene ‘Al’ was epistatic to gene ‘Op’ while concentric branching was manifested due to homozygous recessive state of both the gene pairs (al al op op). Studies on the inheritance pattern of stipule colour revealed presence of two pairs of genes showing complementary (9:7) type of interaction. The proposed gene pairs St$^G$ St$^G$ St$^{BP}$ St$^{BP}$ complimented each other manifesting green colouration of stipule in control while presence of any one of the two (St$^G$ St$^G$ St$^{BP}$ - / St$^{G-}$ St$^{BP}$ St$^{BP}$) or both the genes (St$^G$ St$^G$ St$^{BP}$ St$^{BP}$) in the homozygous recessive state resulted in blackish-purple colour of stipule in the blackish-purple stipule mutant. Variation in seed size in the F$_2$ and backcross progenies segregating into 15:1 and 3:1 respectively of control (normal size of seed) x WFM-II (bold seeded) suggested involvement of two pairs of genes showing duplicate type of interaction. Presumably the gene pairs S$^{NSN}$ and S$^{BBS}$ were identical in activity and regulated seed size without interfering each other. Normal seed size like the control mother cultivar was manifested in all cases having at least one dominant alleles (S$^{NSN}$S$^{BBS}$/ S$^{NS}$S$^{NS}$S$^{BBS}$) but in absence of all the dominant alleles (S$^{NS}$S$^{NS}$S$^{BBS}$) enhancement of seed size was spectacular like the bold seeded mutant line WFM-II. Variation in
peduncle length was contrasting in between true breeding long pedunculate mutant (9.9cms± 0.19) and dwarf mutant (1.22cms± 0.05). Segregation of peduncle length distinctly into longer, long, medium, normal and short sizes in the F₂ progeny of the crosses LPM x DM and LPM x control indicated possibility of polygenic interaction between 2 pairs of genes, Pdl₁ and Pdl₂ controlling the length of peduncle. Each of the effective alleles of the probable gene pairs might have contributed small but cumulative effect manifesting longest and shortest length of peduncle in the mutant lines respectively.

Analysis of joint segregation studied in the present investigation involving two contrasting pairs of characters in the F₂ and backcross progenies raised through intercrossing control with each of the mutant lines separately and individually between different mutant lines revealed independent assortment of the marker characters in most of the cases. Linked association of the traits was, however, detected in certain cases. Among the different traits taken into account normal Mendelian segregation was predominant; but modes of inheritance modified in the dihybrid segregation of stipule colour with winged nature of internode, stem habit and length of tendril possibly due to complementary type of interaction, while duplicate factors might be involved in the modification of seed size with stem habit, leaflet shape, number of corolla and stipule shape. On the other hand, predominant occurrence of some of the parental marker characters vis-à-vis fewer number of recombinants in different dihybrid crosses indicated linked association of the concerned traits. Altogether 14 gene loci were identified and assigned to 3 linkage groups. Positions of the proposed genes controlling different traits were mapped on the basis of cross over value (cov %) following Kosambi's (1944) formula. Thus the genes Phⁿ controlling plant height, Nd for node number, Lftn for leaflet number and Wc for winged nature of internode were mapped in 'linkage group 1'. Similarly gene Prt for stem habit, Lfts
Informations 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 F₂ segregating individuals of *Lathyrus sativus* Chowdhury and Slinkard (1999) constructed a genetic linkage map and 69 markers were assigned to 14 linkage groups comprising 898 cM with an average map distance of 17.2 cM between 2 markers. Subsequently, linkage was also detected between 2 isozyme loci Aat-2 and Skdh (Chowdhury and Slinkard 2000). Gutierrez *et al.* (2001) mapped 6 other isozyme loci in 3 linkage groups of grass pea. Loci Prxl- Pgd2, Pgm2-Est2 and Aat2G – Pgm1 were assigned to linkage group 1, 2 and 3 respectively. Considering RAPD, STS and STS/CAPS markers, a linkage map comprising of 9 linkage groups with a total map distance of 803.1 cM. was constructed from backcross individuals derived through intercrossing between ascochyta blight resistant and susceptible accessions of grass pea (Skiba *et al.* 2004). Two QTLs, namely QTL1 and QTL2 associated with blight resistance were also detected by the authors in linkage group 1 and 2 respectively.

Analysis of variance of yield and component characters revealed significant differences among the mutants and the mother variety BioR-231 of grass pea. Mean values of different yield attributes shifted positively in eight of the 15 different mutant lines isolated in the present investigation. Among them, grain yield was significantly high in 6 mutant
lines and seed ODAP content reduced conspicuously to the standard low range (0.1-0.4%) of ODAP content fixed earlier by Ramanujam et al. (1980) in Indian grass pea germplasms. Four of these mutants were significantly earlier in maturity and black seed coat mutant (BSCM) required minimum days to mature. Yield has increased more than two times over control in BSCM and it was followed by WFM-II (bold seeded mutant), LPM, NWIM, PBM and PVFM. It is thus evident that BSCM showing best performance in yield vis-à-vis earliness in maturity and significantly lower level of seed ODAP content may be assumed to be the most potential genotype among all of the mutant lines isolated in the present investigation. On the other hand, spectacular increase in biological yield provided the white seed coat mutant and blackish purple stipule mutant with the promising fodder yield potentialities. Cultivation of grass pea as a forage crop is a common practice in different countries (Campbell 1997).

High amount of variations for all the traits particularly seed yield per plant was reflected by high values of respective genotypic coefficient of variations (GCV) and phenotypic coefficient of variations (PCV). The magnitudinal differences of GCV and PCV were very low in all the traits but considerably high in days to flower. This has indicated negligible role of environment in the expression of all the traits while its influential role was evidenced in days to flower. High estimates of heritability (broad sense) for all the traits except days to flower also supported this finding. The results of the present investigation were in conformity with the earlier observations on different induced mutant lines of grass pea (Kumar and Dubey 1998, 2001), lentil (Dixit and Dubey 1985) and faba bean (Vandana, Dubey D K1992). The estimate of heritability acts as a predictive instrument in expressing the reliability of phenotypic value and helps in selection. In most of the yield components of the present materials both heritability and genetic advance (as percentage of mean)
were high, but heritability was high and genetic advance was low for
days to maturity, while both heritibility and genetic advance were low in
case of days to flower. Johnson et al. (1955) suggested that heritability
along with genetic advance was more useful than heritability alone in
predicting the resultant effect for selection of best individuals. Panse
(1957) attributed additive gene effect for high heritability with high
genetic advance, and suggested improvement of the concerned trait/s
through individual plant selection; high heritability coupled with
moderate to low genetic advance 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 lines.

Present investigation revealed significantly positive correlation
between yield and number of pods per plant, 100 seed weight, plant
height and number of primary branches per plant suggesting their
positive contribution towards seed yield, and maximum contribution was
due to number of pods per plant as indicated by its high co-efficient
value. Significantly negative association of days to flower with number of
pods and seed yield per plant but positive correlation with number of
seeds per pod indicated favourable influence of earliness towards pod
setting and seed yield per plant although per pod seed number reduced.
Induction of early flowering can, therefore, improve seed yield through
high number of seed setting. Significantly positive correlation of both
plant height and number of branches with per plant number of pods and
seed yield, on the other hand, suggested that these traits contributed
towards yield via increased number of pods per plant. Number of pods,
thus, appeared to be most important yield component, which may be
enhanced due to increase in height and number of branches, although
association of height and number of branches was not significantly
positive. Strong positive association of grain yield with plant height,
number of branches, 100 seed weight and especially with pods per plant and negative association with days to flowering were also observed in different genotypes of grass pea (Kaul et al. 1982, Wolde 1991, Waghmare et al. 1996, Pandey et al. 1997, Biswas 1998, Kumar and Dubey 2001, Das and Kundagrami 2002).

Association of seed ODAP content with number of pods and 100 seed weight was significantly negative, and barring days to flower and seeds per pod its relationship with seed yield and protein content was negative but not significant during all the three years of observations. The observations suggested that early flowering and bold size of seeds might have contributed towards reduced ODAP content in seed. Similar relationships of ODAP content and yield components have been reported earlier in different genotypes of grass pea (Sharma et al. 1997, Pandey et al. 1997, Das and Kundagrami 2001). Kumari and Prasad (2005), however, recorded positive correlation of seed ODAP with yield and all other components except 100 seed weight. Seed ODAP content varied irrespective of flower or seed coat colour without showing any direct relationship in the different mutant lines isolated by the present investigation. This finding was in agreement with earlier observation by Kaul et al. (1986). Quader et al. (1988), however, suggested flower and seed coat colour to be used as genetic markers for identifying lines with low neurotoxin contents in seed. Das and Kundagrami (2001) also mentioned the possibility of using these marker characters for identification of certain genotypes.

Seed protein banding profiles exhibited conspicuous variations in number, width and intensity of bands in the control and different mutant lines. Among the total number of 41 bands identified, band number 33 was unique for PVFM, 39 for RPFM, 19 and 28 for BPWFM, 35 for WFM-I, 12 for WFM-II, 36 for BSCM, 16 for LGSCM, 24 for WSCM, 3 for BPSM, 32
for ESM, 17 for LPM, 20 for DM, 27 for NWIM and 4 for PBM; on the contrary, band number 13 was common to all but in yellow seed coat mutant (YSCM). The mutants shared 14 bands with the mother variety, while some bands were also shared by 2 or more genotypes. Each of the 15 mutant lines was thus characterized by its specific band(s) as well as number, width and intensity of the common bands. SDS-PAGE analysis of seed storage protein was earlier employed to evaluate intraspecific variation in grass pea (Sood et al. 1995). Differences in number, width and intensity of bands were also observed in different cultivars, induced mutant lines and intercrossed segregants of grass pea (Roy et al. 2001, 2004). Presence of specific band was detected in radiation induced mutants of other crops also (Dutta et al. 1987, Sarkar et al. 1990, Nayeem et al. 1999.). Unique banding pattern specific to a cultivar can be used for its identification as well as maintenance of genetic purity; and genotype specific polypeptides can be treated as markers for detection of mutants, hybrids and other breeding lines derived from the genotypes (Mohanty et al. 2001). These genotype specific markers being quite stable, simple and easily recognizable as compared to DNA markers have widely been used to elucidate genetic variation, evolution, pattern of inheritance and linked association in different other legumes (Larsen 1967, Ladizinsky and Adler 1975, Osborn 1988, Kumar and Ram 1989, Panella et al. 1993, Panigrahi et al. 2001, Naik and Kole 2002).

Electrophoretically detectable variations in seed protein banding patterns and identification of genotype specific band(s) in the 15 mutant lines of grass pea cultivar BioR-231 as compared to control has immense importance to be treated as biochemical markers for mass screening of phenotypic mutants and their hybrids. These variations in seed protein profiling may be due to involvement of number of events and several genes might have controlled the expression of polypeptides. According to Osborn (1988) and De Lumen (1990), seed protein expression is governed
by multigene families. Panigrahi et al. (2001) assumed that expression of polypeptides were monogenically controlled showing complete dominance over its absence. Co-dominance of genes controlling different polypeptides patterns was also reported in various legumes including grass pea and these genes governing expression of different polypeptides present in seed protein were found to be closely linked in grass pea (Chandna and Matta 1997) whereas weak linkage and independent segregation of genes for various polypeptides were reported in pea, soybean, french bean and ground nut (Brown et al. 1981, Matta and Gatehouse 1982, Kitamura et al. 1984, Mahmood and Gatehouse 1984, Krishna et al. 1986). On the other hand, lack of expression of certain polypeptides in some genotypes has been attributed to deletion of the structural genes encoding the polypeptides or a mutation at a regulatory locus resulting in inhibition of transcription or translation of those particular genes (Brown et al. 1981).

Seven different types of trisomics evolved in the selfed advanced generations of an induced trisomic plant raised initially in the author’s laboratory (Biswas 1998) through gamma ray irradiation in grass pea cv. BioR-231 were characterized subsequently by their cytomorphological peculiarities as well as by leaf protein and isozyme banding patterns in the present investigation. In general, the trisomic plants were distinguishable from diploid mother cultivar by distinct modifications in leaflet and stipule characters, seed coat colour, high percentage of pollen sterility and presence of an extra chromosome. The different types of trisomics could be isolated easily from one another by specific modifications in the above mentioned morphological traits. Among these marker characters, leaflet modifications being prominently detectable at seedling stage was treated as a unique character for identification and designation of each type of trisomics, and on the basis of which
classification of the complete set of 7 different trisomic types was possible. While the compound leaves of normal diploids possessed a pair of opposite linear-lanceolate leaflets with acute apices in each jugate, the seven different trisomics could be distinguished from one another by the modifications in shape, apex, number, position and arrangement of leaflets. Position of leaflets was opposite in all but trisomic type V showing alternate arrangement. Type I was identified with acicular shaped leaflets with ventrally rolled margins in all the leaves, type II showed bifurcated apex in one of the 2 leaflets of the first jugate, type III possessed ternate (three) leaflets in the first jugate lying on the same lateral side in all the leaves, type IV had 2 leaflets on one lateral side and another leaflet on the opposite side in all the jugates of second leaf, type V exhibited alternate arrangement of linear-lanceolate shaped leaflets in all the leaves; the apices of both the leaflets in the first jugate of leaves were revolute in type VI while the apex of one of the leaflets of the first jugate was revolute but the other was normal in type VII. Ventrally rolled leaflet margin towards the midrib detected in the trisomic type I was also reported in primary trisomic for chromosome 3 (Das and Kundu 1973) and interchange trisomics in 6-rowed barley (Prasad and Das 1975).

Conspicuous absence of stipules distinguished type III as exstipulate but all other types exhibited prominent and distinctive modifications in shape, number and position of stipules. In contrast to persistent, free lateral and foliaceous semi-sagittate shape of stipules in normal diploid plants, both the stipules were thin and elongated in type I, one stipule was spinous and the other was lanceolate in type II and both were caducous in type VI; on the other hand, stipule was solitary and lanceolate in type IV and solitary and curved in both V and VII. Type VII was also distinguishable from others by the presence of a stipel.
Modifications in seed coat colouration being distinctly different, identification of each of the 7 types of trisomics was convenient and easier at post-harvest stage. Brown colour of seed coat in normal diploids changed to blackish brown in type I, gray in type II, deep black in type III, yellowish-brown in type IV, black-striped in type V, brownish gray in type VI and deep brown in type VII. All the trisomic types produced both small sized light seeds and large sized heavy seeds although size and weight of seeds were uniform in normal diploid mother variety. Variations in seed-size and weight were reported in different types of trisomics identified in barley (Tsuchiya 1960), pearl millet (Minocha et al. 1976, Singh et al. 1984), *Sorghum bicolor* (Liang 1979) and rye grass (Meijer and Ahloowalia 1981).

The seven trisomic types isolated in the present material were also recognizable by conspicuous modifications in floral morphology. Compared to diploid plants peduncle was markedly elongated in trisomic types II and III but highly reduced in I, VI and VII. Distichous modification of pedicel was manifested only in trisomic type II in contrast to normal solitary pedicel. Number of sepals and / or petals increased in trisomic types I and III but sepal number reduced in type VI. Blakeslee was pioneer to isolate and differentiate the complete set of primary trisomic on the basis of specific modifications in particular plant part/s along with other morphological features in *Datura* (Avery et al. 1959). Use of distinct morphological modifications for identification of complete set of trisomic has been reported subsequently in several other crop plants including *Sorghum vulgare* (Schertz 1966), pearl millet (Gill et al. 1970, Virmani and Gill 1971, Sai kumar et al. 1982, Vari and Bhowal 1986), rice (Hu 1968, Iwata et al. 1970, Misra et al. 1986), maize (McClintock 1929), tomato (Rick and Burton 1954), pea (Gottschalk and Milutinovic 1973), barley (Tsuchiya 1967, Singh and Prasad 2000) and rye (Fujigaki and Tsuchiya 1988).
Generally the present trisomic plants were poor in growth and vigour manifesting shorter height, earlier maturity and reduced yield performances as compared to normal diploids except trisomic type IV which was bushy in nature with higher number of primary branches and large sized bold seeds. Yield performance in this trisomic was comparable to that in the mother variety. Gottschalk and Milutinovic (1973) obtained a trisomic mutant in pea without showing morphological variations from its diploid initial line; while Lin and Ross (1969) reported better viability in 2 trisomics of *Sorghum bicolor* than in the normal diploids. Vigorously growing primary trisomic plant with large and bold size of seeds were also reported in chromosome II trisomics of *Solanum chacoense* (Lee and Hanneman 1982) and ‘triplo-4 robust’ type in barley (Singh and Prasad 2000).

Specific phenotypic modifications in seven different trisomics isolated in grass pea might be attributed to incorporation of a different chromosome to different trisomic types; since each chromosome of a haploid complement in its turn might be involved in trisomic condition, the extra chromosome with additional set of genes would be quite different manifesting distinct phenotypic modifications in each of the 7 grass pea trisomics. Regular occurrence of unique marker leaflet character with specific variations in stipules and seed coat colouration in different trisomic types suggested linked association of the genes governing these traits and justified the above contention.

The extra chromosome in different trisomic types appeared as a univalent or in trivalent association. The univalent (s) appearing mostly in close proximity gives an indication of homology as suggested earlier in gamma ray induced *Vinca rosea* (Sudhakaran 1971). Gottschalk and Milutinovic (1973) explained that a trivalent can be formed in metaphase I only if at least two chiasmata occurred between the 3 homologous
chromosomes during pachytene. Unmodified nature of the extra chromosome in all the 7 grass pea trisomics was evidenced by linear, frying pan or Y-shaped configurations of the trivalent indicating the characteristic of primary trisomics identical to that of the primary trisomic plant initially recovered through gamma ray irradiation by Biswas and Biswas (2002, 2004). Predominant occurrence of linear or chain shaped trivalent indicated reduced frequency of chiasma in trisomic plants than in diploids. Reduction in chiasma frequency in trisomics was also reported in pearl millet (Virmani and Gill 1971) and black cumin (Dutta and Biswas 1984) although Manga (1976) reported increased frequency of chiasma in some trisomics compared to corresponding diploid plants of pearl millet. Chiasma formation is largely under genetic control and differences in its frequency in the trisomic may be the function of the genetic and other modifying factors carried in the trisome (Rees 1961, Smith 1966, Sybenga 1966). Vari and Bhowal (1986) observed little relevance of the variation in chiasma frequency to the length of the extra chromosome and opined that the decrease in chiasma frequency in trisomic plants was mainly due to reduction in chiasma in the bivalents. Among the 7 trisomics in the present material, chiasma frequency was highest and frying pan configuration was most frequent in type V, where as type IV showed maximum frequency of chain shaped trivalent although frequency of chiasma was minimum. Presumably, longer chromosome involving in frying pan configuration of trivalent resulted in increased number of chiasmata, while shorter chromosomes forming chain shaped trivalent manifested reduced frequency of chiasmata (Biswas and Biswas 2004).

Anaphasic irregularity was the common feature in trisomic plants of grass pea due to unequal separation, bridge formation and lagging of chromosomes bringing about disturbances in gamete formation. Occurrence of trisomic plants in the selfed progenies during succeeding
generations has been possible through fusion of normal (n) and abnormal (n +1) gametes produced by trisomic mother plant, although gametes containing an extra chromosome are usually non-viable and the outcome of which was manifested with high incidence of pollen sterility in the trisomic plants. Vari and Bhowal (1986) were of opinion that frequency of n and n +1 gametes in trisomics of pearl millet was determined by the combined effects of many meiotic events such as frequency of univalent (s), laggard at anaphase I and pollen sterility. In trisomic plants of maize, the univalents occurring at metaphase I were usually left at equatorial plate and appeared as laggard at anaphase I (Einset 1943). Ostergren (1951) suggested that orientation of univalents might determine whether they would be included in one of the polar groups of the chromosomes at telophase I or remained at the equator. The number of laggards at the equator might, therefore, be expected to be lower than the number of univalents at metaphase-I. The present study on grass pea trisomics has reiterated these views and possibly the univalent instead of lying at equatorial region moved to a particular pole resulting in unequal separation. Lata and Gupta (1975) explained that higher frequency of trivalent and univalent formation at metaphase-I might lead to higher number of PMCs showing unequal segregation and hence increased frequency of anaphasic irregularities like laggard and bridge formation took place. These irregularities according to them would result in decreased pollen fertility. On the other hand, in Helianthus trisomics, Whelan (1982) suggested that presence of higher univalent frequency at diakinesis- metaphase-I and frequent elimination of the unpaired univalent/s during subsequent anaphaseI- metaphase II and anaphaseII- telophase II might increase pollen fertility although trivalent formation reduced the chance of chromosome elimination and thereby maintained pollen sterility in trisomic plants. In the 7 different grass pea trisomics, unequal separation of 8-7 chromosomes was more common than 9-6 at anaphase I. Both unequal separation and lagging of
chromosomes (7-1-7 and 8-1-6) might be responsible for unbalanced pollen formation and enhancement of pollen sterility noticed in trisomic types I, II, III, VI and VII; whereas comparatively lower percentage of pollen sterility in types IV and V was presumably due to higher incidence of 7-1-7 separation and loss of the lagging chromosome resulting in restoration of pollen fertility to some extent.

Analysis of leaf esterase and peroxidase isozyme patterns in the 7 different trisomic types as well as control and total leaf protein profiles among the trisomics revealed differences in relative mobility, number, width and intensity of bands in different genotypes. This investigation has provided convincing biochemical evidences suggesting that the variations among the genotypes occurred at genetic level. The study of isozymes is advantageous over conventional morphological markers in genetic analysis because genotypic differences are not always expressed at the gross phenotypic level (Sidhu et al. 1984). According to Suh et al. (1977), variations in the banding pattern can usually be related to variations in gene coding for the variant proteins and trisomics with a unique profile of proteins might be more readily distinguished using electrophoresis than by identifying morphological characters. In the present investigation among the different genotypes, conspicuous variations in molecular weight and net charge of different polypeptides was indicated by their differences in relative mobility in trisomic genotypes. Compared to normal diploids number of isoesterase bands increased in all but trisomic types II and VII; while isoperoxidase bands increased only in type VII, but decreased in types II and V. Both increase and decrease in the number of protein bands have been reported in the trisomic genotypes of barley (McDaniel and Ramage 1970), Sorghum bicolor (Suh et al. 1977) and pearl millet (Sidhu et al. 1984). Addition of different chromosomes in different trisomics might be responsible for suppression as well as induction of certain protein bands (Suh et al.
Presence of conspicuous unique band/s in the isoesterase pattern of trisomic III and V in this investigation was in agreement with the novel band/s reported earlier in different trisomics of barley (McDaniel and Ramage 1970), *Sorghum bicolor* (Suh *et al.* 1977) and pearl millet (Sidhu *et al.* 1984). Allelic differences may result in novel protein bands possibly accompanied by loss of an adjacent protein (Suh *et al.* 1977). The enzymes, being polymers of subunits, may show such deviations where genetic differences between alleles involving only a single base pair may act through trisomy to specify an isozyme with electrophoretically detectable differences (Sidhu *et al.* 1984). In contrast to normal diploids, variations in the intensity of different bands was a common feature in all the seven different grass pea trisomics as reported previously in the trisomics of *Datura* (Carlson 1972, Smith and Conklin 1975), barley (McDaniel and Ramage 1970), tomato (Tanksley 1980, Fobes 1980), pearl millet (Sidhu *et al.* 1984) and *Petunia axillaris* (Pulliah and Padmaja 1998). Increased intensity of a particular band in a trisomic has been attributed to ‘dosage effect’ of a ‘structural gene’ resulting in increased production of specific isozyme and the alleles has possibly specified the product in an additive manner; while decreased intensity of certain band(s) may be due to extra doses of ‘regulatory gene(s)’ responsible for the production of higher amounts of repressor molecule. In diploid plants the activity of structural gene is generally well balanced by production of repressor gene but extra amount of structural or regulatory gene products alter the isozyme patterns in genetically unbalanced trisomic types (Sidhu *et al.*1984).

Occurrence of lower frequency of trisomic offsprings in the selfed and in reciprocally intercrossed progenies was possibly due to fewer number of viable n +1 gametes. In heterozygous state AAa or Aaa can form a normal recessive or a dominant gamete along with three n +1 gametes. The ratio of offsprings in the crosses between normal and
trisomic individuals may be 5:1 or 2:1 depending upon the viability of n +1 gametes (vide Cohn 1964). Besides reduced viability of n +1 gametes, reduction in the rate of transmission of the extra chromosome through the ovule and the pollen to less than the expected 50% has been attributed by Khush (1973) to 1) elimination of the extra chromosome during meiosis, due to lagging or misdivision or both, 2) subnormal development of 2n+1 zygotes, endosperm and embryo, 3) poor and delayed germination of 2n+1 seeds, 4) reduced vigour of 2n +1 seedlings and 5) the effect of genetic background. Frequency of trisomic plants further reduced when the pollen parent was trisomic. Higher rate of trisomy transmission through seed (female) parent than pollen (male) parent was also reported in Datura stramonium (Blakeslee and Furnham 1923), tomato (Rick and Barton 1954), barley (Tsuchiya 1967, Das and Bhowmik 1971), jute (Iyer 1968), sorghum (Liang 1979), rye grass (Meijer and Ahloowalia 1981) and in pearlmillet (Singh et al. 1984); although opposite trend was noticed in maize (McClintock 1929), tobacco (Goodspeed and Avery 1939, 1941), spinach (Tabushi 1958) and Lotus pedunculatus (Chen and Grant 1968). Low rate of transmission of extra chromosome through pollen parent has been due to slower growth of n+1 pollen tube than those of n grain (Buchholz and Blakeslee 1922). Besides, poor ability of n +1 pollen grains to germinate, delayed maturity and pollen sterility might hinder formation of 2n +1 plants (Ramage 1965). Fruit setting percentage was also reduced in 7 grass pea trisomics when used as male parent indicating reduced effectivity of n+1 pollen than n+1 ovule in fertilization.

Seed size reduced markedly in trisomics although all of the trisomic types produced both large and small sized seeds; but transmission of trisomy was most frequent in the selfed progeny of the small seeded trisomic plants, while rather few number of trisomics appeared in the progeny of relatively large seeded trisomic plants. This
observation in the present material showed similarities with the higher transmission of the extra chromosome in the small seeded trisomics of tomato (Rick and Barton 1954), barley (Tsuchiya 1960), pearl millet (Singh et al. 1984, Vari and Bhowal 1986) and sorghum (Liang 1979). Lower rate of germination as well as survivality and poor growth and vigour in trisomic plants may be ascribed to their origin from small seeds developed possibly from underdeveloped endosperm (Liang 1979). Among the 7 trisomics in grass pea under study, only type IV showed higher rate of transmission of trisomics from large and heavy seeds even when they are used as male parent in crossing with normal diploids. Similar situation was encountered in ‘tetracone’ trisomics in sorghum by Liang (1979). According to him the effect of the extra chromosome on endosperm development seemed to depend somewhat on whether the extra chromosome originates from the ovule or the pollen and if the extra chromosome comes from the ovule, the endosperm nucleus of a trisomic seed should have a chromosome number of 3n+2; if it originates from the pollen, this number should be 3n+1. This differential effect due to the origin of the extra chromosome may partially explain high rate of transmission of certain trisomics from heavy seeds even when they are used as pollen parent.

Relationship between chromosome length, trivalent formation and transmission of extra chromosome was earlier studied on maize trisomics and it was opined that larger chromosome had a greater physical chance to pair and form trivalent than the smaller chromosomes (Einset 1943). Lowest transmission rate of extra chromosome was suggested in those trisomics which involved a longer chromosome of the set (Lesley1932, Khush 1973, Khush et al.1984). Among the 7 different types of primary trisomics identified cytogenetically in the present study, transmission of trisomy was highest in type IV showing lowest frequency of frying pan configuration of trivalent, while rate of transmission was lowest in type V
exhibiting highest frequency of this configuration. Presumably, frying pan configuration had some influential role regarding transmission and longer sized chromosome being involved in frying pan configuration resulted in reduced transmission rate of the extra chromosome (through anaphasic disturbances).