ISSN : 0974 - 7532 Volume 8 Issue 9

Research & Reviews in

BioSciences

Review

RRBS, 8(9), 2014 [325-336]

Genetic diversity in *Lathyrus sativus* L. germplasm

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ABSTRACT

Grass pea (*Lathyrus sativus L.*) is food, feed and fodder crop. The total acreage of grass pea is estimated at 1.50 million ha with annual production of 1.20 million ton. *Lathyrus sativus* has extensive tolerance of drought, water-logging and poor semiarid soils; resistance to insects and pests. The crop originated in the Balkan Peninsula. It favours self-pollination. However, there have been indications that some out crossing occurs in the species (from 9.8 to 27.8%). The seeds of *L. sativus* provide a source of protein and carbohydrate that are able to sustain life during periods of famine when other food is unavailable. However, the presence of neuro-toxic ODAP in the different parts of the plant is limiting the use of this crop. A study of genetic diversity and its relation to geographical diversity may contribute information about the center of diversity and origin of domestication of a cultivated crop. The genetic variation can be exploited in breeding programmes aimed at crop improvement. Vast arrays of analyses are used worldwide to estimate genetic variability. *L. sativus* shows great morphological variation, especially in vegetative characters such as leaf length, while floral characters are much less variable. *L. sativus* ecotypes are classified on the basis of flower color, marking on pods, and size and color of seeds, which in many cases is connected with their geographical distribution. These characteristics, as well as yield and also nutritional traits of seeds have been estimated to describe the great variability of accessions of both, *L. sativus* and *L. cicera*. Cytological investigations have shown that the basic chromosome number of x = 7 is constant throughout the genus and that most of the species are diploid, with polyploidy as rare exceptions. Despite this stability in chromosome number, large variations in chromosome size have played an important role in the evolution of *Lathyrus* species. SDS-PAGE analysis of reduced seed globulins of *lathyrus* species showed intra-specific variation due to individual variation and/or differences among accessions. It has been repotted that SDS-PAGE of albumins and globulins of different grass pea even of the same geographic origin, have variation in number, width and intensity of bands, concluding that geographical origin does not influence specific seed protein contents and its polymorphism. In grass pea, the literature on both genetic diversity and intra and inter-specific-relationships among collections based on the electrophoretic analysis of isoenzyme is quite poor. Molecular markers including AFLPs, RFLP, RAPD, ISSR and EST-SSRs have proved to be useful for assessing genetic relationships, taxonomic and phylogenetic relationships within and between the sections and the species of the genus *Lathyrus*.

INTRODUCTION

Grass pea (*Lathyrus sativus L.*) is a food, feed and fodder crop belonging to the family Leguminosae (= Fabaceae), subfamily *Papilionoideae*, tribe *Vicieae*. It is placed in the section *Lathyrus* along with 33 other species[1].

Lathyrus sativus is an annual leguminous crop cultivated throughout the arid regions of the Near East, North Africa, West Asia, and Indian subcontinent, China, and grown on a small scale in South America, Canada and Middle East for animal or human consumption[2,3]. The total acreage of grass pea is estimated at 1.50 million ha with annual production of 1.20 million ton, with

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0.92 million ha in South Asia and 0.63 million ha in Sub-Saharan Africa[4]. Its area has significantly decreased in India and Nepal, Following the ban of its cultivation by governments[4].

L. sativus has a number of advantageous biological and agronomic characters, namely extensive tolerance of drought, water-logging and poor semiarid soils; resistance to insects and pests; nitrogen fixation; high grain-yielding capacity and high protein content of its seed[5]. This has made it a popular crop in subsistence farming in certain developing countries that have extreme weather conditions[6].

It is grown mainly for food in India, Bangladesh, Nepal, Pakistan and Ethiopia, and for feed and fodder in other countries[7]. Thousands of breeding lines are developed yearly in *Lathyrus sativus* L. hybridization programmes over the world. The developing of these breeding lines increased genetic uniformity in the frame of *Lathyrus sativus* L. Therefore, the genetic basis of these released cultivars is rather narrow. To widen the genetic basis of these cultivars, we must introduce new sources of genetic variation. To do this, criteria for parental stock selection need to be considered not only by agronomic value, but also from the point of view of their genetic dissimilarity. Therefore, the evaluation of genetic variation in *Lathyrus sativus* L. is a very important task not only for population genetics but also for plant breeders. The study of genetic variation has fallen within population genetics which has focused on analyzing, measuring and partitioning genetic. The genetic variation can be analyzed by agronomic and biochemical traits, and molecular marker polymorphisms. Its analysis enables estimation of the mating system and monitoring of genetic changes caused by factors affecting the reproductive biology of a species. Utilization of exotic germplasm for characteristics such as disease resistance or agronomic traits is the ultimate goal of assessing genetic diversity in plant crops including *Lathyrus sativus* L.

ORIGIN AND GEOGRAPHICAL DISTRIBUTION

It has been reported by several authors that the origin of *L.* sativus was unknown as it was thought that the natural distribution had been completely obscured by cultivation, even in southwest and central Asia, its presumed centre of origin[8]. However, it is now suggested that the crop originated in the Balkan Peninsula. There are reports of wild *L. sativus* in Iraq[9] but it is not clear if these are indeed wild or escapes from cultivation. As reported by Jackson and Yunus[10], some of the earliest archaeological evidence comes from Jarmo, in Iraqi Kurdistan, dated at 8000 BC. Remains of *Lathyrus* species have been found at Ali Kosh (9500-7600 BC) and TepeSadz (7500-5700 BC) in Iran and are among the most common foods recorded at these sites[10]. At Azmaska Moghila, in Bulgaria, remains dated at ca. 7000 BC have been tentatively identified as *L. cicera*[11]. Remains of *L. sativus* also have been reported in India dating back to 2000-1500 BC by Saraswat[12] who indicated the possibility of diffusion of the crop from West Asia. Vavilov[13] described two separate centers of origin of the crop. One was the Central Asiatic centre which includes northwest India, Afghanistan, the Republics of Tajikistan and Uzbekistan and western Tian-Shan. The second was the Abyssinian centre. In addition, Vavilov noted trends in diversity similar to those found in other pulses, such as lentils and broad beans, in that smaller-seeded forms were found in southern and south west Asia, whereas around the Mediterranean region, almost all were highly cultivated forms with large white seeds and flowers[10].

Chowdhury and Slinkard[14] suggested that the Near East and North Africa regions included the most variability for isozyme systems, which can indicate the grass pea area of origin.

All grass pea lines appear to divide into two geographical groups: one group derives from the Indian subcontinent and another from the Mediterranean/European region, which typically has higher yields and larger seeds[15]. There are now widely distributed throughout Eurasia, North America, temperate South America and East Africa with a small amount being cultivated in Australia[16].

BREEDING SYSTEM

The floral biology of *L. sativus* is such that it favours self-pollination. However, there have been indications that some out crossing occurs in the species, which is dependent on environmental or genetical factors. The extent of natural out crossing that can occur in *L. sativus* has been a concern of several plant breeders over the

past 10 years. Rahman *et al*.[17], in a study using four flower colours for which the genetics were known, found out crossing from 9.8 to 27.8%. This was determined by planting red, white and pink (all recessive to blue) flowered lines and then surrounding them with a blue-flowered line, the blue flower being dominant. Evaluation of the flower colour of individual plants was used to compute the out crossing that occurred between lines based on natural pollinating mechanisms. It did not attempt to determine the amount of pollination that occurred within the genotypes.

It is not known if wind or insects are the major vector in the transfer of pollen, which can rapidly increase the heterogeneity in different populations.

Male sterility has been reported in many plant species but has only been reported to a limited amount in *L. sativus*. The first report of male sterility was by Srivastiva and Somayajulu[18], in which they found that some plants had reduced stamens and the anthers did not produce pollen. No seed set was observed on selfing these plants although open-pollination gave good seed set. Quader[19], in a study involving 40 sterile plants and 40 pollinator lines, found that 26 pollinator lines produced sterile plants.

SEED AND ODAP CHARACTERISTICS

The seed of *L. sativus* provide a source of protein and carbohydrate that are able to sustain life during periods of famine when other food is unavailable[20]. In fact, these seeds have good protein content (relatively rich in lysine) and a high level of polyunsaturated fatty acids[21]. Seeds contain 18.2-34.6%, 0.6% fat, 58.2% carbohydrate (about 35% starch)[22,23]. The seeds also contain 1.5% sucrose, 6.8% pentosans, 3.6% phytin, 1.5% lignin, 6.69% albumin, 1.5% prolamine, 13.3% globulin, and 3.8% glutelin. The essential amino acids are (in mg per 16 grams of nitrogen): arginine 7.85, histidine 2.51, leucine 6.57, iso leucine 6.59, lysine 6.94, methionine 0.38, phenylalanine 4.14, threonine 2.34, tryptophane 0.40, and valine 4.68 (like other cool season food legumes, grass pea are deficient in methionine and tryptophane)[23].

The harmful potential of grass pea dependency was known to ancient Hindus and to Hippocrates (460–377 BC)[24]. Physicians from ancient Greece also knew of the disease and warned against the danger of eating grass pea[25]. Centuries later, in 1671, Duke George of Wurttemberg banned consumption of *Lathyrus* flour in his principality because of its ability paralyze the legs, an edict that was subsequently twice enforced by his successor Leopold in 1705 and 1714[26]. Cantani[27] coined the name latirismo (lathyrism) to describe the disease. Outbreaks of neurolathyrism occurred throughout Europe, northern Africa, the Middle East, Afganisthan, and India during the 18th, 19th and 20th centuries. The Lathyrism problem is arising from the over-consumption of ODAP neurotoxin[28]. In particular, B-diamino-propionic acid (B-ODAP), neurotoxic secondary metabolite, is a non-protein amino acid which causes neurolathyrism; this pathology appears when this molecule is ingested in large quantities over a three-to-four month's period[29].

Environmental factors such as drought, zinc deficiency, iron oversupply and the presence of heavy metals in the soil can considerably increase the level of β-ODAP in the seeds grown in farmers' fields as compared to more optimal experimental fields[30]. Flower and seed coat colour could be useful genetic markers for identifying lines with low neurotoxin content[31]. Dahiya[32] reported that genotypes with light cream colour seed contained low neurotoxin content. But Quader *et al*.[31] reported that white-flowered plants had increased toxin compared with blue-flowered plants.

GENETIC DIVERSITY

Genetic variation is defined as the variation of individual genotypes within and among species. It is important trait for long term survival of species and enables a population to adapt to new conditions brought by environmental change[33]. Genetic diversity plays a very important role in survival and adaptability of a species because when specie's environment changes, slight gene variations are necessary to produce changes in the organisms' anatomy that enables it to adapt and survive. A species that has a large degree of genetic diversity among its population will have more variations from which to choose the fit alleles. Increase in genetic diversity is also essential for a species to evolve. Species that have very little genetic variation are at a great risk. With very little gene variation within the species, healthy reproduction becomes increasingly difficult, and offspring often deal with similar problems to those of

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inbreeding.

It is revealed that plant species with different breeding systems, seed dispersal mechanisms, geographic ranges and life forms tended to maintain different mean levels of genetic diversity within their populations[34]. Interspecific comparisons between species demonstrated that genetic distance statistics were generally predictive of phylogenetic relationships. For example, progenitor-derived species pairs tended to be more genetically distinct than populations within species but less genetically distinct than well-defined congeners[35]. Hamrick[36] and Loveless and Hamrick[37] used several life history and ecological traits to determine whether interpopulation genetic heterogeneity was related to the species' characteristics. They found that life form, geographic range, breeding system and taxonomic status had significant effects on the partitioning of genetic diversity within and among plant populations.

A study of genetic diversity and its relation to geographical diversity may contribute information about the center of diversity and origin of domestication of a cultivated crop. Issues, like whether or not genetic variation is being lost with progressive domestication or how the variation is distributed among populations, can be addressed by a study of genetic diversity[38].

A major goal of genetic resource conservation is to conserve as wide a representation as possible of the array of extant genetic variations of target taxa[39]. This is irrespective of the relative frequency of any gene or linked gene complex in germplasm. Satisfying this objective is dependent in part on the efficiency of selection of species and location for the sampling of the genetic diversity. Most species display a complex of genetic variations along their range of distribution. For landraces, this is a function of species characteristics, such as breeding system, migration and dispersal mechanisms, which determine the movement of genes among populations[40]; biotic pressure, for example, competition, predation and local anthropogenic influence and biotic selection intensities determined by location[39].

Genetic conservation strategies are initially concerned with understanding of the genetic variation within species and then by the geographical distribution of genetic variation. Such a study will increase sampling efficiency for meeting genetic resource management[39].

Estimation of genetic variability is based on morphological, cytological, biochemical and molecular traits. However, the estimation of genetic variability based on morphological and cytological traits has the disadvantages of being influenced by both environmental and genetic factors and may therefore not provide an accurate measure[41].

Genetic diversity based on morphological traits

Morphological variation in population has been described for characters controlled by a single or multiple gene systems. The greater of gene loci number that determine a trait, the more continuous the variation will be. The expression of quantitative traits is influenced by the environment and the variation pattern in these traits is generally considered to be the result of both genetic and environment attributes.

Highly heritable morphological traits such as leaf color, flower color, seed color, and seed size were among the earliest genetic markers used in scientific investigations and are still in use in germplasm management[42]. *L. sativus* shows great morphological variation, especially in vegetative characters such as leaf length, while floral characters are much less variable[10].

L. sativus ecotypes are classified on the basis of flower color, marking on pods, and size and color of seeds, which in many cases is connected with their geographical distribution. These characteristics, as well as yield and also nutritional traits of seeds have been estimated to describe the great variability of accessions of both, *L. sativus* and *L. cicera*[43].

L. sativus is a highly variable species in terms of seed weights and flowers color[10]. Seed weight heritability estimates have been reported[44]. The feature that differentiated the accession lines under study the most was seed size. Large-seed forms were typical of the Mediterranean region (Italy and Spain), medium-seed for the lines from northern France and Germany, and the smallest seed was characteristic of the Polish cultivars. The weight of 100 seeds of some of the large seeded Italian lines exceeded 40 g, and the value of that trait in the Polish cultivars did not exceed 15 g[45]. Seed weight and total seed protein have shown positive correlation in *L.sativus*[46]. The environment exerts a strong effect on the mean seed weight of grass pea, and the effect is stronger as seed weight increases. The outsized variance of extra-large-seeded parents contributes powerfully in dropping the estimates of the genotypic variance and heritability[47].

Sammour *et al.*[48] evaluated sixty-six accessions

representing eighteen species of the genus *Lathyrus* collected from different geographic regions for variations of quality traits (100 seeds weight, ash, total seed proteins and 3-(-N-oxayl)-L-2,3 diaminopropoinc acid – ODAP contents). High variability of ODAP levels was exhibited at both inter-specific and intra-specific levels. Sammour *et al*.[49] evaluated eighteen grass pea (*Lathyrus sativus* L.) accessions (donated from USDA germplasm) collected from different geographical regions for variations of seed weight, and seed protein content. They found that environmental factors may not be most appropriate for explaining variations in seed weight and inferred that Eastern Africa sub-region is possibly a new center of origin of *L. sativus* due to the presence of small-seeded accessions. The data also revealed that there were no correlations between protein content and seed weight.

Sedehi *et al*.[50] evaluated morphological traits of the grass pea landraces. Analysis of variance indicated highly significant differences among 20 grass pea landraces for the morphological traits.

Lioi *et al*.[51] assessed genetic relationships among 13 grasspea (Lathyrus sativus L.) landraces mainly collected in Southern Italy using agronomic traits. The agronomic data obtained provided useful information for the choice of the best grasspea landraces for southern Italian marginal areas.

Barika et al.[52] analyzed eleven accessions of grass pea (*L. sativus* L.) for seed storage proteins, 100 seeds weight and protein content to measure genetic variation. Frequency distribution of polypeptide bands in *L. sativus* L. has not shown clear correlation with seed characteristics (seed weight, seed protein content) of the studied accessions.

Genetic diversity based on karyological traits

Cytological investigations have shown that the basic chromosome number of x = 7 is constant throughout the genus and that most of the species are diploid, with polyploidy as rare exceptions[53,54]. Despite this stability in chromosome number, large variations in chromosome size have played an important role in the evolution of *Lathyrus* species which are associated with a fourfold variation in 2C nuclear DNA amount[55-57].

Many karyotypic studies have been performed on Old World members of *Lathyrus*, but there is a paucity of data for American species, with the karyotypes of only five South American entities described so far[58]. From the available information, a number of conflicting observations have arisen. Some authors claim that, in addition to the numerical constancy, *Lathyrus* species display morphological uniformity of chromosomes and homogeneous karyotype arrangement[58,59]. However, others have found enough interspecific karyotype differences to allow Species characterization[60]. Such discrepancy was also observed at the infraspecific level, mainly in the widely studied *L. odoratus L.* and *L. sativus L.*[61].

B chromosomes are additional passengers found in the karyotypes of about 15% of eukaryote species. They are best understood as genome parasites exploiting the host genome because of their transmission advantage, and are frequently deleterious for the organism carrying them[62-63]. The significance of B chromosomes is to be found in their wide spread occurrence in hundreds of flowering plants, and also in gymnosperms and in some lower forms such as ferns, bryophytes and fungi (they are also common in animals, including mammals)[64]. Owing to their particular properties, B chromosomes have been used to elucidate the function of post-translational histone modifications, such as histone H3 phosphorylation[65] and methylation[66-68].

L. sativus has satellites chromosomes in some accessions. Satellite numbers varied between 1 to 2 pairs.[54].

Genetic diversity based on biochemical traits

A. Proteins (SDS-PAGE)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDSPAGE) is the most widely used analytical method to resolve separate components of a protein mixture. It is almost obligatory to assess the purity of a protein through an electrophoretic method. SDS-PAGE simultaneously exploits differences in molecular size to resolve proteins differing by as little as 1% in their electrophoretic mobility through the gel matrix. The technique is also a powerful tool for estimating the molecular weights of proteins[69-73]. The success of SDS-PAGE as an indispensable tool in protein analysis has been attributed to three innovations that permitted the correlation of electrophoretic mobility with a protein's molecular mass[74-76].

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed storage proteins has proven a simple and effective method for distinguishing

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among cultivars of the largely cross-fertilized pasture grasses and legumes despite their high innate genetic variability[77]. Similar techniques have been used very extensively for cultivar identification in breeding crops but to a lesser extent for the differentiation of cultivars of out breeding species[78-80]. Seed protein electrophoresis has also become a useful tool in evolutionary studies to determine species relationships. The seed protein profiles reflect genetic affinities within a taxon and even between different biological entities[81].

The validity of seed protein electrophoresis apart from morphological traits as a powerful tool for cultivar identification, solving taxonomic and evolutionary problems and studying genetic diversity[46-66].

SDS-PAGE (Sodium Dodecyl Sulphate Polyacrilamide Gel Electrophoresis) have provided valid evidence for detecting intraspecific variation and assessing interspecific relationships[82]. Many studies based on the electrophoretic analysis of seed proteins have been used to examine genetic variability and systematic problems in several legumes such as the genus Astragalus[83], genus *Lupin*[84], genus *Pisum*[85], genus *Lathyrus*[86,87], genus *Onobrychis*[86], genus *Phaseolus*[88] and genus *Vicia*[86].

Sodium dodecyl sulphate-polyacrylamide gel electrophoresis of reduced seed globulins covered *Lathyrus sativus, L. amphicarpos, L. blepharicarpus, L. cicera, L. gorgoni, L. marmoratus, L. pseudocicera and L. stenophyllus*. This study showed intraspecific variation due to individual variation and/or differences among accessions and species showed to be distantly related taxa except for the rather closely allied L. cicera and L. marmoratus[89,90].

A few groups have studied in genotype specificity, inter-specific variation, and genetic diversity in relation to geographical origin among accession of *L.sativa* by means of seed storage protein; globulins, albumin, total seed proteins[89-91]. They showed that SDS-PAGE of albumins and globulins of different grass pea even of the same geographic origin, have variation in number, width and intensity of bands, concluding that geographical origin does not influence specific seed protein contents and its polymorphism. Sammour *et al*.[48] evaluated eighteen grass pea (*Lathyrus sativus* L.) accessions (donated from USDA germplasm) collected from different geographical regions for variations of seed proteins analysis. Multivariate analysis (cluster and factor analysis) based on protein analysis data showed a high genetic variability among the accessions of different geographical regions and a low variability among the accessions of the same region.

B. Isozymes

Isozymes were defined as structurally different molecular forms of an enzyme with, qualitatively, the same catalytic function. Isozynes originate through amino acid alteration, which cause changes in net charge, or the spatial structure (conformation) of the enzyme molecules and also, therefore, their electrophoretic mobility. After specific staining, the isozyme profile of individual samples can be observed[79,92,93].

Isozyme analysis is quick and effective method for the determination of genetic diversity[94,95]. Isozymes are used as genetic markers to observe the recombination and segregation of linked qualitative and quantitative characteristics[96-98].

The electrophoretic analysis of isoenzyme variation has proved to be particularly useful in defining more precisely the size and structure of genetic diversity in the gene pools of different grain legumes[99]. In grass pea, the literature on both genetic diversity and intra and interspecific-relationships among collections is quite poor[100,101].

Some work has been published about the variability affecting *L. sativus*[102,103].

Yunus and Jackson.[104] also observed the absence of correlation of isozymes with morphological data in grasspea. The absence of correlation between markers indicates that there is no one best marker that can be used for diversity study. Hence, it is important to study diversity by using both morphological and molecular markers. Similar results have been reported by many authors in other crops[105,106].

Talukdar[107] and Sammour *et al*.[108] investigated genetic basis of different leaf esterase and root peroxidase isozymes by analyzing their zymogram phenotypes in selfed and intercrossed progenies of two local varieties (used as control) and three induced true breeding *dwarf mutant* lines of grass pea (*Lathyrus sativus* L.). Two non-allelic genes, *df1/df2* and *df3* controlling dwarfism in grass pea were included in the present linkage studies with different isozyme loci. The dwarf mutants could be distinguished from one another and also from control varieties by the presence of unique allozyme/s coded by allele/s in different loci.

Genetic diversity based on molecular traits

During last decades, molecular markers have proven to be powerful tools for assessing genetic variation within and among populations of plants. Several criteria should be considered in choosing molecular techniques for genetic diversity studies including the following: whether the techniques are highly reproducible between laboratories and whether the data that is generated can be reliably transferred; whether markers are dominant or codominant, allowing homozygotes and heterozygotes to be distinguished; the amount of genomic sequence information required; and whether the markers detect highly polymorphic loci[109-111]. At present, various molecular techniques are available for assessing genetic diversity in plants including identification of amplified fragment length polymorphism (AFLP), restricted fragment length polymorphism (RELP), internal transcribed spacer (ITS-1), random amplified polymorphic DNA (RAPD) and microsatellites or inter simple sequence repeat (ISSR).

A. AFLP

Amplified Fragment Length Polymorphisms (AFLPs) detect polymorphism at a great number of loci, require no prior sequence knowledge and have proved to be useful for assessing genetic relationships among grasspea landraces[112]. Amplified Fragment Length Polymorphism (AFLPs), along with the morphologic characters were used to clarify the taxonomic and phylogenetic relationships within and between the sections and the species of the genus *Lathyrus*[113,114].

Lioi *et al.*[51] assessed genetic relationships among 13 grasspea (*Lathyrus sativus* L.) landraces mainly collected in Southern Italy using AFLP markers. AFLP markers provided useful information on genetic variation and relationships among landraces. Even though the number of polymorphic fragments detected by AFLP technique was low, it was sufficient to discriminate all the accessions.

B. RELP

At section level, Badr *et al.*[63] and Sammour & Shanshoury[115] examined systematic relationships in *Lathyrus* sect using RFLP and note that all trees clearly show a close relationship among accession of the same species, confirming the monophyly of species examined.

Chtourou-Ghorbel *et al.*[116] and Sammour[117] assessed the genetic diversity of five Lathyrus species belonging to the Sect. Lathyrus and Clymenum. Results indicated that L. sativus is more closely related to L. cicera. This relationship supports studies of morphological variation which identified L. cicera as closely related to L. sativus. These two species may be a result of hybridization or common ancestry. Some interspecific crosses between the two have been successful.

C. RAPD

Randomly Amplified Polymorphic DNA markers (RAPDs) have been widely applied to investigate population genetic structures, diversities and distances in plant taxa[118-120], despite having some restrictions - e.g. a dominant nature which makes it impossible to distinguish homozygote and heterozygote genotypes at individual loci. In highly inbred species e.g. grass peas the dominance effect of RAPD markers is minimal, and monolocus approaches for RAPD data are generally considered appropriate for measuring the genetic structure of populations[39].

Random amplified polymorphic DNA (RAPD) is a PCR-based technique which provides a virtually unlimited number of anonymous DNA markers[121,122] As such, it has been promoted as an alternative technology to allozymes and RFLPs. The RAPD markers have found application in many fields including assessment of genetic diversity, linkage mapping[123], systematics[124] and estimation of population genetic parameters[125-127].

RAPD analysis is a quick and easy technique for examining genetic relationships; however, for estimating interspecific relationships it has been observed that RAPD analysis may be less reliable than RFLP analysis due to the possibility of non-homology of RAPD products scored as identical[128]. However, results from the analysis of the genus *Lathyrus* using RAPD analysis concurred with variations in morphological characters, as has been observed in other species[129,130], thus it appears that RAPD analysis may be used successfully in conjunction with othermore traditional methods to study the evolutionary relationships within the genus Lathyrus and also to assist in classifying L. sativus germplasm[131,132].

Chtourou-Ghorbel *et al.*[116] concluded that RAPDs are equivalent to RFLPs in assessing the genetic diversity of five Lathyrus species belonging to the Sect. *Lathyrus and Clymenum*, in addition to their simplicity and low costs.

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Nosrati *et al.*[133] assessed the relationship between genetic similarity and larger geographical distance among five accessions of selfing legume *Lathyrus sativus* L. (grass peas, Fabaceae) using RAPDs by including 10 randomly selected individuals from each accession. Five primers produced 73 clear, reproducible and scorable polymorphic bands. The percentage polymorphic bands ranged from 20.6% in German to 60.3% in Polish accessions. The range of Nei's within-accession genetic diversity was wide, ranging from 0.075 in German to 0.25 in Polish accessions. Partitioning of total genetic diversity by AMOVA indicated 76.44% genetic diversity among accessions and 23.56% within accessions, indicating that *L. sativus* is a selfing species. The shortest genetic distance was detected between German and Iranian accessions (0.202), while the greatest genetic distance was revealed between Iranian and Polish accessions (0.5102), indicating that in selfing species genetic similarity among accessions is not correlated with geographical distance.

Barika et *al.*[52] applied randomly amplified polymorphic DNA (RAPD) technique to assess the genetic variability among five selected genotypes of grasspea. A total of 257 loci were amplified of which 159 were polymorphic including 57 genotype-specific unique bands. Majority amplicons were shared by most of the genotypes which indicated a very narrow genetic gap between them. The investigation showed that though all the genotypes of grasspea were of apparently similar morphology there exists polymorphism at the molecular level, which can be exploited in breeding programmes aimed at crop improvement.

Sedehi *et al.*[50] (2008) evaluated the genetic diversity of 20 grass pea landraces from various locations in Iran using 32 RAPD and ISJ primers. Average of polymorphism percentage of RAPD primers was 73.9%.among used primers, 12 random primers showed polymorphism.

D. ISSR

Among molecular markers, the inter simple sequence repeats (ISSRs) have been successfully applied in many crop species[134,135]. To date, fewer than 40 microsatellite (simple sequence repeat [SSR]) markers have been published for grasspea, and only 17 of them were characterized for size polymorphism[136].

Belaid *et al*[137] have used the ISSR markers as tools for assessing genetic variation and determining the relationships among different populations from a wide range of geographical origins, representing *L. sativus,L. cicera and L. ochrus* of the genus *Lathyrus*. The data provide evidence of a large genetic diversity among and within the tested populations.

Lioi *et al.*[51] assessed genetic relationships among 13 grasspea (*Lathyrus sativus* L.) landraces mainly collected in Southern Italy using SSR markers. SSR markers provided useful information on genetic variation and relationships among landraces. The use of SSR to detect polymorphic sites in grasspea showed that most landraces were clearly grouped in two sub-clusters. One comprised two landraces from most northern localities, while all the other landraces were clustered together at a very narrow genetic distance.

D. EST-SSR

Shiferaw *et al.*[138] evaluated genetic variation among and within the populations 320 genotypes of Ethiopian grass pea collected from different geographical regions of Ethiopia, by 21 Expressed sequence tagged (EST), and 19 EST-SSR markers. Out of the 21 STS markers 11 gave RAPD-like profiles, and 10 gave monomorphic bands which were converted to CAPS markers. From the total markers analyzed 7 RAPD-like, 6 CAPS and 8 EST-SSRs showed polymorphism among and within accessions.

Sun et al.[139] characterized 24 grasspea accessions from worldwide sources for size polymorphism using three hundred EST–simple sequence repeat (SSR) primer pairs and loci. Among them 139 SSR loci produced no PCR product, 117 SSR loci were monomorphic, and 44 SSR loci were polymorphic. The mean number of alleles per locus ranged from two to 11. The observed heterozygosity and expected heterozygosity ranged from 0.000 to 1.000 and 0.042 to 0.836, respectively.

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