ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

ASSESSMENT OF DIVERSITY, MORPHOLOGICAL VARIATION AND DESCRIPTION OF GRASSPEA (*LATHYRUS SATIVUS*) AND OTHER RELATED SPECIES

A Thesis submitted in partial fulfillment of the requirements for the Degree of Master of Science in Biology (Botanical Science)

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ABSTRACT

Lathyrus sativus (grasspea) has been widely cultivated in South Asia and Ethiopia for over 2500 years and is used as a food and feed. It is rich in protein content, around 30 g/100 g edible seeds. Agronomically, the species is able to withstand both severe drought as well as water logging. Although seeds of grasspea are tasty and protein rich, excessive consumption of the seeds causes a motor neuron disease called neurolathyrism which is characterized by the paralysis of the lower limbs. The neurotoxic causal agent of this disease is believed to be a non protein aminoacid called Oxalyl Di aminopropionic Acid (ODAP).

Morphological marker analysis and molecular analysis have been used widely to estimate genetic variability of populations. These methods have useful in addressing questions on population genetic structure and genetic conservation. Knowledge of genetic diversity of species is particularly important, since modern breeding practices have narrowed the genetic diversity of cultivated crops. In the case of grasspea, the problem of Lathyrism is leading to the banning of its production which in turn aggravates genetic erosion and loss of diversity of the crop.

Fifty one grasspea accessions which were selected from the genebank collection of International Livestock Research Institute (ILRI) were evaluated and characterized for different qualitative and quantitative morphological characters. Cluster analysis was performed to estimate differences between accessions. Randomly Amplified Polymorphic DNA (RAPD) was also used to study the nature of variation. In addition to *L. sativus,* three other species of the genus (*L.cicera*, *L. clymenum* and *L. ochrus*) and seventeen unidentified populations of *Lathyrus* were also evaluated for morphological and biochemical characters and characterized accordingly. Cluster analysis of both the morphological and the RAPD data showed that all of the unidentified *Lathyrus* populations were found to be *L. sativus*. The result also showed that two of the accessions (5295 and 5296) represented by *L. ochrus* and one accession (5282) represented by *L. cicera* were found to be *L. sativus*. The results would suggest that germplasm evaluation is important for proper characterization of populations.

Key words: *Lathyrus sativus*, grasspea, genetic diversity, morphological characters, RAPD, cluster analysis

1. Introduction

Humanity relies on a diverse range of cultivated species; at least 7000 such species are used for a variety of purposes (Robert and Wieneke, 1996). It is often stated that only a few staple crops produce the majority of the food supply. This might be correct but the important contribution of many species with modest amount should not be underestimated. Agricultural research has traditionally focused on few staples, while relatively little attention has been given to minor (underutilized or neglected) crops (Siddique *et al*., 1999). Such crops have, therefore, generally failed to attract significant research funding. Unlike most staple crops, many of these neglected species are adapted to various adverse growing conditions in marginal lands such as those of the Andean and Himalayan highlands, arid areas, salt-affected soils, etc (Smart, 1984). Furthermore, many crops considered neglected at a global level are staples at national or regional level, and contribute considerably to the local food supply and are important for a nutritionally well-balanced diet. The limited information available on many important and basic aspects of neglected and underutilized crops limits their sustainable conservation and development. One major factor hampering this development is that the information available on germplasm is scattered and not readily accessible. Therefore, the existing knowledge of the genetic potential of neglected crops is limited. This results frequently, in uncoordinated research efforts for most neglected crops, as well as in inefficient approaches to the conservation of these genetic resources (Robert and Wieneke, 1996).

The increase in agricultural productivity resulting from research carried out in recent decades might have offered hope for greater food availability in developing countries in general, and in African countries in particular. Unfortunately, the present situation is quite different. In a number of African countries, food security is far from being achieved. In 1996, even though production increased in a number of areas in the continent, millions of victims of natural disasters still needed a considerable amount of food relief. In mid-1997, no less than 29 countries in the world, half of which are situated in Africa, suffered from severe food shortages necessitating exceptional or emergency food assistance (FAO, 1997). The reasons for this situation are numerous. First, population growth outstrips food production. Secondly, year after year, the situation worsens as a result of the deterioration of arable land due to desertification, salinity, floods, urbanization or civil wars, as well as to high and persistent crop losses before and after harvest mainly due to resistance of disease and insects to pesticides (Senghor, 2005). Different studies have shown that the valuable genetic diversity of several crops is under constant danger of being irretrievably lost due to natural calamities such as drought, replacement of landraces by genetically uniform crop varieties and change and development in land use (Siddique *et al*., 1999).

The grasspea (*L.sativus* L., in the family Leguminosae/Fabaceae) has over the past decade received increased interest as a plant that is adapted to arid conditions and contains high levels of protein, a component that is increasingly becoming hard to acquire in many developing areas. The genus *Lathyrus* is large with 187 species and subspecies recognized (Allkin *et al*. 1983). The species are found both in the Old World and the New World. The centers of diversity for Old World species in the genus are in Asia Minor and the

Mediterranean region (Zeven and de Wet, 1982). However, only one species *Lathyrus sativus* is widely cultivated as a food crop (Jackson and Yunus, 1984), while other species are cultivated to a lesser extent for both food and forage. Some species are valued as ornamental plants, including the sweet pea (*L. odoratus*).

Grasspea (*L.sativus*) is an erect or ascending, much branched, bushy or slender legume reaching a height of 30-90 cm. It has a deep, much branched well developed taproot, with flowers that are axillary, solitary, about 1.5 cm long, and can be bright blue, reddish purple, red, pink, or white and have short and slender pedicel (3-5) cm (IPGRI, 2000). The pinnate leaves are opposite, consisting of one or two pairs of linear-lanceolate leaflets, 5-7.5 x 1 cm, and a simple or much-branched tendril. Leaflets are entire, sessile, cuneate at the base and acuminate at the top. Cytogenetic and biosystematic studies conducted on some of the main pulse crops have focused attention on wild species genetic resources and their more efficient utilization in crop improvement. The extent of the morphological variation of *L. sativus* also has received attention, with Jackson and Yunus (1984) finding great variation, especially in vegetative characters within the species.

Grasspea is an important crop of economic significance in India, Bangladesh, Pakistan, Nepal and Ethiopia (Robert and Wieneke, 1996). It is cultivated and extensively naturalized in Central, South and Eastern Europe (from Germany south to Portugal and Spain and east to the Balkans and S. Russia), in Crete, Rhodes, Cyprus and in West Asia (Syria, Lebanon, Palestine, Iraq and Afghanistan) and North Africa (Egypt, Morocco and Algeria).

This plant is endowed with many properties that make it an attractive food crop in drought-stricken, rain-fed areas where soil quality is poor and extreme environmental conditions prevail (Palmer *et al*., 1989). Despite its tolerance to drought it is not affected by excessive rainfall and can be grown on areas subjected to floods (Kaul *et al.*, 1986; Rathod 1989; Campbell *et al*., 1994). It has a very hardy and penetrating root system and therefore can be grown on a wide range of soil types, including very poor soils and heavy clays. This hardiness, together with its ability to fix atmospheric nitrogen, makes the crop one that seems designed to grow under adverse conditions (Campbell *et al*. 1994). Compared with other legumes, the grasspea is resistant to many pests including storage insects (Palmer *et al*., 1989).

The association of grasspea with lathyrism is becoming a discouraging factor for grasspea production. Furthermore, the cereal based extension program in Ethiopia coupled with the absence of improved varieties for grasspea production is another factor leading to the reduction of grasspea production and genetic resources (Wuletaw Tadesse, 1999). Hence, there is a great need for germplasm collection and maintenance to promote grasspea breeding program for developing varieties with high grain yield and low toxin content.

Grasspea (*L. sativus*) is a food, feed and fodder crop belonging to the family Leguminosae (Fabaceae), subfamily Papilionoideae, tribe Vicieae. Other economically important species include *L. cicera* L. and *L. tingitanus* L. for grain and *L. ochrus* L., *L. latifolius* L. and *L. sylvestris* L. as forage species. A newly described species, *L. amphicarpus* is presently found in the Middle East and has the potential of becoming important as a self-seeding forage species (IPGRI, 2000).

Wild relatives and progenitors of cultivated plants represent a strategic part of germplasm collections. As the genetic base and variability of agricultural crops are relatively narrow, an introgression of genes from wild species can substantially influence the breeding progress. Thus, knowledge of their taxonomy, morphological variability, crossing ability and other features is not only important from a botanical point of veiw, but also accelerates and increases their potential successful utilizations (Foote, 1997).

Therefore, the further exploration of minor and underutilized species, the collection of these genetic resources and the assessment of genetic diversity within and between landraces should have priority in genebank activities. At the same time, it is necessary to develop better methods of characterization and evaluation of germplasm collections, to improve strategies for conservation and collection of germplasm, and to increase the utilization of plant genetic resources (Hammer *et al*., 2000).

Hence this study is aimed with the following objectives.

2. Objectives

General objective

- To assess diversity in *L. sativus* and related species.

Specific objectives

- To study the extent of morphological variation in *L. sativus*
- To study the extent of variation in biochemical characters (RAPD analysis) for *L. sativus* and three related species (*L. cicera, L. ochrus and L. clymenum*)
- To identify unidentified populations of *Lathyrus* using the data generated both in morphological and molecular studies.

3. Literature

3.1. Status of Grasspea (*Lathyrus sativus*)

Grasspea is an annual vine closely resembling field pea (*Pisum sativum L.*) in growth habit, but its leaflets are long and grass shaped, hence its name grasspea. In Ethiopia, grasspea is known by different local names as: *guaya* in Amharic, *sebere* in Tigrigna and *gayo* in Afan Oromo (Wuletaw Tadesse, *et al*., 1997). In India, Nepal and Bangladesh *Lathyrus sativus* is commonly called *khesari* (Kaul *et al*., 1986). Its unique feature of drought tolerance makes it a golden crop for the arid regions of the world. Nevertheless, the presence of a toxic chemical in the seed, which causes paralysis of the limbs when consumed excessively, makes it a threatening crop (Smart *et al*., 1994).

In Ethiopia, grasspea is produced in the off-season under residual moisture. It is planted from the end of August to the beginning of October depending on the amount of the main season rainfall (Wuletaw Tadesse, *et al*., 1997). Harvesting is carried out 4-5 months after planting in January or February when the leaves begin turning yellow and before the pods get completely ripen to avoid loss of seeds in the field. The crop is usually grown in Ethiopia after teff or barley, the common rotation being: teff-grasspea-teff, but noug and finger millet can also be used as preceding crops. In all grasspea-producing countries including Ethiopia, there is no application of fertilizer, intensive ploughing or weeding unlike other crops (Wuletaw Tadesse, 1999).

3.2 Distribution in Ethiopia

Grasspea is widely grown in Ethiopia. However, its production is more common in the north-western (58%), the central (16%) and the north-eastern (13%) parts of the country. The northern as well as southern parts of the country account for the remaining 13% of the area under grasspea (Woldeamlak Araia and Alellign Kefyalew, 1990).

In Ethiopia, grasspea ranks fifth among legumes in terms of area planted (CSA, 2006). Its production area increased from 70,400ha in 1992 to 115,475ha in 2006 with an average annual growth rate (in terms of area planted) consistently positive. The factor underlying this growth is the relatively good performance of grasspea under adverse environmental conditions, such as moisture stress, water logging, insufficient soil fertility, diseases, and pest damage (Asgelil Didabe *et al*., 1994).

3.3 Utilization of *Lathyrus sativus*

Grasspea *(L. sativus*) is cultivated in a number of countries for human food, animal feed and fodder. The crop is well adapted to areas that are suited for rice growth as it tolerates flooding at early stages and drought later in the season (Kaul *et al.*, 1986).

Rotter *et al*. (1991) reported chemical compositions of four samples of *L. sativus*, which showed very high protein content. It also shows a higher level of trypsin (which hydrolyzes proteins) inhibitor activity (Campbell, 1997).

The major uses of *L. sativus* include: green fodder, pasture, and dried stover, seed as feed and as human food. In South Asia, Ethiopia and China the crop has a dual purpose and in other regions it is mostly used as fodder or feed (Kaul *et al*., 1986).

In Ethiopia, *L. sativus* is eaten in different ways (Reda Tekle Haimanot *et al.*, 1997). Boiled seed (*nifro*) is often eaten. This preparation is not considered harmful as much of the toxin is destroyed during boiling. Unleavened bread (*Kitta*) is used in times of acute food shortage. Roasted seeds (Kollo) are used as snacks. *Kitta* and *Kollo* preparations do not destroy much of the toxin, having therefore a toxic potential.

Shiro is flour prepared from legumes including *L. sativus* and is used in making *Wott*, an Ethiopian sauce. The pan-cake like unleavened bread-*enjera*, made out of teff, wheat, barely, maize or sorghum is eaten with *Wott*. The role of *Wott* in Lathyrism is not documented.

In India, Pakistan, Bangladesh and Nepal the most common use of *Lathyrus sativus* is a *dhal*. In India, the grains are sometimes boiled whole, but are most often processed through a *dhal* mill to obtain split *dhal*. *Dhal*, a soup-like dish, is the most common method of retailing the crop in the Indian subcontinent. Some people soak the seeds or *dhal* overnight and decant the water before cooking. This system of preparation eliminates about 90% of the toxin. Nearly 10% of the *dhal* is mainly used for culinary purposes (Kaul *et al*., 1986).

The use of *L. sativus* as leafy vegetable, green pods, a green seed as snacks or as cooked vegetable is also common and appears to have less contribution in causing lathyrism as cooking reduces the toxicity by changing the β ODAP to α ODAP which is less toxic (Mehtha, 1997). Snacks made out of *L. sativus* can be identified from their taste. It was suggested from Bangladesh that using vitamin C rich foods along with *L. sativus* reduces chances of lathyrism (Kaul *et al*., 1986).

3.4 Production Constraints

Fear of Lathyrism might be the major production constraint of grasspea. But there are other numerous constraints, which hinder the exploitation of grasspea's potential (Mehtha, 1997). Some of these are as follows:

- Poor productivity of the traditional varieties
- Non-availability of quality seed to grasspea growers
- Low income status of farmers in grasspea growing areas
- Absence or ineffectiveness of rhizobium strains in soils
- Unawareness of input utilization
- Non-availability of grasspea production technology
- Poor cultivation practices
- Pre and post harvest mechanization difficulties
- Lack of trained man power in research institutes and extension departments
- Marketing and storage problems.

3.4.1 Lathyrism

Lathyrism is a motor neuron disease affecting population in Indian sub-continent, Africa and China (Mehtha, 1997). The causative agent has been identified as β-N-oxalyl diaminopropionic acid (ODAP) or β-N-oxalyl amino alanine (BOAA).

Lathyrism and its relation to *L. sativus* has been known in Ethiopia for many years. The disease has been given descriptive ethnic names such as *Sebre* and *guaya beshita*, both implicating the leg breaking (paralytic) nature of its effect. The distribution of neurolathyrism in Ethiopia is fairly widespread (throughout the arable parts) of the country with the greatest number of cases being reported in the northern and central highlands, particularly in the administrative regions of Tigray, Gondar, Gojam, Wollo and Shoa (Wuletaw Tadesse, 1999).

According to Reda Tekelehaimanot *et al*., (1997), the highest incidence of lathyrism was recorded in the Fogera and Dembiya plains of the Gondar region and west Gojam zone. Farmers in Ethiopian *Lathyrus* endemic areas believe that the grasspea is more toxic when consumed in the row form, particularly if taken with milk (Reda Tekelehaimanot, 1989).

Although lathyrism was periodically encountered, attention from Ethiopians was drawn to it only in 1976-77, when the Gondar college of medical sciences and Ethiopian Nutritional Institute (ENI), set out to study an unprecedented epidemic outbreak that occurred in the Dembiya and Fogera plains, around lake Tana (the source of the Blue Nile) with prevalence rates of 0.5-2.3% (Wuletaw Tadesse, 1999).

3.4.2 Lathyrism and Poverty

The occurrence of lathyrism is intricately linked to drought that caused famine, poverty and malnutrition. The hardy *L. sativus* may provide most of the food for survival during drought where lathyrism is prevalent (Enneking, 1998).

The disease also causes serious psychological problems among the affected peasants. Figure 1 shows Lathyrism affected family. Most of the lathyritic people are productive with help from the family (Haileyesus Getahun and Reda Teklehaimanot, 1997). Few of them are forced to be beggars, many of them shifted into less physically strenuous duties such as embroiding and weeding after developing this disease.

Figure 1. Members of a family affected by neurolathyrism in Ethiopia Source: Haileyesus Getahun, 2001

According to Reda Teklehaimanot (1989), males were affected much more than females with the ratio of 2.6:1. It can be inferred from this that lathyrism has great economical and psychological impact.

Detoxification techniques for strains with high Beta-Oxalyl Diamino Propionicacid (β-ODAP) provide some measure to reduce the impact of neurolathyrism (Enneking, 1998). Governments need to pay attention to the potential threat of neurolathyrism and provide contingency plans to deal with high-risk areas during famine periods. Reduction of poverty and malnutrition in lathyrism prone areas are likely to provide the best solution to this problem.

The development of *L. sativus* as a safe food crop, not only in Bangladesh, India, Nepal and Ethiopia where it is currently cultivated but also in other countries with suitable climates to grow this crop necessitates a good understanding of the conditions and predisposing factors which lead to the onset of neurolathyrism (Mehtha, 1997).

3.4.3 Research Efforts Carried out to Prevent Lathyrism

The grasspea production has been banned and replaced by best alternative crops by many governments of the *Lathyrus* growing countries. However, as a form to combat Lathyrism this has failed to be adapted by farmers due to their preference for grasspea (Wuletaw Tadesse *et al*., 1997).

Identification of zero/low ODAP grasspea strains is a long lasting and dependable solution to combat the menace of lathyrism in order to exploit the immense potentials of this hardy pulse (Wuletaw Tadesse, 1999). The crop improvement program at Adet Research Center, Ethiopia, identified two low toxin varieties with ODAP levels of 0.25 and 0.27% (Wuletaw Tadesse *et al*., 1997).

The development of biotechnology and its application in *L. sativus* has resulted somaclones with ODAP content of less than 0.01% in India (Mehtha, 1997). Microbe strains, which can degrade ODAP and utilize it as a sole source of carbon and nitrogen, are already isolated (Mehtha, 1997).

3.5 Genetic Diversity

Genetic diversity is usually thought of as the amount of genetic variability among individuals of a variety, population or species (Brown, 1983). Plant genetic resources with highest potential for genetic diversity are represented primarily by landraces, wild relatives of cultivated species and wild/weedy species that often contain genes for disease and pest resistance, and characters of adaptation to changing environments. Accordingly, the enormous diversity of biological resources mainly crop plants in Ethiopia is accounted due to its wide ranges of agro climatic conditions (Melaku Worede, 1988; Vavilov, 1951). These are highly prized for their potential value as sources of important variations for crop improvement programs. And can serve as valuable means to fill the gaps that still exist in the available base of genetic diversity in the world collection of many major crop species. Among the most important traits, which are believed, to exist in these materials are early maturing, disease and pest resistance, nutritional quality, resistance to drought and other stress conditions and characteristics especially useful in low input agriculture (Melaku Worede, 1988).

The nature and extent of genetic diversity in grasspea is yet to be assessed for its use in breeding programs. Its exceptional value lies in developing new crop varieties to meet modern agricultural needs. This involves a series of breeding procedures to combine desirable genes (Mehtha, 1997). According to Campbell *et al*., (1994), the preservation and conservation of genetic diversity from breeding lines, primitive cultivars, indigenous landraces and wild species of *L. sativus* is needed to:

1. Develop the tolerance to diverse climatic conditions such as drought and flooding.
2. Exploit variability in plants for maximum inert atmospheric nitrogen fixation by rhizobium, which is a major nutrient for plant growth.
3. Breed varieties for greater biomass, grain yield and low ODAP content.
4. Change crop maturity and plant canopy architecture to better fit within the overall existing cropping grasspea patterns.
5. Improve the yield potential and stability within a range of environments.

Therefore, diversity is important to small farmers so that they exercise economic self-sufficiency in the context of their ecological and socio-economic environment.

3.5.1 Challenges to Crop Genetic Diversity

Farmers have traditionally depended upon their own skills and resources to develop the crops that they need. The process began with the earliest domestication of wild species and has continued through the careful selection of plant materials adapted to variable (and often changing) growing conditions and preferences. The result has been a complex and continually evolving collection of local crop varieties, often referred to as landraces, that reflects interactions with wild species, adaptations to changing farming conditions, and responses to the economic and cultural factors that shape farmers priorities. The richness and range of the diversity of these landraces is now under threat because of the changing nature of agricultural production. One important factor is the widespread adoption of modern varieties (MVs) that are the products of formal plant breeding programes. These MVs often provide yield increases and other advantages that result in their being sown over large areas. Technological change, such as the use of fertilizer and irrigation, also lowers the demand for landraces adapted to difficult growing conditions. In addition, crop production is increasingly market- oriented, making farmers less inclined to select for crop characteristics that once were important for local customs and culture.

Many advances of modern plant breeding have been possible because of the wide range of genetic source material provided by landraces. Ironically, the success of modern plant breeding now threatens the source of genetic diversity on which further progress depends, as farmers find it less rewarding to maintain the diverse mixture of landraces developed by their ancestors.

In addition, the widespread use of MVs raises questions about the stability of crop production and the threat of disease or pest attack. The threat to stability does not necessarily derive from the MVs themselves. MVs are more uniform than landraces, which may increase their susceptibility to pests or pathogens, but many MVs owe their acceptance to the fact that they are more resistant than the varieties they replace. The principal threat to yield stability from MV use is the increasing uniformity and continuous cropping, that their use engenders. Large areas planted with a single variety are potentially a cause of concern, no matter what the source of the variety.

Furthermore, the preservation and utilization of crop genetic diversity is of particular importance to the more marginal, diverse agricultural environments where modern plant breeding has had much less success. Farmers in these areas tend to be poorly served by public research and extension systems. These areas are often centers of diversity for many crop species, but increasing poverty is forcing many of these farmers to place more dependence on non-farm sources of income, with consequent reduction in their capacity to grow and maintain the range of local varieties they have been accustomed to manage.

This hazard should be prevented. Some plant materials may not appeal now for our present requirements but may prove to be paramount importance in the future. Plant material has to be therefore, collected while it exists for utilization in the present and future breeding programs.

3.5.2 Genetic Maintenance

Unwise exploitation of natural resources has caused an irreversible loss of genetic variability and become the major cause of world wide genetic erosion. The seriousness and rapid expansion of the problem has created a universal need to collect and conserve genotypes that would no longer be available if not conserved today.

The loss of crop genetic resources in the developing countries was so crucial that it attracted the attention of many scientists and nations which led to the establishment of the International Board for Plant Genetic Resources (IBPGR) in 1974 so as to coordinate a global network of gene banks and provide plant breeders with diverse genetic resources necessary for sustained food production.

Although it is possible to conserve all available genetic resources, a practical and successful strategy has been to identify germplasm categories and from each of them to collect and conserve a representative sample. Methods for maintenance of genetic resources vary according to several factors, including the species, its geographical distribution, breeding system and seed behavior (Perrino, 1992). However, two main strategies that address the conservation of plant genetic diversity have so far been used.

3.5.2.1 *Ex-situ* Conservation:

The conventional solution to the conservation of plant germplasm has been through gene banks. These are based on collections of genetic material, from centers of crop origin and elsewhere that are stored in controlled conditions and periodically regenerated. Populations, individuals,. tissues and organelles and DNAs can be conserved in genebanks. Genebanks have been important sources of material for plant breeding programs and other research activities, and will continue to be a basic element in conservation programs.

Some of the largest genebanks contain impressive numbers of accessions; however, the costs of characterizing and cataloguing genebank material are considerable. Only a small proportion of genebank material is actually used by plant breeders, in part because of inadequate characterization of accessions. In addition, farmers usually do not have easy access to the materials they have donated. The control of genebanks is also an issue of debate; there are concerns that a country that hosts an important international collection can deny another country access for political reasons.

These issues of control are beginning to hamper efforts at germplasm collections, as countries are increasingly concerned with the possibility that their local landraces may be collected, utilized commercially, and even legally protected, without any compensation. More resources are needed to support national plant genetic conservation efforts, and more attention needs to be devoted to making these collections relevant to farmers' interests and capacities. In some cases, national genebanks are being redirected to encourage more farmer participation in their development and utilization. For example, the Institute of Biodiversity Conservation (IBC) in Ethiopia is involved in several activities that involve farmer participation in conservation activities. The Unitarian Service Committee of Canada helped support an IBC project in which materials collected from nearby areas are given to farmers to plant and to carry out simple mass selection to improve their characteristics (Melaku Worede, 1993).

Status of Collection

The germplasm collection of *Lathyrus* species maintained by International Center for Agricultural Research in Dry Areas (ICARDA) contains 44 species from 46 countries (Table 1) with a total of 3038 accessions. These accessions are held in trust under the auspecies of the Food and Agriculture Organization (FAO) under a Consultative Group on International Agricultural Research (CGIAR) agreement with the FAO. All germplasm is distributed under a Material Transfer Agreement (MTA) to protect the rights of the country of origin of the gemplasm (Larry and Ali, 1998).

Table 1. ***Lathyrus*** **species germplasm accessions maintained at ICARDA**
Source: Larry and Ali, 1998

Regions/Countries	***L. sativus***	***L. cicera***	***L. ochrus***	**Others**
North Africa	*17*	*45*	*45*	*117*
Morocco	14	31	9	94
Algeria	-	12	6	18
Tunisia	3	2	30	4
Egypt	-	-	-	1
West Asia	*70*	*79*	*39*	*810*
Turkey	13	53	4	299
Syria	4	24	11	457
Cyprus	20	1	22	-
Jordan	1	1	-	33
Palestine	-	-	-	2
Iraq	-	-	-	10
Iran	13	-	1	8
Afghanistan	19	-	-	1
Ethiopia	110	-	-	-
South Asia	*1 285*	*3*	*2*	*12*
Pakistan	82	2	-	3
Nepal	81	-	-	9
Bangladesh	1 115	-	-	-
India	7	1	2	-
Others	*78*	*58*	*52*	*117*
Total	1 560	185	138	1 056

3.5.2.2 *In-situ* Conservation

An option that is attracting increasing attention is the possibility of carrying out genetic resource maintenance in the field. This is often referred to as *in-situ* conservation, in contrast to the *ex-situ* alternative of genebanks. Arguments in support of *in-situ* conservation include the importance of recognizing the roles of both environmental factors and farmer intervention in landrace development. In areas of high crop genetic diversity, landraces often evolve through crossing with wild or weedy relatives, and farmers play a crucial role in selecting and adapting new material. It is this process of landrace development that *in-situ* conservation attempts to enhance and preserve. It is also argued that *in-situ* conservation programmes would provide more opportunities for community conservation, where farmers would be better able to recognize and utilize the plant genetic resources that are available in the community.

But there are several decisions that must be made in designing *in-situ* conservation projects. First, because landraces are not static entities, decisions have to be taken with regard to the nature of human intervention in the selection process.

In addition, farmers generally seek germplasm from other sources to complement their own landraces. Resources and information are provided to farming communities to empower them to make more informed decisions about the management of local varieties and the utilisation of MVs.

In-situ conservation projects will undoubtedly approach these decisions in a variety of ways. At this stage it is important to encourage a range of *in-situ* programs and to learn from experience. The International Plant Genetic Resources Institute (IPGRI) in Rome is currently developing a large multi-country collaborative project to test and develop techniques for *in-situ* germplasm conservation.

3.5.2.3 Farmer-participatory plant breeding programs

There are a number of efforts under way to encourage a wider scope for farmer participation in formal plant breeding. Possibilities include greater farmer representation in priority setting for crop breeding programs, more explicit attention to the crops and varietal characteristics of importance to these farmers, the transfer of significant aspects of plant breeding research to farmers fields, and the enlistment and training of farmers to take a more active part in variety testing and selection process.

There is a growing literature on methods to encourage farmer participation. The innovations include Rapid Rural Appraisal Techniques to understand farmer varietal preferences, the organization of various types of adaptive on-farm research to test varieties under farmers' conditions, the wider use of landraces in formal breeding programs, and the establishment of mechanisms for contact between farmers and experiment station personnel. Some plant breeders see the possibility of an integrated system that incorporates the strengths of both formal and informal plant breeding techniques (Robert and Wieneke, 1996).

3.6 Morphological Variation

Highly heritable morphological traits such as leaf color, flower color, seed color, seed size were among the earliest genetic markers used in scientific investigations and are still in use in germplasm management (Plucknett *et. al*., 1987). Vavilov's determination of centers of diversity for various crop species was based on his extensive field studies and observation of phenotypic traits.

L. sativus shows great morphological variation, especially in vegetative characters such as leaf length, while floral characters are much less variable (Jackson and Yunus, 1984). This array of variation is undoubtedly the result of geographical separation as well as selection by man. The pattern of variation in *L. sativus* based on flower color and seed color agrees with that described by Vavilov (1951), who also noted that forms of *L. sativus* with white flowers and white seeds were highly selected by farmers. Such forms were typical of a more western distribution around the Mediterranean basin. One can postulate that the forms with blue flowers speckled seeds are more primitive, and the pattern of variation found in *L. sativus* is like that found in other pulses such as lentils and broad beans (Jackson and Yunus, 1984).

Genetic variation studies using morphological traits, which are of interest to the breeder's, are important to speed up a breeding program. They are also inexpensive, simple and rapid to score. However, morphological markers have also weaknesses such as many of the traits are controlled by many genes, they are influenced by environment, the phenotype of most morphological markers can only be determined at the whole plant level; allele frequencies at morphological marker are much lower; alleles at morphological loci interact in a dominant recessive manner that limits the identification of heterozygous genotypes, and require growing of plants to a suitable stage before certain characters can be scored (Seifu Tilahun, 1997).

3.7 Molecular Markers

In the past, morphological and physiological characters of *L. sativus* have been studied. However, the usefulness of phenotypic traits to study the genetic variation of the divergence between populations is less. Classification using genetic markers provides a large unbiased basis for the measurement of average population similarities and differences.

Molecular markers are used in plant genome studies to assess genetic diversity, identify plants at cultivar level, for construction of genetic maps, as specific probes for screening of traits in breeding programs and to tag genetic traits (Lawson *et al*., 1994). For example, Randomly Amplified Polymorphic DNA (RAPD) analysis has been used in cluster analysis studies for classification of two *Lathyrus* species, *L. odoratus* and *L. latifolium* (Hanada and Hirai, 2000).

RAPD analysis is conducted mainly to see the similarity among different somaclones and the parent as well as to get finger print of the somaclones at DNA level, which could Be used further for identifying a particular somaclone (Mehta and Santha, 2001).

The development of the Polymerase Chain Reaction (PCR) has revolutionalized the analysis of nucleotide sequence variability. Williams *et al*., (1990), described the procedure for the identification of polymorphism in plants based on PCR. Both RAPD and RFLPs (Restricted Fragment Length Polymorphism) are being used extensively for the study of genetic diversity. DNA markers are considered ideal for diversity study and enable to overcome the short comes of other markers, even though they are expensive (Powel, 1992).

4. Materials and Methods

4.1 Field experiment

4.1.1 Description of the experimental site

The field study was carried out in Debre Zeit (Bishoftu), which is located at 8° 44` N, 39° 02` E in the Oromia National Regional State. Debre Zeit is 47 Km from Addis Ababa. It is located at 1980 m a.s.l characterized by moderate rainfall (851 mm average annual rainfall) and 17.9°C average mean temperature. The experimental site is dominantly vertisol soil type, which is ideal for grasspea production.

4.1.2 Experimental procedures

A total of 50 grasspea accessions including one local accession (*Wassie*) were used for this study. The materials were obtained from ICARDA. Table 2 shows the list and origin of the populations used for this study. Figure 2 shows the distribution of the origins of the populations in temperature, elevation and rainfall respectively.

The experiment was laid out in Randomized Complete Block Design (RCBD) with three replications. The spacing between rows was 30cm and distance between plots and between blocks was 1 & 2m respectively. Five different individual plants were selected randomly from each plot and were used for evaluation. Fifteen individuals per population and a total of 765 individual plants were evaluated for the following characters using International Plant Genetic Resource Institute (IPGRI) descriptor:

1. Leaflet size (length and width)

- Leaflet length (cm): maximum length of leaflet recorded from the middle area of the main branch
- Leaflet width (cm): maximum width of leaflet recorded from the middle area of the main branch

2. Days to flowering (d): Number of days from sowing to stage when 50% of plants have begun to flower in a row.
3. Flower color and size: Score on fresh open flowers to score standard, wing and keel colors separately. Blue, white, white mixed with other colors and pink colors were recorded as 1, 2, 3 & 4 respectively.
4. Plant height (cm): Height of plant at physiological maturity measured from ground to the tip the longest branch.
5. Number of pods per plant: Mean number of pods, recorded from randomly selected plants at physiological maturity.
6. Number of seeds per pod: Mean number of seeds counted on randomly selected pods. Recorded at physiological maturity.
7. Seed size: This was rated as 1 for small, 2 for medium and 3 for large seeds by observing the total harvested seeds per plant.
8. Seed length (mm): Maximum mean length of randomly selected seeds.
9. Seed width (mm): Maximum mean width of randomly selected seeds.
10. Seed thickness (mm): Maximum mean thickness of randomly selected seeds.
11. Hundred seed weight (g): 100 seeds were randomly taken from each plant and weighed using sensitive balance in g.

12. Seed Color: was observed from harvested seed of each individual plant and recorded as 1 for grey mottled, 2 for yellow white, 3 for grayed white, 4 for green mottled and 5 for brown mottled.
13. Pod length (cm): Maximum mean length of randomly selected mature pods. Recorded at physiological maturity.
14. Pod width (cm): Maximum mean width of randomly selected mature pods. Recorded at physiological maturity.
15. Stipule length (cm): Maximum length of stipules recorded from the middle area of the main branch.
16. Stipule width (cm): Maximum width of stipules recorded from the middle area of the main branch.

The materials used in this experiment were: Meter and ruler for measuring the different quantitative characters mentioned above, Royal Horticultural Society (RHS) color chart for comparing the different flower and seed colors and Hand lens for observing hairiness

Table 2. List and origin of the 51 grasspea populations used in the field experiment.

accno	genus	species	origin	province	site	longitude	latitude
5280	*Lathyrus*	*sativus*					
5311	*Lathyrus*	*sativus*	TUR	Aydin	Zeylim (Aydin)	E27 51	N37 51
5312	*Lathyrus*	*sativus*	TUR	Aydin	Zeylim (Aydin)	E27 51	N37 51
5313	*Lathyrus*	*sativus*	TUR				
5315	*Lathyrus*	*sativus*	TUR				
5316	*Lathyrus*	*sativus*	GRC	Macedonia	bei Rodopi region, 700m		
5318	*Lathyrus*	*sativus*	GRC	Epirus	Ioannina, Agr. Exp. Stn.	E20 51	N39 40
5319	*Lathyrus*	*sativus*	GRC	Epirus	Ioannina, Agr. Exp. Stn.	E20 51	N39 40
5320	*Lathyrus*	*sativus*	GRC	Aegean Islands	Finiki	E 27 7 0	E 35 28 59
5321	*Lathyrus*	*sativus*					
5322	*Lathyrus*	*sativus*	GRC	Peloponnesus	Supena	E 22 0 0	N 37 30 0
5323	*Lathyrus*	*sativus*	GRC	Peloponnesus	Supena	E 22 0 0	N 37 30 0
5324	*Lathyrus*	*sativus*	GRC	Peloponnesus	Boleta	E22 20	N37 28
5325	*Lathyrus*	*sativus*	GRC				
5326	*Lathyrus*	*sativus*	GRC	Peloponnesus	Palaiogrtsanon region		
5328	*Lathyrus*	*sativus*					
5329	*Lathyrus*	*sativus*	DEU				
5330	*Lathyrus*	*sativus*	DEU				
5331	*Lathyrus*	*sativus*					
5332	*Lathyrus*	*sativus*	DEU				
5333	*Lathyrus*	*sativus*	DEU				
5334	*Lathyrus*	*sativus*	GRC	Crete	Ambelouzos	E24 53 53	N35 04 25
5335	*Lathyrus*	*sativus*					
5337	*Lathyrus*	*sativus*	GRC	Crete	Ambelouzos	E24 53 53	N35 04 25
5338	*Lathyrus*	*sativus*		Crimea	Cherson	E34 21	N45 19
5339	*Lathyrus*	*sativus*					
5340	*Lathyrus*	*sativus*	DEU				
5342	*Lathyrus*	*sativus*					
5343	*Lathyrus*	*sativus*	HUN				
5344	*Lathyrus*	*sativus*	DEU				
5345	*Lathyrus*	*sativus*					

accno	**genus**	**species**	**origin**	**province**	**site**	**longitude**	**latitude**
5346	*Lathyrus*	*sativus*	DEU				
5348	*Lathyrus*	*sativus*	POL				
5349	*Lathyrus*	*sativus*	POL				
5350	*Lathyrus*	*sativus*					
5351	*Lathyrus*	*sativus*					
5352	*Lathyrus*	*sativus*	PRT				
5354	*Lathyrus*	*sativus*	TUN				
5357	*Lathyrus*	*sativus*	PRT				
5358	*Lathyrus*	*sativus*	DEU				
5360	*Lathyrus*	*sativus*					
5361	*Lathyrus*	*sativus*					
5362	*Lathyrus*	*sativus*		Krasnodar Territory			
5363	*Lathyrus*	*sativus*	HUN				
5378	*Lathyrus*	*sativus*		Nizk Tatry	Tekovske Luzany	E18 33	N48 06
5380	*Lathyrus*	*sativus*		Carpathian mountains	SW Senohrad, Dolne MladHonice	E19 04	N48 19
5396	*Lathyrus*	*sativus*	AFG	Badakhshan	Barak, 38 Km from Faizabad	E70 51 26	N36 55 53
5404	*Lathyrus*	*sativus*					
5415	*Lathyrus*	*sativus*					
5420	*Lathyrus*	*sativus*					
Wassie	*Lathyrus*	*sativus*	ETH				

TUR= Turkey, GRC= Greece, DEU= German, POL= Poland, PRT= Portugal, TUN= Tunisia, HUN= Hungary, AFG= Afghanistan, ETH= Ethiopia

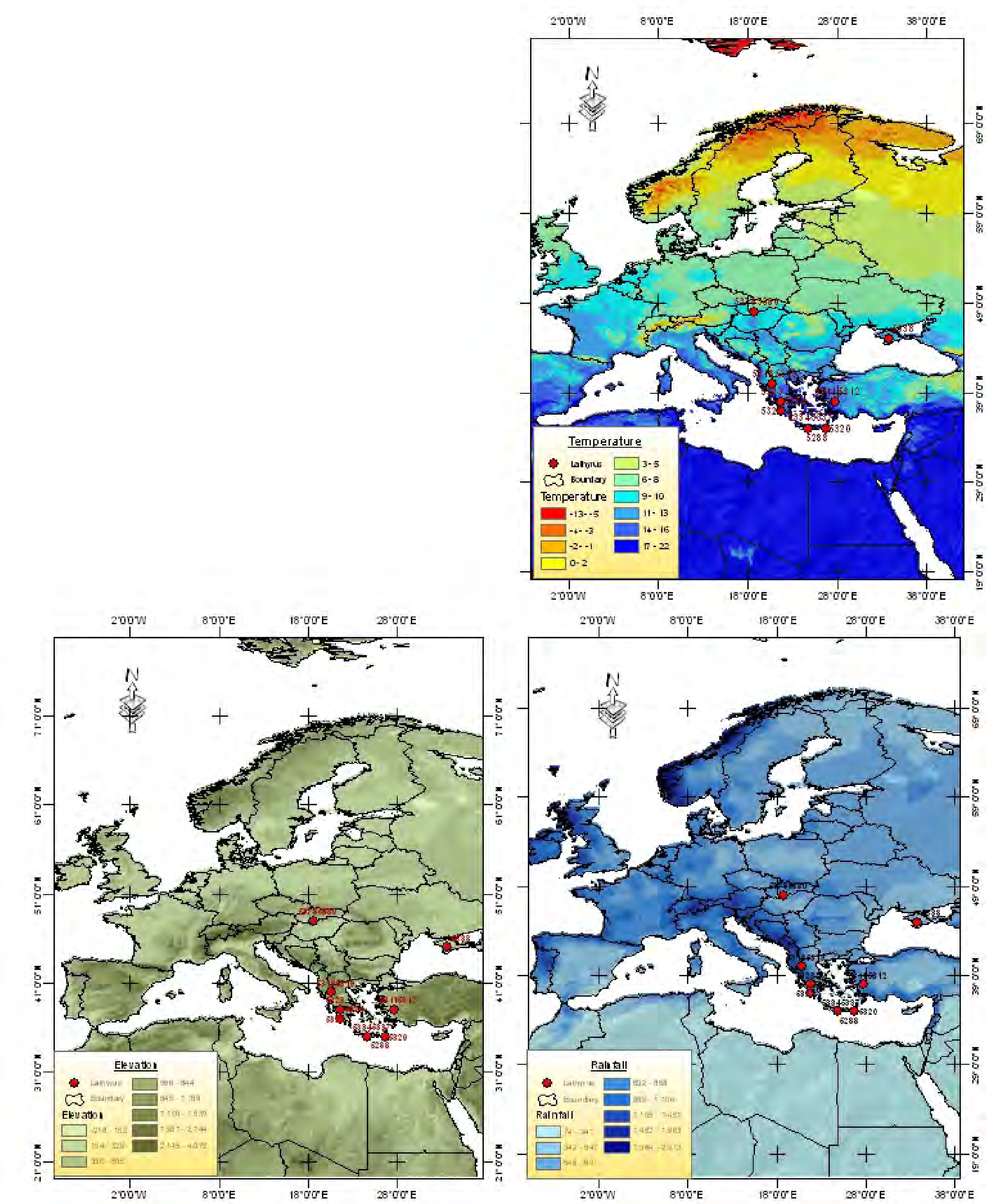


Figure 2. Distribution of the populations used for this study in temperature, elevation and rainfall

4.2 Green House Experiment

The green house experiment was carried out in six *Lathyrus* species (*L. sativus, L. cicera, L. ochrus, L. clymenum, L. crassipes and L. aphaca*) and seventeen unidentified *Lathyrus* populations were used for morphological description and evaluation. But unfortunately two of the *Lathyrus* species (*L. aphaca* and *L. crassipes*) died in the green house because of powdery mildew and spider mite diseases and were excluded from the analysis. Thus only four species were included in this study (*L. sativus, L.clymenum, L. ochrus and L.cicera*). Table 3 shows list and origin of the populations used for this study. The morphological characters used for this study were the following:

1. Corolla color: score on fresh open flowers for standard and wing colors separately
2. Upper leaflet number: number of leaflets per leaf on the upper part of the leaf (one pair, two pairs and more than two pairs).
3. Lower leaflet number: number of leaflets per leaf in the lower part of the leaf (absent, one pair, two pair and more than two pairs).
4. Leaf tendrils present or absent
5. Standard length (cm): mean length of standard petal measured on randomly selected, fully expanded flowers
6. Standard width (cm): mean width of standard petal measured on randomly selected, fully expanded flowers
7. Wing length (cm): mean length of the wing measured on randomly selected, fully expanded flowers
8. Wing width(cm): mean length of the wing measured on randomly selected, fully expanded flowers

9. Peduncle length (cm): the mean length of randomly chosen peduncles at maturity
10. Stem winged or not: assessed at the middle of the main branch at 50% flowering
11. Stem hairiness: whether stem is hairy or glabrous
12. Pod length (mm): maximum mean length of randomly selected matured pods recorded at physiological maturity
13. Pod width (mm): maximum mean width of randomly selected matured pods recorded at physiological maturity
14. Seed length (mm): Maximum mean length of matured seed.
15. Seed width (mm): Maximum mean width of matured seed.
16. Seed color: was observed from harvested seed of each individual plant and recorded as 1 for grey mottled, 2 for yellow white, 3 for grayed white, 4 for green mottled and 5 for brown mottled.

Table 3. List and origin of the populations used in the green house study.

accno	genus	species	origin	province	site	longitude	latitude
5282	*Lathyrus*	*cicera*					
5288	*Lathyrus*	*clymenum*	GRC	Crete	Ambelouzos	E24 53 53	N35 04 25
5295	*Lathyrus*	*ochrus*	GRC	Peloponnesus	Kryoneri	E22 27 58	N36 44 08
5296	*Lathyrus*	*ochrus*	GRC	Peloponnesus	Markovonion	E22 22 31	N37 33 16
5330	*Lathyrus*	*sativus*	DEU				
5331	*Lathyrus*	*sativus*					
5387	*Lathyrus*	*sp.*					
5390	*Lathyrus*	*sp.*					
5391	*Lathyrus*	*sp.*					
5392	*Lathyrus*	*sp.*					
5395	*Lathyrus*	*sp.*					
5398	*Lathyrus*	*sp.*					

5405	*Lathyrus*	*sp.*						
5407	*Lathyrus*	*sp.*	CAN					
5410	*Lathyrus*	*sp.*						
5413	*Lathyrus*	*sp.*						
5422	*Lathyrus*	*sp.*	DEU					
5424	*Lathyrus*	*sp.*						
6515	*Lathyrus*	*clymenum*						
7679	*Lathyrus*	*sp.*	ETH					
9936	*Lathyrus*	*crassipes*	ARG					
15606	*Lathyrus*	*sp.*	ETH					
15607	*Lathyrus*	*sp.*						
15608	*Lathyrus*	*sp.*						
15609	*Lathyrus*	*sp.*	ETH					

GRC= Greece, DEU= German, CAN= Canada, ETH= Ethiopia, ARG= Argentina

4.3 Molecular Study

4.3.1 RAPD Analysis

In this analysis ten different accessions of *Lathyrus sativus* which showed highest variation in morphological characters from the field study and the plant populations from the green house experiment were used. The different steps involved were described as follows.

4.3.1.1 Preparation of DNA extraction buffer

The following DNA extraction buffers were prepared according to Khan and Majid (2001). 2% CTAB; 1.4M NaCl; 20mM EDTA, pH 8; 100mM Tris-HCl, pH 8 and 0.2% mercaptoethanol.

4.3.1.2 DNA extraction

Fresh leaf samples were prepared for DNA extraction. Small-scale extraction of DNA from plant tissues for PCR was done. Genomic DNA was extracted from leaf samples according to Khan and Majid (2001) with some modifications on the amount of leaf.

300 mg of fresh leaf tissues were grind to powder in liquid nitrogen and transferred to 2 ml capacity eppendorph tube while still frozen and then 800 µl hot (65ºC) CTAB buffer was added and vortexed so that they could be mixed. After this, equal volume of chloroform: Iso amyl alcohol (24:1) were added and centrifuged at 4000 rpm for 10 minutes. Upper layer was carefully removed to clean the tube and 800 µl of cold Iso propanol was added and left overnight at -20ºC. The solution was centrifuged at 14000 rpm for 30 minutes to pour off supernatant and pellet was washed with 70% ethanol and then dried in vacuum desiccators. Finally the pellet was re- suspended in 100 µl Tris EDTA and stored at -20°C. Materials used in the above experiment were: Scissors for cutting leaves, Alcohol to avoid contamination when cutting, Plastic bags for collecting the leaf samples and Pistol and mortar for grinding the leaves with liquid nitrogen

4.3.1.3 PCR Amplification

The extracted DNAs were PCR amplified. PCR conditions were used as described by Khan *et. al*., 2000. PCR was performed in a 0.2 ml tube for use on GeneAmp PCR systems 9700 (PE Biosystems). The reaction consisted of 50 ng of DNA, 0.2 µM primer, 1.5mM $MgCl_2$, 2.5 µ 10 × PCR buffer (Applied Biotechnologies), 100 µ M of dNTPs and 1 unit of Taq polymerase (*Taq*) in 25 µl reaction volume.

The thermocycler was set at 94°C for 5 min. and repeated 50 sec. at 94°C, 1 min. for annealing (36°C), and 2 min. at 72°C. This was followed by a final cycle of 72°C for 10 min.

The PCR amplification was done using ten RAPD primers. Table 4, 5 and 6 show primer details.

From 20 *Lathyrus* primers tested, the following 10 gave PCR products with clear pattern for all populations studied and showed repeatable pattern in separate amplification experiments.

Table 4. List of primers, their sequence and products generated through amplification

No.	Code		Products	Fragment size range (bp)
1	OPJ04	CCGAACACGG	15	300-1850
2	OPJ09	TGAGCCTCAC	15	400-1900
3	OPJ10	AAGCCCGAGG	16	530-3000
4	OPJ11	ACTCCTGCGA	9	350-1800
5	OPJ12	GTCCCGTGGT	16	400-3000
6	OPJ13	CCACACTACC	17	400-2000
7	OPJ14	CACCCGGATG	11	350-1850
8	OPJ18	TGGTCGCAGA	13	350-1800
9	OPJ19	GGACACCACT	8	500-1600
10	OPJ20	AAGCGGCCTC	9	350-1700

Table 5 Details of the ten primers used in the PCR amplification

Primer	Concentration (Volume 1 ml)	Molecular Weight	Temperature	Amount of DNA	Optical density	GC content
Primer 1	106 pmol/ µl	3022 g/mol	34.0 ºC	320 µg	11.8	70%
Primer 2	113 pmol/ µl	2988 g/mol	32.0 ºC	335 µg	11.5	60%
Primer 3	109 pmol/ µl	3062 g/mol	34.0 ºC	334 µg	12.6	70%
Primer 4	82 pmol/ µl	2988 g/mol	32.0 ºC	245 µg	8.4	60%
Primer 5	125 pmol/ µl	3035 g/mol	34.0 ºC	379 µg	12.1	70%
Primer 6	148 pmol/ µl	2917 g/mol	32.0 ºC	433 µg	14.9	60%
Primer 7	131 pmol/ µl	3013 g/mol	34.0 ºC	396 µg	13.8	70%
Primer 8	103 pmol/ µl	3068 g/mol	32.0 ºC	315 µg	11.4	60%
Primer 9	91 pmol/ µl	2997 g/mol	32.0 ºC	273 µg	9.9	60%
Primer 10	115 pmol/ µl	3013 g/mol	34.0 ºC	347 µg	12.1	70%

Table 6 List of different volumes of TE buffer added to make up 100-µM solution of each primer.

Primer	Volume of TE buffer needed for 100 µM Solution of primer
Lathyrus 1	1060 µl
Lathyrus 2	1121 µl
Lathyrus 3	1091 µl
Lathyrus 4	819 µl
Lathyrus 5	1249 µl
Lathyrus 6	1484 µl
Lathyrus 7	1313 µl
Lathyrus 8	1027 µl
Lathyrus 9	910 µl
Lathyrus 10	1151 µl

4.3.1.4 Preparation of electrophoresis buffer and gel

The quality of genomic DNA, if present, was tested using agarose gel electrophoresis. Stock solution of 10X Tris Borate EDTA (TBE), commonly used electrophoresis buffer: 108 gm Tris base; 55 gm Boric acid; 7.44 gm EDTA, pH 8.57 components per liter was prepared and stored at room temperature. From the stock, working solution of 10X TBE enough both to pour the gel and fill the electrophoresis tank was prepared. 0.6 gm molecular grade agarose to prepare 1.5% agarose concentration was measured and 40 ml of 10X TBE was then added. The slurry was heated in a microwave for 1 minute. After the agarose solution cooled down to about 55^{o}C, 15μl of ethidium bromide was added for better visualization of the gel and mixed very well. Then it was poured on to the gel tray and the comb was inserted immediately after the agorose was poured and the gel was left for 30 minutes to dry properly. The comb was carefully removed and put the gel tray in to the electrophoresis tank properly filled with electrophoresis buffer.

4.3.1.5 Loading genomic DNA ample

15 μl of the amplified DNA sample was added to 1 μl loading dye (Bromophenol blue) and mixed very well. The mixture was loaded to the well of the gel. The electrode was connected and the power supply was turned on; the voltage was adjusted at 70 V and left for 80 minutes.

4.3.1.6 Visualization of the gel

The electrophoresed gel was photographed using UV transluminator, BiodocAnalyzer™, and polaroid camera, cannon, connected with computer. Different photograph using different lens apparture were taken and saved for scoring.

4.4 Data Analysis

4.4.1 Morphological data

All the morphological data collected from the field experiment were subjected to analysis of variance using SAS software (SAS institute, 1987). Simple correlation coefficients between all possible traits were also computed. Cluster analysis was computed based on both quantitative and qualitative morphological characters and from this, ten most diverse populations were selected and used for further molecular study (RAPD) analysis.

The morphological data collected from the green house experiment were analyzed using SAS software. Cluster analysis was performed and compared with the morphological identification of each species.

4.4.2 Molecular data

4.4.2.1 Data Scoring

Although a large number of fragments were generated from each primer, only clearly distinguishable and reproducible bands were selected and data were entered in a computer file as a binary matrix “0” coded for absence and “1” for presence of a band.

4.4.2.2 Data analysis

- Numbers of total and polymorphic loci were obtained by direct count, and the size of bands was compared with 100 bp DNA ladder. Illustrative figure of the bands is shown in Figure 3.

- Percent polymorphic loci were calculated as follows:
  - Percent polymorphic loci over the ten primers was calculated as:

PPL = (TNPL/TNL) x 100

Where, PPL = Percent polymorphic loci over all primer

TNPL = Total number of polymorphic loci over the ten primers

TNL = Total number of loci observed

  - Percent polymorphic loci per primer was calculated as:

PPLP = (NPLP/NLP) x 100

Where, PPLP = Percent polymorphic loci per primer

NPLP = Number of polymorphic loci per primer

NLP = Number of total loci per primer

- The genetic variability was calculated according to Nei (1987) using the ARLEQUIN 3.01 software package (Excoffier, 2006).

- The degree of genetic differentiation averaged over all populations and population pairwise comparison for genetic differentiation and their significant test over all loci were tested using ARLEQUIN version 3.01 (Excoffier, 2006).

- Analysis of molecular variance (AMOVA) (Excoffier *et al*., 1992) was used to separate the variance between and within populations and F values were computed using the ARLEQUIN version 3.01 program package (Excoffier, 2006).

- Population pairwise genetic distance matrix was calculated using POPGENE version 1.31 (Yeh *et* al., 1999) based on Nei's (1978) genetic distance.

- Phylogenetic tree was constructed using Unweighted Pair Group Method using Arithmetic Averages (UPGMA) and was expressed as dendrogram. NTSYS software version 2.1 was used to construct the tree (Sneath and Sokal, 1973).

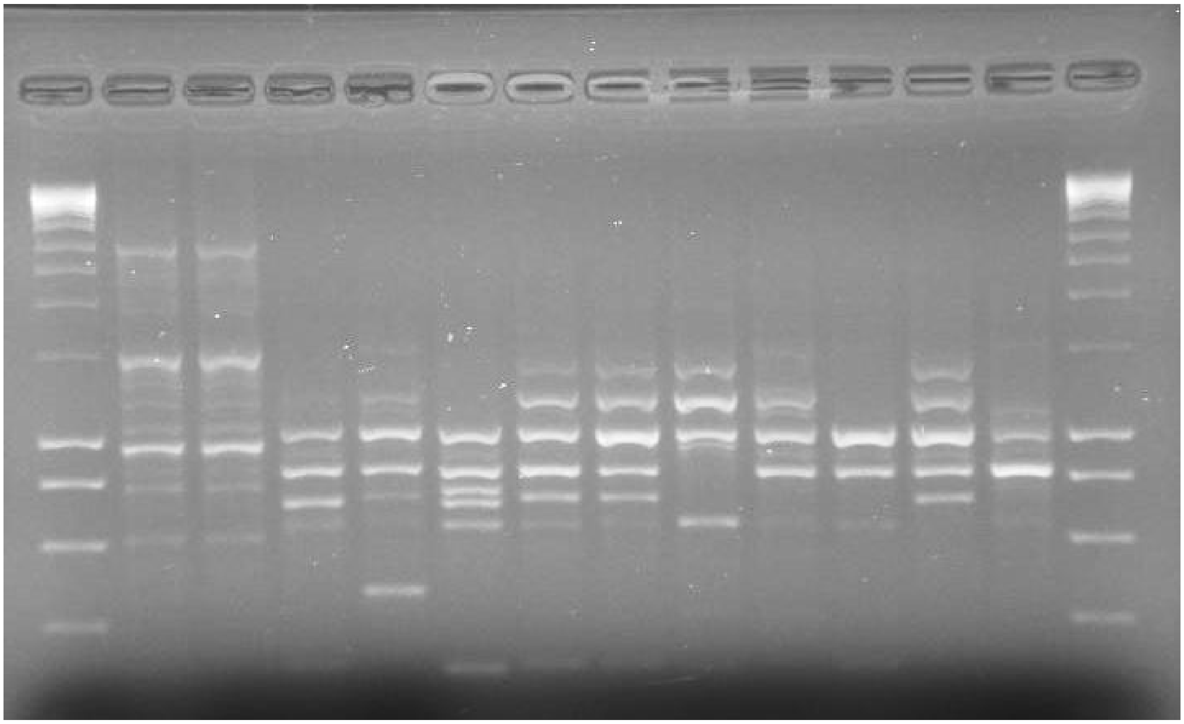


Figure 3. Representative photograph of the bands using primer OPJ04

5. Results and Discussion

5.1 Morphological Diversity

5.1.1 Mean and Coefficient of Variation for Quantitative Characters

Mean, range and coefficient of variation in morphological traits are widely used to determine variations within and between populations (Endashaw Bekele, 1996, Sharma, *et al*, 1995).

Summary statistics (mean, minimum, maximum and coefficient of variations) over the entire individuals of the 51 grasspea populations are presented in Table 7.

Table 7. Mean, minimum and maximum values, and coefficients of variation (CV), observed in 51 grasspea populations

Descriptor	**Mean**	**Minimum**	**Maximum**	**CV%**
PLH (mm)	387.25	316.67	466.67	9.99
DF	52.42	43.67	56.67	3.55
PDL (mm)	35.86	28.33	40.67	6.02
PDW (mm)	11.79	9.33	14.00	7.63
PPP	38.63	24.67	89.33	24.4
SPP	2.95	2.33	3.67	11.48
SW (g)	0.22	0.11	0.34	14.63
SDL (mm)	8.05	5.67	11.00	15.63
SDW (mm)	7.28	5.00	10.33	17.17
SDT (mm)	5.05	4.5	6.13	7.32

PLH= plant height, DF= days to 50% flowering, PDL= pod length, PDW= pod width, PPP= pods per plant, SPP= seeds per plant, SW= seed weight, SDL= seed length, SDW= seed width, SDT= seed thichness

As indicated in Table 7 the character that showed the highest coefficient of variation is number of pods per plant followed by seed width, while days to 50% flowering showed the lowest coefficient of variation. The mean values for each of the 51 grasspea populations in each character are indicated in annex 1.

Analysis of variance over the entire data showed that highly significant differences between populations were observed for stem thickness, leaflet width, keel length, wing width, days to flowering and pods per plant (Table 8). Two factor analysis of variance was also carried out to determine variation within a population. Significant difference was observed between individuals in a given population for plant height, stem thickness, leaflet width, stipule width, seed weight, days to 50% flowering, keel length, wing length and width and sepal length and width (Table 9).

Table 8. Mean squares for 8 quantitative morphological traits of 51 grasspea populations

Character code	Mean square for factor A	Mean square for error	CV	SE
STT	2.97*	0.2219	8.45	0.4719
PLH	1443.738	1501.56	10.01	38.749
LLW	4.56*	0.3079	10.97	0.5549
SW	0.005	0.0027	14.64	0.0523
DF	27.86*	1.89	3.55	1.375
KL	2.89*	0.1242	4.49	0.352
WW	6.38*	0.2548	8.16	0.5048
PPP	949.36*	34.215	24.39	5.849

STT= stem thickness, PLH= plant height, LLW= leaflet width, SW= seed weight, DF= days to 50% flowering, KL= keel length, WW= wing width, PPP= pods per plant
*indicates significance at 5%

Table 9. Mean squares for the 51 grasspea populations and 3 replications within the populations.

Source	D.F	PLH	STT	LLW	STW	SW	DF	KL	WL	WW	SPL	SPW
Acc	50	4494.27*	1.16*	1.68*	0.383	0.008	10.35	0.87	1.61	1.83*	1.41*	0.32*
Rep	2	7401.96	5.05*	3.15*	1.08*	0.006*	7.86*	1.83*	2.13*	3.13*	2.83*	0.6*
Error	100	3013.29	0.45	0.532	0.343	0.001	2.26	0.22	0.39	0.45	0.57	0.19

D.F= Degrees of freedom, PLH= plant height, STT= Stem thickness, LLW= leaflet width, STW= stipule width, SW= seed weight, DF= days to 50% flowering, KL= Keel length, WL= wing length, WW= Wing width, SPL= sepal length, SPW= sepal width
***** indicates significance at 5%

5.1.2 Correlation Analysis of Quantitative Characters

Thorpe (1976) indicated that character associations are important to indicate common elements of epigenetic control and/or similar response of characters to selection pressure.

Table 10. Correlation among 10 different characters of 51 grasspea populations

	PLH	PDL	PDW	SPP	PPP	SW	SDL	SDW	SDT	DF
PLH		0.239	0.251	-0.209	0.061	0.286*	0.281*	0.358**	0.005	0.023
PDL			0.719***	0.022	-0.249	0.436**	0.411**	0.313**	0.188	0.429**
PDW				-0.321	-0.230	0.578***	0.614***	0.517***	0.329**	0.304**
SPP					-0.011	-0.308	-0.292	-0.535	-0.104	0.189
PPP						-0.204	-0.184	-0.095	-0.243	-0.511
SW							0.926***	0.793***	0.623***	0.054
SDL								0.804***	0.590***	-0.005
SDW									0.369***	-0.016
SDT										0.021
DF										

PLH= Plant height, PDL= Pod length, PDW= Pod width, SPP= Seeds per pod, PPP= Pods per plant, SW= Seed weght, SDL= Seed length, SDW= Seed width, SDT= Seed thickness, DF= Days to 50% flowering.
* indicates significance at P< 0.05, ** indicates significance at P< 0.01 and *** indicates significance at P< 0.001

Table 10 shows pod length was highly correlated with pod width and seed weight and size and also with days to 50% flowering. Similarly there was highly significant correlation between seed size (length and width) and pod length and width. The presence of persistent correlation among most characters considered could be due to natural selection between individuals within a population (Endashaw Bekele, 1996).

5.1.3 Cluster Analysis

Based on the mean morphological values of both qualitative and quantitative characters, the 51 grasspea populations were grouped into 4 clusters (Table 11 and 12).

Cluster 1: consisted the maximum number of populations (35) collected from different countries.

Cluster 2: twelve populations collected from seven countries were included in this cluster.

Cluster 3: consisted three populations collected from Turkey, Slovakia and Russia.

Cluster 4: this cluster consists of one population which is collected from Ethiopia.

Table 11. Average linkage clustering of 50 grasspea populations based on quantitative morphological characters

Cluster	Accession Number
I	5334, 5344, 5338, 5340, 5322, 5316, 5332, 5315, 5328, 5320, 5350, 5326, 5357, 5313, 5329, 5415, 5333, 5342, 5324, 5280, 5345, 5325, 5420, 5354, 5319, 5323, 5360, 5318, 5335, 5351, 5362, 5330, 5321, 5343, 5352
II	5346, 5358, 5331, 5363, 5337, 5339, 5361, 5349, 5311, 5380, 5396, 5404
III	5312, 5378, 5348
IV	*Wassie*

Table 12. Average linkage clustering of 51 grasspea populations based on qualitative morphological characters

Cluster	Accession Number
I	5311, 5312, 5316, 5322, 5331, 5333, 5335, 5337, 5343, 5345, 5348, 5357, 5358, 5361, 5362, 5378, 5380, 5326, 5354
II	5315, 5318, 5320, 5313, 5324, 5328, 5330, 5334, 5338, 5339, 5346, 5350, 5363, 5323
III	5321, 5325, 5319, 5329, *Wassie*, 5351, 5352, 5280, 5396, 5404, 5332, 5420
IV	5340, 5342, 5344, 5349, 5360, 5415

Table13. Significant difference among the four clusters for mean values of the different characters

Cluster	**No**	**PLH**	**STMT**	**LW**	**SW**	**DF**	**KLL**	**WW**	**PPP**
1	35	391.52^a	5.37^b	4.75^b	0.22^a	52.39^a	7.72^b	5.84^b	38.95^b
2	12	379.17^a	6.28^a	5.47^b	0.22^a	53.03^a	8.47^a	7.28^a	35.14^b
3	3	387.78^a	5.56^b	6.44^a	0.25^a	53.33^a	6.89^c	6.11^b	32.11^b
4	1	333.33^b	4.33^c	6.67^a	0.11^b	43.67^b	7.00^c	5.33^b	89.33^a

PLH= Plant height, STMT= Stem thickness, LW= Leaflet width, SW= Seed weight, DF= Days to 50% flowering, KLL= Keel length, WW= Wing width, PPP= Pods per plant.
* Means with the same letter are not significantly different

Clustering of the populations based on quantitative morphological characters is indicated in dendogram (Figure. 4). Four major clusters are resulted. Cluster 1 contains 35 populations. It is however, sub-divided into different sub-clusters indicating the great interclass variation. Cluster 2 contains twelve populations collected mainly from Europe. Cluster 3 contains three populations. The last cluster (Cluster 4) contains only one population (*Wassie*) indicating that this population is different from all other populations. This is the only population collected from Ethiopia. This population was grouped in Cluster 3 together with most of the populations when qualitative morphological characters were used as classifying variables (Table 12).

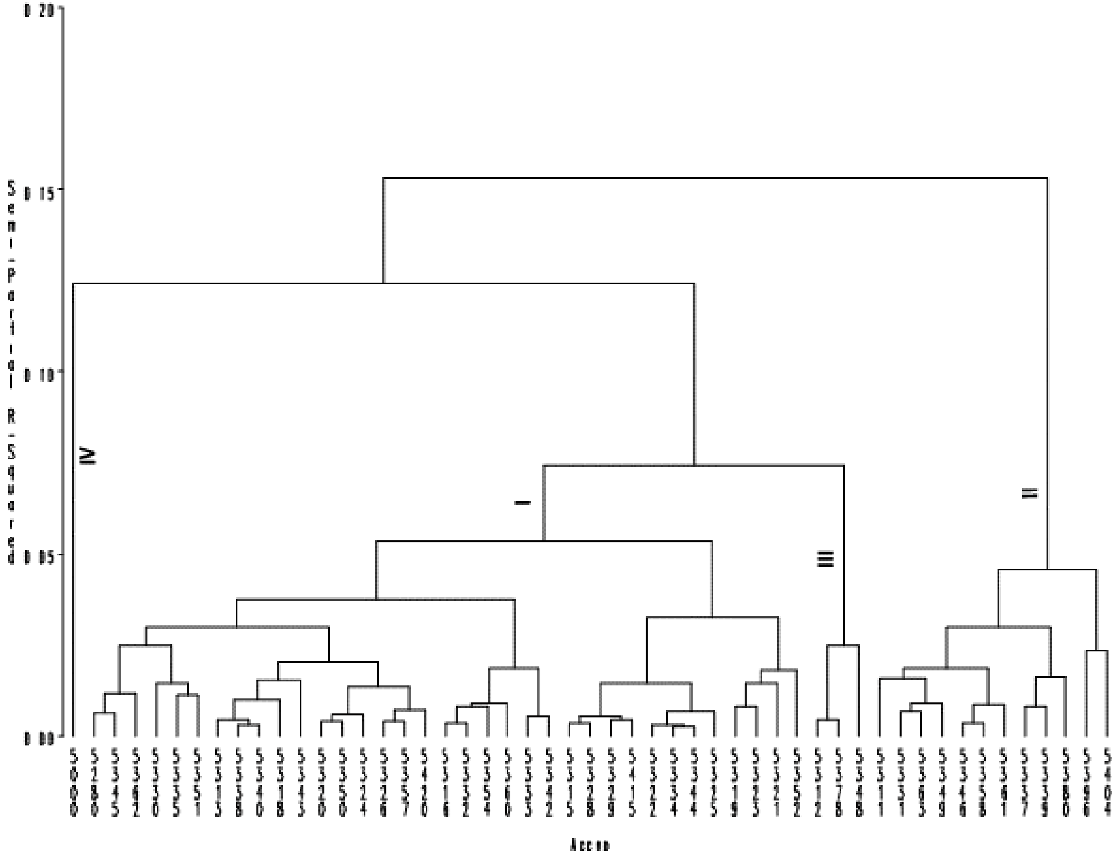
As indicated in Table 13, cluster 4 represented by only one accession (*Wassie*) is significantly different from the other three clusters in mean plant height, seed weight, days to flowering and number of pods per plant. This accession is characterized by small plant height, seed weight and took few days for flowering where as the highest number of pods (89) per plant was observed in this accession indicating that it can give more seeds which are relatively smaller in size. It can also be inferred that, it needs few days for flowering implicating that yield can be collected within a short period of time. Mean values for each of the clusters for the different characters is indicated in Annex 2.

The overall clustering result indicates the lack of consistency in classification of the populations when different classifying variables are considered.

In general, the variation based on qualitative traits is much less among populations of the different accessions. Similar result was obtained by Jackson and Yunus (1984). Dendogram of the 51 accessions by ward method cluster was performed for qualitative morphological characters (Figure. 5).

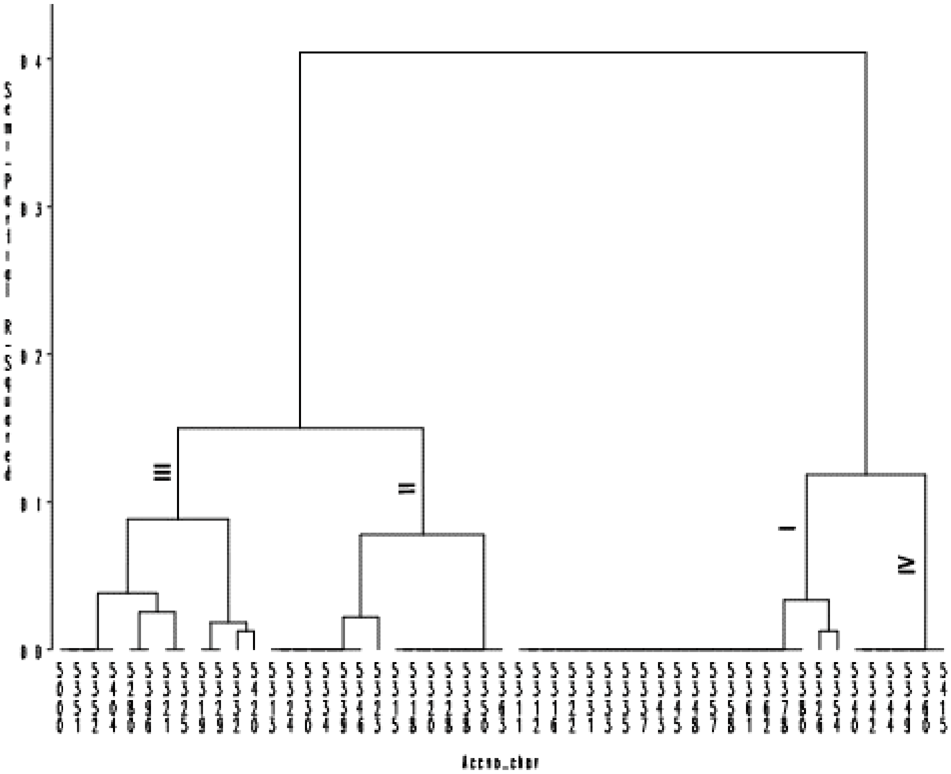
White mixed with other colored flowers was dominantly expressed in most of the accessions comprising about 37.3%. Where as white flower color comprise only 11.8%.

The other colors, blue and pale blue comprise 23.5% and 27.4% of the population respectively. Variation in flower colors in all the populations is indicated in Figure 6. Seed colors were highly related to flower colors. The blue colored flowers gave rise to grey mottled seed colors, where as the pale blue flowers gave green and brown mottled seeds. White flowers gave yellow white seeds while white mixed with other colors gave greyed white seeds. This is also indicated in the dendogram (Figure. 5). The different accessions clustered together are characterized by specific flower and seed colors. Variation in the different seed colors in all the accessions is shown in Figure 7.



5000= *Wassie*

Figure. 4 Dendogram of 51 accessions of *Lathyrus* by WARD method cluster for quantitative characters



5000= *Wassie*

Figure. 5 Dendogram of 51 accessions of *Lathyrus* by WARD method cluster for qualitative characters


A= White

B= Dark blue

C= Pale blue


D= Pink

Figure. 6 Variation in flower colors in all the accessions

A= Yellow white B= Brown mottled C= Grayed white

D= Grey mottled E= Light green mottled F= Black

G= Light brown mottled H= Green mottled I= *Wassie*

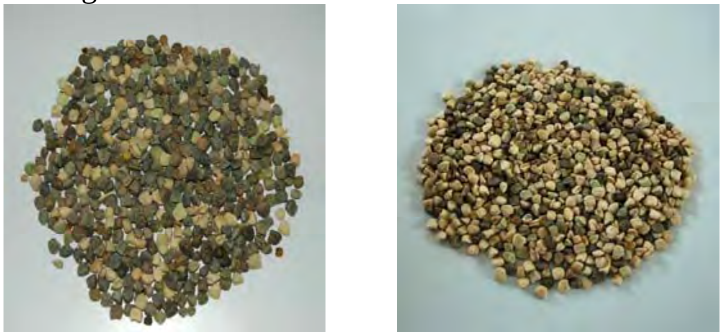


Figure.7 Variation in Seed colors and size in all the accessions

5.2 Morphological Study on the different species of *Lathyrus*

5.2.1 Morphological Characterization and identification of the 24 *Lathyrus* accessions

This study involved morphological identification of the *Lathyrus* species by comparing them with type specimens. Results indicate that accessions 5295 and 5296 which were initially grouped as *Lathyrus ochrus* were found to be *Lathyrus sativus* because of the following characters: leaflet width is much smaller ranging from 4-8 mm which is mainly the characteristics of *L. sativus* and in the case of *L. ochrus* the corolla is characterized by light yellow color where as these accessions are mainly composed of blue colored corolla which is the main feature of *L. sativus*. Furthermore all leaves are with one or more pairs of leaflets which are linear-lanceolate and 2 in number unlike *L. ochrus* which is characterized by leaves with a flattened stalk inflated as leaf blade, twisted on its base and lower leaves are without leaflets but with one or several tendrils; the upper leaves consist of 1-4 small leaflets which are ovate and alternate. All these features combined with the results obtained from the molecular study make these two accessions (5295 and 5296) to be classified under *L.sativus* (Table 14).

Among the accessions which were grouped under *L. clymenum*, only one accession (6515) turned out to have the characteristic feature of *L. clymenum* possessing the following characteristics: lower leaves without leaflets, the rachis broadly winged and resembling a leaf; upper leaves with 2-4 pairs of leaflets; the corolla with purple standard color and wings violet or whitish. One the other hand, the second accession, 5288 did not possess the above mentioned characters which are typical for *L. clymenum*. Thus, it is distinctly separated from all other accessions possessing the following characteristics: lower leaflets smaller than the upper leaflets and ovate in shape whereas upper leaflets resemble that of *L. sativus* having a pair of leaflets which are linear-lanceolate; corolla color is light orange (both the standard and the wing). So it might belong to a different species possibly *L. articulatus*.

Accession number 5282 which was identified as *L. cicera* characterized by the rather conspicuous salmon red color flowers (a color which is not shared in many other flowers of this genus) turned out to have blue colored flowers which mainly resemble that of *L. sativus*. In addition, *L. cicera* leaves consist of a pair of leaflets which are linear (or slightly lanceolate) in shape and up to 100cm long and 5mm wide and the presence of a set of 3 curly tendrils, about 4 cm long or more. The tendrils have a common stalklet which joins them to the leaf petiole. The stalklet of the tendrils is the longest, followed by that of the flower (the peduncle), while the leaf petiole is the shortest. But in the case of this accession, the stalklet of the tendrils joins the almost sessile stipule which is again a common feature of *L. sativus*. All these features point out to the fact that this accession can be classified under *L. sativus*.

The other two accessions (5330 and 5331), grouped under *L. sativus*, were also confirmed to have the characteristics of *L. sativus* possessing all the features.

The rest of the seventeen accessions which were initially referred as *Lathryus* spp. turned out to be *L. sativus* possessing all the floral (white, pink or blue colored) and leaf characteristics (pair of leaflets which are linear to lanceolate and upper leaflets modified into three curly tendrils). The size and color of seeds were also similar to *L. sativus* ranging from 3-15 mm long and consisting of yellowwhite, greyedwhite, grey, green and brown mottled seed color. These accessions were also grouped in the same cluster both in the molecular and morphological cluster analysis.

5.2.2 Cluster analysis

Based on the mean morphological values of each species the 24 accessions of *Lathyrus* species were grouped into three main clusters. Clustering of the populations based on vegetative characters is indicated in dendogram (Figure. 8). Mean values for each of the clusters for the different characters is indicated in Annex 3

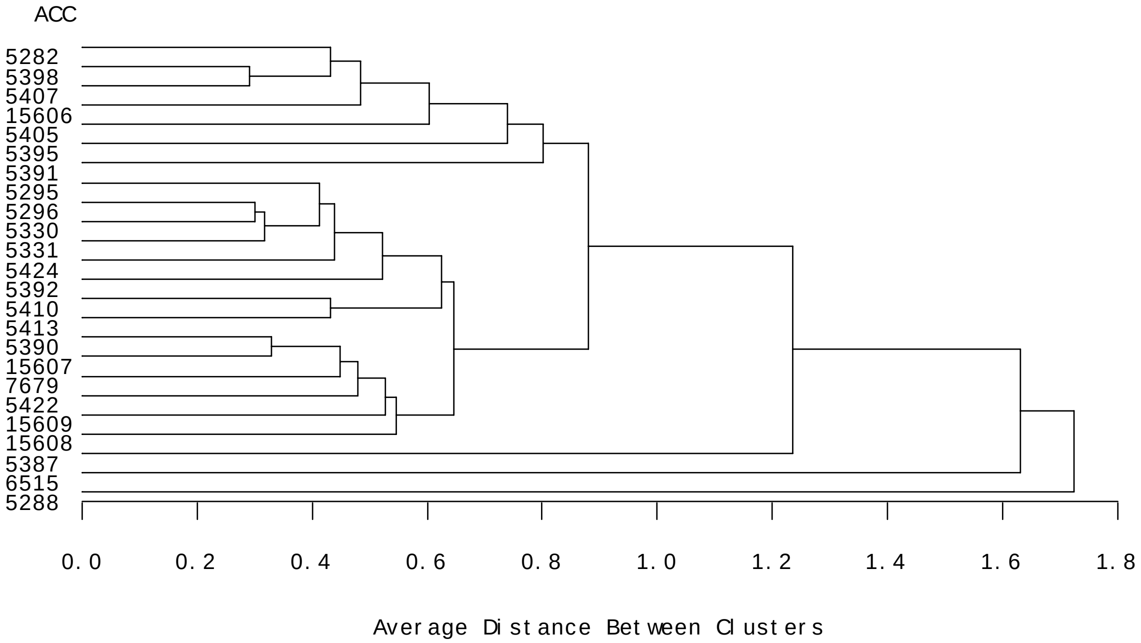


Figure 8. Dendogram of 24 accessions of *Lathyrus* by average linkage cluster for quantitative characters

As indicated in the dendogram three major clusters are resulted. Cluster I and II consist of only one accession 5288 and 6515 respectively where as Cluster III consist of 21 accessions which are again divided into different sub-clusters. As confirmed by the morphological identification accession 6515 represents a distinct accession which is grouped under *L. clymenum* , this result also supports the previous morphological based conclusion which grouped this accession separately. The other accession 5288 is also grouped in a distinct cluster confirming again the previous conclusion that it is grouped in a different species category as *L. articulatus*. Table 14 shows the previously grouped populations and the populations after characterization.

Cluster III which consists of 21 accessions which are grouped under *L. sativus* are divided into different sub-clusters based on their flower and seed colors and seed size. Accession 5387 is the only accession which is characterized by pure white flowers which are relatively larger in size with large yellow white seeds. Accessions 5282, 5398, 5407, 15606, 5405, 5395 and 5391 which are grouped into a different sub-cluster are characterized by blue colored flowers which are larger in size with large grey mottled seeds where as accessions 5390, 15607, 7679, 5422, 15609, 15608 and 5387 are characterized by blue colored flowers with relatively smaller seeds. The remaining accessions which are grouped under a different sub-cluster 5295, 5296, 5330, 5331, 5424, 5392, 5410 and 5413 are distinguished by their mixed white colored flowers which have large greyed white seeds.

Table 14. List of the initially described and the characterized populations of Lathyrus after the study.

Initially described			**Characterized**	
Accession	Genus	Species	Genus	Species
5282	*Lathyrus*	*cicera*	*Lathyrus*	*sativus*
5288	*Lathyrus*	*clymenum*	*Lathyrus*	*articulatus*
5295	*Lathyrus*	*ochrus*	*Lathyrus*	*sativus*
5296	*Lathyrus*	*ochrus*	*Lathyrus*	*sativus*
5330	*Lathyrus*	*sativus*	*Lathyrus*	*sativus*
5331	*Lathyrus*	*sativus*	*Lathyrus*	*sativus*
5387	*Lathyrus*	*sp.*	*Lathyrus*	*sativus*
5390	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
5391	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
5392	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
5395	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
5398	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
5405	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
5407	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
5410	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
5413	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
5422	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
5424	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
6515	*Lathyrus*	*clymenum*	*Lathyrus*	*clymenum*
7679	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
15606	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
15607	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
15608	*Lathyrus*	*sp*	*Lathyrus*	*sativus*
15609	*Lathyrus*	*sp*	*Lathyrus*	*sativus*

5.3 Molecular study

5.3.1 RAPD markers

The ten primers produced a total of 88 loci with 1040 bands ranging from 200-1000 base pairs. Primer OPJ13 produced the highest number of loci (13) followed by primer OPJ04 which produced 12 number of loci and the least (6) from primer OPJ20 (Table 15).

Fifty nine polymorphic loci, constituting 67.05% of the total loci, were investigated (Table 15). When percent polymorphic loci per primer is considered, the highest percentage was observed from OPJ13 (76.92%) followed by OPJ19 (75%) and the least from OPJ11 (55.56%).Among the populations studied, the highest number of polymorphic loci (43) was observed in accession 5352, followed by accession 15607 (42) and the least (7) was observed in accession 5288.

Table 15. Characteristic summery of RAPD primers

	Characteristic information				
Primer	TNL	NPL	PPL	PS	GC content
OPJ04	12	8	66.67	CCGAACACGG	70%
OPJ09	7	4	57.14	TGAGCCTCAC	60%
OPJ10	11	7	63.63	AAGCCCGAGG	70%
OPJ11	9	5	55.56	ACTCCTGCGA	60%
OPJ12	7	5	71.42	GTCCCGTGGT	70%
OPJ13	13	10	76.92	CCACACTACC	60%
OPJ14	7	5	71.42	CACCCGGATG	70%
OPJ18	8	5	62.5	TGGTCGCAGA	60%
OPJ19	8	6	75	GGACACCACT	60%
OPJ20	6	4	66.67	AAGCGGCCTC	70%
Total	88	59	67.05		

TNL= Total number of loci, NPL= Number of polymorphic loci, PPL=percent polymorphic loci, PS= Primer sequence, GC= Guanine Cytosine content.

As far as number of polymorphic loci per primer is concerned, it more or less fall in the ranges of polymorphism(4-10) that can be detected under optimal conditions which varies from 1 to 10 polymorphic loci per gel (Parker *et al*., 1998). Nei and Kumar (2000) also explained that the existence of two or more alleles with substantial relative frequencies in a population (usually more than 1 %) is called genetic polymorphism.

5.3.2 Genetic variability with in accessions

Within species genetic variability was estimated over polymorphic loci. The gene diversity value for each population vary from 0.0329 in accession 5288 to 0.1858 in accession 15607 and the remaining populations were found between this range.

The gene diversity within population was estimated based on the mean allelic frequency of the fifty nine polymorphic loci. Lynch and Milligan (1994), Nei (1973) and Nei and Chesser (1983) reported that with the estimates of the allelic frequency in hand, it is possible to estimate the gene diversity (hetrozygosity) within population.

The relative lower hetrozygosity value obtained in this study possibly be associated with less number of individuals used and the dominance property of the RAPD markers. Lynch and Milligan (1994) reported that because of the dominance property of RAPDs, gene frequency estimates for such loci are necessarily less than those obtained with co dominant markers such as isozymes and RFLPs. The down ward estimate and less accuracy may be associated with the rather relatively higher sampling variance of the gene frequency in the case of RAPDs (Lynch and Milligan, 1994) and they suggested that increase in sampling efforts (2-10 times more individuals) needs to be applied to a RAPD locus in order to achieve a genetic parameter as accurate as would be acquired in a locus with co dominant markers.

The lowest diversity value observed in accession 5288 revealed the distinctness of the accession as it was classified in to a different species (*L.clymenum*) while the rest were classified under *L.sativus*.

5.3.3 Analysis of molecular variance (AMOVA)

When data are available from more than one population, it is usually of interest to evaluate the degree to which the total gene diversity partitions into its within and between population components (Lynch and Milligan, 1994). In addition to its implication to conservation, partitioning the genetic variation in to its components has significant impact in the future breeding plan. According to present study, the interspecies difference accounted for 19.67 % of the total variation (Table 16).

Results of the Analysis of Molecular Variance (AMOVA) of the four species of *Lathyrus* tested are presented in Table 16, and showed that although there is a considerable genetic variation between the different species, the within species variation is much higher. Percent of variation between species accounts for 19.67% and within species accounts for 24.60% where as within accession variation is 55.72%. This result is in consistent with previous studies on *Lathyrus* (Croft *et. al*., 1999).

Table 16. Analysis of molecular variance (AMOVA) obtained from haplotype frequencies of four *Lathyrus* species.

Source of variation	df	Sum of squares	Variance components	Percentage of variation
Among species	3	199.299	3.21061	19.67
Among accessions within species	26	409.753	4.01546	24.60
Within accessions	23	209.167	9.09420	55.72
Total	52	818.218	16.32027	
Fixation Index FST : 0.44277				

5.3.4 Genetic distance

The unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) in pairwise comparison are presented in Table 17. The smallest distance was found between accessions 5295 and 5282 (0.1536) and the largest was between accessions 5407 and 5288 (0.7200). The smallest distance observed between the accessions is expected since both were grouped under the same species (*L.sativus*) in the previous morphological based classification. The highest distance between accessions 5395 and 5288 is also expected since both were grouped under different species categories, the former under *L.sativus* and the latter under *L.clymenum*. List of labels for population samples used in Table 17 are presented in annex 4.

Table 17. Unbiased measures of genetic identity (above diagonal) and genetic distance (below diagonal) among the different accessions of *Lathyrus*

pop ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	****	0.5513	0.6651	0.6228	0.5975	0.5860	0.6138	0.5907	0.5628	0.5860	0.6254	0.5676	0.5166	0.4867	0.5840	0.5803	0.6138	0.6576	0.5397	0.5860
2	0.5955	****	0.5879	0.5929	0.5682	0.4886	0.5682	0.5227	0.5114	0.5341	0.6023	0.6364	0.5795	0.5568	0.4318	0.6233	0.5000	0.6376	0.5341	0.6705
3	0.4079	0.5311	****	0.8374	0.6596	0.6377	0.8576	0.7283	0.7740	0.7810	0.7950	0.8028	0.7431	0.6348	0.6834	0.7086	0.6974	0.8123	0.5900	0.6266
4	0.4736	0.5228	0.1774	****	0.7024	0.6637	0.7431	0.7024	0.7382	0.7971	0.8127	0.6884	0.6834	0.6258	0.6377	0.7206	0.7263	0.7514	0.5821	0.6834
5	0.5150	0.5653	0.4162	0.3533	****	0.6250	0.7045	0.6591	0.6705	0.6477	0.6477	0.5682	0.5341	0.6023	0.6364	0.7040	0.7045	0.7409	0.6023	0.6477
6	0.5345	0.7161	0.4498	0.4100	0.4700	****	0.6250	0.7841	0.6818	0.7045	0.6591	0.6250	0.5909	0.6136	0.6023	0.6571	0.6932	0.7326	0.7045	0.6591
7	0.4880	0.5653	0.1536	0.2969	0.3502	0.4700	****	0.7500	0.7386	0.7159	0.7386	0.7273	0.7159	0.6477	0.6818	0.6998	0.7273	0.8139	0.6250	0.6250
8	0.5264	0.6487	0.3170	0.3533	0.4169	0.2432	0.2877	****	0.8295	0.7841	0.7386	0.7273	0.7386	0.7614	0.6591	0.6765	0.7500	0.7629	0.7614	0.6932
9	0.5748	0.6707	0.2562	0.3035	0.3998	0.3830	0.3029	0.1869	****	0.7955	0.7727	0.6932	0.6591	0.6591	0.5795	0.6284	0.6932	0.7595	0.6364	0.6364
10	0.5345	0.6272	0.2472	0.2268	0.4343	0.3502	0.3342	0.2432	0.2288	****	0.7727	0.6932	0.7045	0.6591	0.6477	0.6224	0.6477	0.7166	0.7045	0.6818
11	0.4694	0.5070	0.2294	0.2073	0.4343	0.4169	0.3029	0.3029	0.2578	0.2578	****	0.7614	0.6591	0.6364	0.6023	0.6529	0.7159	0.7584	0.6136	0.6136
12	0.5663	0.4520	0.2196	0.3734	0.5653	0.4700	0.3185	0.3185	0.3665	0.3665	0.2726	****	0.7841	0.6250	0.6364	0.6693	0.6364	0.8246	0.6705	0.6477
13	0.6605	0.5455	0.2969	0.3806	0.6272	0.5261	0.3342	0.3029	0.4169	0.3502	0.4169	0.2432	****	0.7045	0.6023	0.6541	0.6023	0.7411	0.6818	0.6136
14	0.7200	0.5855	0.4544	0.4687	0.5070	0.4884	0.4343	0.2726	0.4169	0.4169	0.4520	0.4700	0.3502	****	0.6250	0.7378	0.6477	0.7503	0.7045	0.6818
15	0.5379	0.8398	0.3806	0.4498	0.4520	0.5070	0.3830	0.4169	0.5455	0.4343	0.5070	0.4520	0.5070	0.4700	****	0.7112	0.7273	0.7576	0.7159	0.5568
16	0.5443	0.4728	0.3445	0.3277	0.3509	0.4199	0.3569	0.3908	0.4646	0.4742	0.4264	0.4014	0.4245	0.3040	0.3408	****	0.8051	0.8347	0.6338	0.7193
17	0.4880	0.6931	0.3603	0.3198	0.3502	0.3665	0.3185	0.2877	0.3665	0.4343	0.3342	0.4520	0.5070	0.4343	0.3185	0.2168	****	0.7987	0.6477	0.6705
18	0.4192	0.4500	0.2079	0.2859	0.2999	0.3112	0.2059	0.2706	0.2751	0.3332	0.2765	0.1929	0.2996	0.2872	0.2776	0.1806	0.2248	****	0.7663	0.7570
19	0.6167	0.6272	0.5277	0.5410	0.5070	0.3502	0.4700	0.2726	0.4520	0.3502	0.4884	0.3998	0.3830	0.3502	0.3342	0.4560	0.4343	0.2661	****	0.6818
20	0.5345	0.3998	0.4674	0.3806	0.4343	0.4169	0.4700	0.3665	0.4520	0.3830	0.4884	0.4343	0.4884	0.3830	0.5855	0.3295	0.3998	0.2784	0.3830	****

Nei's genetic identity (above diagonal) and genetic distance (below diagonal).

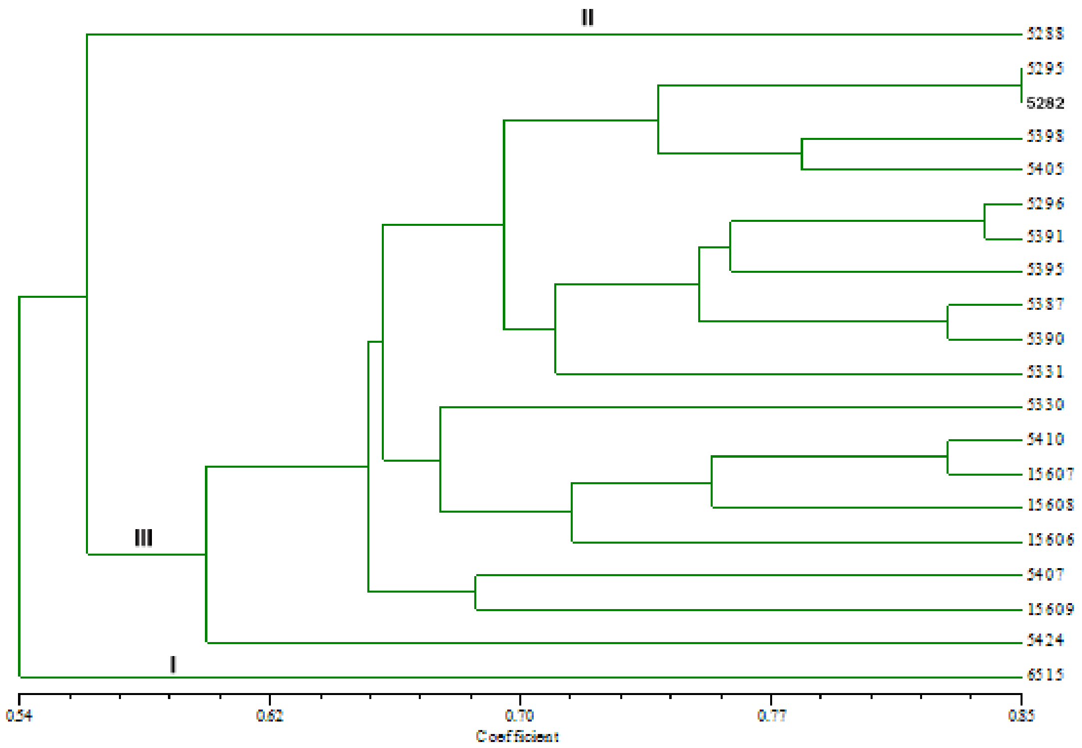


Figure 9. Dendrogram generated by the UPGMA method for Nei (1978) genetic distance using NTSYS program.

The resulting genetic distance was used to construct a dendrogram using Unweighted Pair-Group Method using Arithmetic Averages (UPGMA). The resulting tree and the coefficients are presented in Figure 9. The accession 6515 formed a distinct branch from the rest of the accessions followed by accession 5288 which also formed a single cluster. All the other accessions fall under one cluster except accession 5424 which was grouped together with the other accessions previously when morphological characters were used as a classifying parameter. As indicated in the previous morphological study, accession 6515 is a distinct accession which is grouped under the species *Lathyrus clymenum*. This result also confirms the distinctiveness of this accession. It also verifies that accession 5288 is a different species from all the others (*L. articulatus*) by grouping it in a different single cluster.

5.3.5 Cluster analysis for the ten *L. sativus* populations using molecular data

The ten representative *L. sativus* populations selected from each cluster from the morphological study were subjected to RAPD analysis and the data generated from this were used to construct a dendrogram using Unweighted Pair-Group Method using Arithmetic Averages (UPGMA) in the NTSYS program (Fig. 9).

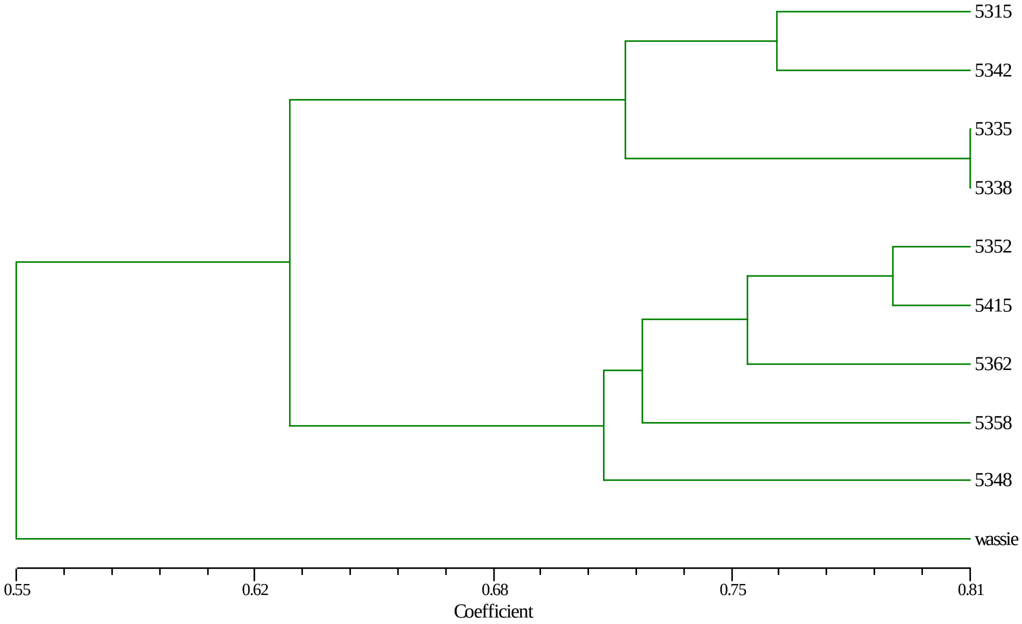


Figure 10. Dendrogram of ten *L. sativus* populations generated by UPGMA method using molecular data.

As shown in Figure 10, the local accession *Wassie* is again grouped in a different single cluster confirming the morphological result which also grouped this accession separately. Accession 5358 and 5348 which were taken from cluster II and III respectively from the morphological study are grouped in the same big cluster but in two different sub-clusters. The fact that they are grouped in the same cluster implies their close relationship in morphological characters especially in flower and seed color and size. Whereas their difference in sub-cluster implies their being originated from different sources, the former from Poland and the latter from Germany. The other remaining accessions were taken from cluster I of the previous classification (morphological data) which are again grouped based on their morphological characters; higher plant height, narrow leaflet width and medium flower and seed sizes.

We can infer from the above result that molecular markers are important for a better classification of populations.

6. Conclusion and Recommendations

The present study was undertaken to investigate the morphological and molecular variation of *Lathyrus sativus* and its wild relatives and aid future germplasm selection.

The morphological diversity study showed that the local accession (*Wassie*) has been identified as a different accession characterized by smaller plant height, seed weight and took little days for flowering. It is also characterized by highest number of pods per plant indicating that it can give more seeds which are relatively smaller in size. It also possesses smaller and grey mottled seeds, a character which is considered harmful since these seeds are assumed to possess higher amount of ODAP. So it is not advisable to use this accession for food and breeding work has to be done.

On the other hand, accessions (5312, 5378 and 5348) were characterized by larger leaflet width, stipule length and large white seeds which implicate that these large leafed varieties may have emerged from selection of forage types. Germplasm with large white seeds (which are assumed to have low ODAP) and high yield are of particular interest, since these characters are attractive to both consumer and producer and the future of grasspea as a protein-rich food and feed depends on the development of high-yielding varieties with low seed β-ODAP.

The morphological study also showed that there was highly significant correlation between seed size and pod length and width; pod length and seeds per pod among the different accessions used for this study. The presence of persistent correlation among most characters considered could be due to natural selection between individuals within a population.

The morphological study carried on the different species of *Lathyrus* revealed that all accessions which were considered to be *Lathyrus ochrus*, *Lathyrus cicera and Lathyrus* species turned out to be *Lathyrus sativus*. This clearly indicates the need for germpalsm evaluation as an important parameter for proper characterization of accessions in genebanks.

The molecular study disclose that the RAPD markers used for this study were found to be moderately polymorphic and were found to be useful for the genetic characterization of the different populations of *Lathyrus*. This study also confirms the previous results obtained from the morphological study by clearly defining the distinct accessions.

Generally, we can conclude that molecular markers can be used along with morphological markers to estimate genetic variability of the different populations of *Lathyrus* giving a better result than a single marker. Further study should also focus on the identification and measuring diversity of wild relatives using the possible available markers in order to improve the crop through conventional breeding and biotechnological techniques.

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Annex 1. The mean values for each of the 51 grasspea populations in each character

Acc	PLH	STMT	LL	LW	STPL	STPW	KLL	WW	SPP	PPP	SW	SDS	SWT	DF
5352	317	4	63	6	17	5	8	5	4	30	13	4	0.2	53
5348	397	6	60	7	22	5	6	6	2	34	19	6	0.2	54
5343	383	6	63	5	18	4	8	6	3	43	17	6	0.2	51
5312	383	5	67	6	20	5	7	6	3	31	15	5	0.2	52
5378	383	6	63	6	20	4	7	6	3	31	19	7	0.3	54
5331	317	6	59	6	18	5	8	7	3	39	18	5	0.2	52
5349	400	7	60	6	18	5	8	7	3	33	18	4	0.2	51
5380	433	7	59	5	19	5	9	7	2	39	21	7	0.3	52
5361	433	6	60	5	15	5	8	7	3	44	16	6	0.2	53
5404	383	6	58	7	18	4	10	8	3	34	12	5	0.2	54
5337	367	7	56	6	16	5	8	8	3	36	19	6	0.2	54
5339	383	7	59	5	19	5	8	8	3	43	16	5	0.3	54
5311	333	6	57	5	16	5	8	7	2	29	16	6	0.2	50
5358	400	6	60	5	16	4	8	7	3	35	17	5	0.2	53
5346	417	6	62	5	17	5	8	7	3	42	15	5	0.2	53
5345	417	6	65	5	16	5	9	7	3	40	13	4	0.3	54
5363	367	6	60	6	17	5	8	7	3	25	17	6	0.2	53
5338	367	5	63	5	17	5	8	6	3	39	18	5	0.2	53
5335	433	5	64	5	19	5	8	6	3	55	18	6	0.2	53
5315	383	6	60	4	18	5	8	7	3	41	15	4	0.2	53
5330	367	6	58	4	17	5	8	6	3	44	15	5	0.2	51
5328	400	5	60	5	17	5	8	6	3	37	15	5	0.2	53
5324	433	6	63	6	17	5	8	6	3	36	17	6	0.2	51
5350	450	5	65	5	19	5	7	5	3	39	15	5	0.2	51
5322	400	6	63	5	15	5	7	5	3	38	15	5	0.2	50
5415	417	6	60	4	17	5	8	5	3	35	17	6	0.1	53
5321	333	6	59	5	17	5	8	5	3	26	14	3	0.3	51
5329	367	5	59	4	18	5	7	6	3	39	17	7	0.2	53
5318	350	6	64	6	19	5	7	6	3	35	15	5	0.3	54
5344	367	5	64	4	16	5	7	5	3	44	15	6	0.1	51
5000	333	4	53	7	14	4	8	6	3	89	9	3	0.2	44
5362	433	5	61	4	15	4	8	5	2	38	16	4	0.2	51
5319	333	5	61	4	17	4	8	6	4	41	15	5	0.3	53
5316	400	5	63	4	17	5	7	5	3	33	19	7	0.2	51
5323	333	5	55	4	16	5	7	6	3	33	13	5	0.2	52
5357	383	5	64	5	17	5	8	6	3	47	19	5	0.3	54
5332	400	6	62	5	17	5	8	5	3	36	18	7	0.3	53
5334	400	5	63	4	16	5	8	6	3	35	15	6	0.3	51
5313	417	6	64	5	19	5	8	6	3	32	16	6	0.3	53
5354	450	5	60	4	16	5	8	5	3	33	16	7	0.3	50
5325	417	5	64	5	16	4	8	6	3	46	17	6	0.2	51
5320	383	6	64	4	18	5	7	6	3	33	13	5	0.2	51
5326	337	6	63	5	18	6	8	6	3	38	18	6	0.2	51
5351	450	5	60	5	18	4	8	5	4	41	16	5	0.2	54
5333	367	6	55	5	16	5	8	6	3	42	19	5	0.2	54
5280	400	5	61	5	16	5	8	6	3	48	13	4	0.3	53
5360	467	5	61	5	15	4	9	7	3	42	18	6	0.1	53
5396	317	5	50	5	16	5	8	6	4	25	10	4	0.2	57
5340	400	5	62	5	19	5	7	5	3	44	18	5	0.2	53
5420	417	5	67	5	19	5	8	6	3	37	17	5	0.3	54
5342	333	5	57	5	17	5	8	6	2	41	19	7	0.2	53

Annex 2. Mean values for each of the four clusters for the different characters in 51 grasspea populations

CLUSTER

	1				2				3				4			
	N	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max
Plantheight	35	392	317	467	12	379	317	433	3	388	383	397	1	333	333	333
StemThickn	35	5.4	3.7	6.0	12	6.3	5.0	7.0	3	5.6	5.3	5.7	1	4.3	4.3	4.3
Leafletlength	35	61.7	55	67	12	58.4	50	62	3	63.3	60	67	1	53.0	53	53
Leafletwidth	35	4.8	4.0	6.0	12	5.5	4.7	7.0	3	6.4	6.0	7.3	1	6.7	6.7	6.7
Stipulelength	35	17.1	15	19	12	17.2	15	19	3	20.8	20	22	1	14	14	14
Stipulewidth	35	4.9	4.0	5.7	12	4.9	4.3	5.3	3	4.6	4.3	5.0	1	4.3	4.3	4.3
Standardlength	35	12.3	11	14	12	13.2	12	14	3	13.4	13	15	1	10.0	10	10
Keellength	35	7.7	7.0	8.3	12	8.5	8.0	9.7	3	6.9	6.3	7.3	1	7.0	7.0	7.0
Keelwidth	35	10.0	9.0	11	12	10.8	10	12	3	8.6	8.3	8.7	1	9.7	9.7	9.7
Winglength	35	10.4	9.7	11	12	11.7	10	12	3	10.3	9.7	11	1	9.0	9.0	9.0
Wingwidth	35	5.8	5.0	7.5	12	7.3	6.7	8.3	3	6.1	5.7	6.3	1	5.3	5.3	5.3
Sepallength	35	6.1	5.3	7.5	12	6.5	5.7	7.7	3	7.7	7.7	7.7	1	4.3	4.3	4.3
Sepalwidth	35	1.4	1.0	2.0	12	1.5	1.0	2.0	3	1.3	1.0	2.0	1	1.0	1.0	1.0
Calyxtubelength	35	4.1	3.3	4.7	12	4.3	3.7	5.0	3	4.0	4.0	4.0	1	3.7	3.7	3.7
Podlength	35	35.8	31	39	12	36.6	33	41	3	35.9	35	36	1	28.3	28	28
Podwidth	35	11.7	10	14	12	12.2	11	14	3	12.1	12	13	1	9.3	9.3	9.3
SeedsperPod	35	3.0	2.3	3.7	12	2.9	2.3	3.7	3	2.8	2.3	3.0	1	3.0	3.0	3.0

Annex 3. Mean, Minimum and Maximum of each of the 4 clusters for each of the variables in different accessions of Lathyrus species.

	CLUSTER															
	1				2				3				4			
	N	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max	N	Mean	Min	Max
uplfltno	21	2.0	2.0	2.0	1	2.0	2.0	2.0	1	3.3	3.3	3.3	1	2.0	2.0	2.0
lowlefltno	21	2.0	2.0	2.0	1	2.0	2.0	2.0	1	0.0	0.0	0.0	1	2.0	2.0	2.0
tendrilno	21	3.0	3.0	3.0	1	4.5	4.5	4.5	1	2.3	2.3	2.3	1	3.0	3.0	3.0
stlength	21	13.4	12	16	1	11.8	12	12	1	15.7	16	16	1	8.3	8.3	8.3
stwidth	21	19.7	17	22	1	17.3	17	17	1	21.0	21	21	1	9.3	9.3	9.3
kl	21	8.2	7.0	9.7	1	7.3	7.3	7.3	1	9.0	9.0	9.0	1	8.0	8.0	8.0
kw	21	9.5	8.0	11	1	8.5	8.5	8.5	1	8.7	8.7	8.7	1	8.0	8.0	8.0
wl	21	11.1	10	13	1	10.0	10	10	1	11.0	11	11	1	8.0	8.0	8.0
ww	21	5.9	5.0	7.3	1	5.3	5.3	5.3	1	7.0	7.0	7.0	1	3.8	3.8	3.8
pdcl	21	19.9	12	23	1	19.5	20	20	1	27.7	28	28	1	10.0	10	10
nofl	21	1.0	1.0	1.0	1	1.0	1.0	1.0	1	1.0	1.0	1.0	1	1.0	1.0	1.0
pdl	21	31.1	25	35	1	34.8	35	35	1	31.7	32	32	1	31.3	31	31
pdw	21	12.2	10	15	1	14.3	14	14	1	11.0	11	11	1	9.3	9.3	9.3
sdl	21	8.0	5.3	11	1	10.0	10	10	1	6.7	6.7	6.7	1	4.7	4.7	4.7
sdw	21	7.0	4.7	9.3	1	9.3	9.3	9.3	1	6.0	6.0	6.0	1	4.0	4.0	4.0

Uplfltno= Upper leaflet number, lowlefltno= Lower leaflet number, tendtilno= Tendril number, stlength= Standard length, stwidth= Standard width, Kl= Keel length, Kw= Keel width, wl= Wing length, ww= Wing width, pdcl= Peduncle length, nofl= Number of flower per peduncle, pdl= Pod length, pdw= Pod width, sdl= Seed length, sdw= Seed width

Annex 4. List of labels for population samples used in Table 17.

Label	Population name
1	5288
2	6515
3	5295
4	5296
5	5330
6	5331
7	5282
8	5387
9	5390
10	5391
11	5395
12	5398
13	5405
14	5407
15	5410
16	5424
17	15606
18	15607
19	15608
20	15609