INTRODUCTION

The genus *Lathyrus* belonging to the family Fabaceae, subfamily Papilionoideae, tribe Vicieae is distributed both in the New World and the Old World (Allkin *et al.* 1983). Of the nearly 200 species and subspecies of this genus, only *Lathyrus sativus* L. is widely cultivated as a food crop and commonly known as grass pea; while *Lathyrus odoratus* (sweet pea) is grown commercially as an ornamental crop due to its floral morphology and perfume (Razdan *et al.* 1980, Jackson and Yunus 1984, Ochatt *et al.* 2007). The common names of grass pea are a matter of intense discussions and have been of interest for researchers around the world for quite some time (Campbell 1997). Grass pea is now believed to have originated in the Balkan peninsula in the early Neolithic period, dated to the beginning of the 6th millennium BC (Jackson and Yunus 1984, Kislev 1989). Quoting archaeological evidences, Jackson and Yunus (1984) also reported remains of *Lathyrus sativus* at Ali Kosh (9500-7600 BC) and Tepe Sadz (7500-5700 BC) in Iran and at Jarmo in Iraqi Kurdistan (8000 BC). In India, earliest remains of *Lathyrus sativus* was recorded at Atranjikhera, a small village situated at the north bank of Kali river at Etah district of western Uttar Pradesh dated back to 2000-1500 BC and also at Chirand (1900-1400 BC) and Narmada Valley (1657-1400 BC) by Saraswat (1980). Vavilov (1951) described two separate centres of origin- central Asiatic and Abyssinian centres from which it spread towards Eurasia through Mediterranean region and to South America, East Africa and Australia. Ochatt *et al.* (2007) advocated that one group of grass pea would have originated from the Indian subcontinent and was composed mainly of genotypes with coloured flowers and seeds, the shape of which being round and wrinkled; while a second group originating from Mediterranean basin comprised mainly of white flowered genotypes with white, flat smooth and large seeds coupled
with high yield. In India this crop possibly diffused from west Asia (Chowdhury et. al 1971) and has many common names like ‘khesari’, ‘chickling vetch’, ‘lakhodi’, ‘batura’ etc. It has wild relatives in Iraq and is probably a derivative from the genetically nearest wild species L. cicera (Townsend and Guest 1974, Hopf 1986). Cultivated for more than 8000 years, grass pea is one of the oldest pulse crop (Smartt 1984). It is widely grown in different parts of the world including Bangladesh, India, Nepal, Pakistan and Ethiopia and to a limited extent in South America, northern Europe, Australia, some parts of Russia, Ukraine, China and also in south-west Asia for its seeds as dietary constituents and vegetative parts as fodder as well as green manure while its main use as a forage crop was restricted to central and south Europe and Mediterranean region (Smartt 1984, Jackson and Yunus 1984, Singh and Misra 1985, Dahiya 1985, Siddique et al. 1999, Mera et al. 2003, Rybinski 2003, Yang et al.2005). According to Milczak et al.(1997), grass pea was introduced to Poland in the 17th century, probably by Tatars and it is cultivated as a vegetable and locally known as ‘soczewica podlaska’ (podlaska lentil).

Grass pea (Lathyrus sativus L.) possessing a rare combination of many desirable properties relating to grain as well as vegetative yield is potential enough to be an important protein rich dual purpose crop. Its tolerance to severe drought, excessive flooding and fluctuations in temperature (10-40°C) coupled with its ability to grow well in almost all types of soils from coastal areas to high elevation showing resistance against insect, pest, downy and powdery mildew and the capacity to ameliorate soil fertility through high rate of biological N₂ fixation make this pulse a dependable cropper in developing and underdeveloped countries (Duke 1981, Smartt et al. 1994, Campbell et al. 1994, Robertson et al. 1996, Vaz Pato et al. 2006). It is grown in India as a
winter pulse crop and the seeds are generally sown in September-October. The plant is a vine, much branched, sub-erect, straggling or climbing herbaceous with moderately winged procumbent stem and well developed tap root system. Leaves are alternate, pinnately compound usually with two (in a jugate) opposite linear-lanceolate shaped leaflets. Often the apices of the upper leaflets are modified into tendrils. Stipules are free lateral, foliaceous, navicular in shape and semi-sagittate at base. The flowers are solitary, axillary and bright blue, reddish purple, red or white and are borne on peduncles, corolla typically papilionaceous, stamens diadelphous (9+1) with vexillary stamen free, filaments free with uniform length, anthers are elliptoid and yellow. Pods are oblong, (2.5-4.5)cm. x (0.6-1.0)cm., flat and slightly curved with 3-4 seeds, grayish brown, mosaic spotted or mottled. The crop is harvested within 4-5 months when leaves begin to turn brown because fully ripe pods often dehisce resulting in loss of seeds.

Seeds of *Lathyrus sativus* L are mainly consumed as pulse meal ('dal') and its flour is sold as 'basan' which is frequently used to adulterate pigeon pea 'dal' and chick pea 'basan' mainly in Bangladesh, Nepal and some parts of India. The young boiled, salted pods are used as snack whereas tender leaves are often consumed as green vegetables in these countries. A pan-cake like preparation of 'badi' or 'pakoda' from seed flour is sold by street vendors in Nepal (Rahman *et al.* 1974, Bharati and Neupane 1989, Yadav 1995). In Ethiopia and in Eritrea, grass pea is consumed as 'Wott', a sauce prepared from seed flour, 'nifro' (boiled grain) and 'kitta', an unleavened bread (Tekele-Haimanot *et al.* 1993). It is easy to cultivate, cheap and often used as an excellent green manure. For cattle and poultry feeding also grass pea seeds are mixed with oil cakes and salts. A new variety 'AC Greenfix' of grass pea has recently been released in U.S.A for its high potentiality as green manure, particularly in organic farming (Krause and Krause 2003).
Notwithstanding rich protein content, minimum agronomic input and wide scale use by a vast population in various purposes, cultivation of *Lathyrus sativus* has not been discouraged only but banned also in many countries because its seeds contain the neurotoxin compound β-N-oxalyl-L-α,β-diamino propionic acid (ODAP also known as BOAA) first identified by Bell (1962). Consumption of grass pea seeds over a long period of time as a sole source of food can cause an irreversible motor neuron disease Neurolathyrism resulting in paralysis of lower limbs in humans and hind legs in animals accompanied with general weakness in skeletal muscles and increased stiffness. This disease can lead to sudden death as a consequence of aorta rupture (angiolathyrism) or to a chronic crippling syndrome. The toxins may also cause metabolic disturbances through synthesis of elastic components from mesenchymal tissues and the skeletal systems which brings about disorders in the growth and development of cartilages and bones, symptomatized as ‘osteolathyrism’ (Grela et al. 2000). Since the time of Hippocrates, lathyrism was recognized among human and other vertebrates (Stockman 1932). The disease has broken out epidemically from time to time in north western region of Bangladesh (Jahan and Ahmad 1993), eastern Terai region of Nepal (Acharya and Pathak 1990), rural Ethiopia (Haimanot and Kidane 1990, Tekele et al. 1993) and some parts of Uttar Pradesh and remote tribal belt of Madhya Pradesh in India (Watt and Breyer-Brandwijk 1962, Misra et al. 1993). Dr. Arthur Kessler, an interned in the Vapniarca concentration camp, also known as ‘house of the dead’, during world war II reported an outbreak of lathyrism due to inclusion of high proportion of grass pea in food items consumed by the jailed inmates (Radovici 1945).

Not only seeds but also leaf extracts of grass pea was reported to be cytotoxic on living cells due to the presence of the active neurotoxic
principle ODAP. Various types of mitotic and meiotic abnormalities were recorded in *Vicia faba* treated with leaf extracts of *L. sativus* (Raj and Reddy 1971). Neurotoxin content was fixed for low from 0.1- 0.4% (Ramanujam *et al.* 1980), obviously lower seed neurotoxin content within this limit is, most desired in grass pea. Need for development of improved lines with low seed neurotoxin (ODAP) content has been of prime importance and most urgent in grass pea but compared to other pulse crops, it was neglected by geneticists and breeders for decades experiencing only a little evolutionary progress as a pulse crop although it was cultivated for its grain for more than 8000 years. Its dual use as grain as well as forage yielding crop attracts counter veiling selection pressure which might have cancelled each other out and maintained the status quo of the crop over this long time period (Smartt 1984).

Increased yield has been one of the important criteria for selection in most of the crop improvement programmes. In India, grass pea occupies about 4% of total pulse crops constituting about 0.3% of the total pulse production and being cultivated in nearly 1.6 million hectares it produces about 0.5 million metric tons of seeds. At an average seed rate of 40 Kg/ha it yields about 925 Kg/ha of pulse and 0.5 metric tons of straw (FAO 2002). Desired improvement in its yield has not been achieved through conventional breeding methods possibly having narrow range of variation due to self-pollination and interspecific incompatibility (Senn 1938, Liener 1967). Moreover the features like prostrate habit, indeterminate growth, late maturity, pod shattering and of course seed neurotoxin are rather undesirable for broader introduction of grass pea as grain crop in different environmental conditions and these were mentioned as limiting factors by Rybinski (2003). However, informations about research activities for improvement of seed yield in this crop were available first time during 1966 in India (Lal *et al.* 1985), 1967 in Canada (Campbell and Briggs 1987), 1980 in Bangladesh (Kaul and Islam 1981)
and 1986 in Nepal (Yadav and Prasad 1993). Development of few germplasm lines with low seed ODAP content through conventional methods has also been reported (Lal et al. 1985, Campbell and Briggs 1987). Biomass yield of *Lathyrus sativus*, on the other hand, has started to receive attention in breeding programmes only during the past few years although as a source of forage, fodder and straw it has huge potential in south Asian region (Campbell 1997). To coordinate the research work on grass pea world wide, three international networks namely, INILSEL (now ILLRA- International Lathyrus and Lathyris Research Association) based in France, ICARDA in Syria and CLIMA, Australia were established providing valuable data on descriptors for collection, germplasm evaluation and its improvement.

Endeavour to explore the possibility of improvement through biotechnological approaches has been initiated recently only in the end of 20th century (Sinha et al. 1983, Sukanya et al. 1993, Van Dorrestein et al. 1998, McCutchan et al. 1999, Durieu and Ochatt 2000, McCutchan 2003, Barik et al. 2004). Isolation of germplasm with low seed ODAP content by exploiting somaclonal variations through in vitro regeneration was accomplished by Santha and Mehta (2001). Successful development of somaclone BioL 212 or BioL12 (Ratan) with high seed and biomass yield vis-à-vis extreme reduction in ODAP content has been reported. It was characterized phenotypically and biochemically at the molecular level and released as the variety BioL212 (Ratan) for the cultivation in the north eastern plains and central zones of India.


Inheritance and linked association of different stable marker phenotypes are important aspects in genetical research which can provide adequate informations about the linkage groups and pattern of transmission of different traits including seed ODAP content in grass pea. Beside morphological markers, different molecular and isozyme markers have been used recently to construct linkage maps and to detect the locus/loci associated with disease resistance in grass pea.

Compositions of isozyme and seed storage proteins are genotype specific and are not likely to be affected by environmental and seasonal fluctuations (Larsen 1967). Genotype specific band has paramount importance as stable biochemical markers for identification and maintenance of genetic purity (Ladizinsky and Adler 1975, Osborn 1988, Mohanty et al. 2001, Naik and Kole 2002). Being easily detectable and less expensive these reliable parameters may be used conveniently for accurate identification and characterization of different genotypes. Isozymes may be considered as useful genetic and biochemical markers as well as good estimators of genetic variability in grass pea (Tadesse and Bekele 2001). These aspects are rather less explored in grass pea (Chandna and Matta 1997, Roy et al. 2001, 2004).

Grass pea is an ideal material for karyological observations having relatively low number (2n=14), medium-size and good stainability of its chromosomes. Being annual and self-pollinated it is rather convenient for cytogenetic investigations. A trisomic plant (2n=15) identified in the post-irradiated M$_2$ progeny of grass pea has been of adequate cytogenetic importance (Biswas 1998, Biswas and Biswas 2002, 2004).

Considering all these perspectives, limitations and prospects in the present investigation endeavour was made to create variations through induction of mutation by gamma ray irradiation in grass pea (*Lathyrus sativus* L.). The mutants were screened and isolated from control mother variety BioR-231 and characterized phenotypically as well as
electrophoretically by analyzing seed protein banding profiles. Tracing mode of inheritance, genetic background of induced mutations was ascertained and linked association as and when detected was estimated. Performance of yield attributes and their relationships were evaluated in the advanced generations of 15 different stable mutant lines by estimating variability, heritability, genetic advance and coefficient of correlation. Neurotoxin content was also determined by analyzing percentage of the amino acid ODAP (β-N-oxalyl-L-α,β-diamino propionic acid) in the seeds of mother strain and different mutant lines. Raising selfed progenies successively in the following generations of the trisomic stock, seven different types of trisomics were identified by their marker phenotypes at the very seedling stage as well as at the post-harvest stage by unique modifications in seed coat colour and characterized cytogenetically. Variations in leaf protein and isozymes banding profiles of the trisomic types were also studied. Some of these observations have already been published in different scientific journals.