

Genetic Diversity Assessment of Grass Pea (*Lathyrus sativus* L.) Varieties from Southern Bangladesh Using RAPD Markers

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Abstract

Grass pea (*Lathyrus sativus* L.) is a promising crop for sustainable agriculture due to its low water demand and drought resistance. Its wider cultivation is constrained by low yield and the neurotoxin oxalyl diamino propionic acid (ODAP). To facilitate genetic improvement, this study assessed the genetic diversity of ten grass pea varieties (V1-V10), including five Bangladesh Agricultural Research Institute (BARI) lines and five local lines from southern Bangladesh, using 12 RAPD primers. The RAPD analysis generated a clear genetic fingerprint for the varieties, producing 549 bands (300 bp-5000 bp) with an overall polymorphism rate of 84.35%. UPGMA cluster analysis grouped the varieties into two major clusters, indicating substantial genetic variability. Genetic distances ranged from 0.1371 to 0.6292, with the highest similarity between V7 (Madaripur) and V8 (Barishal) and the greatest divergence between V1 (BARI Khesari-1) and V8 (Barishal). These findings provide molecular insights that can guide breeding programs to enhance agronomic traits, reduce neurotoxin ODAP levels, and develop DNA-based profiling systems for variety registration.

Introduction

Grass pea (*Lathyrus sativus* L.), locally called Khesari, an annual cool-season legume, is a promising candidate for sustainable agriculture and plays a vital role in regional food security in countries like Bangladesh, India, Pakistan, Nepal, and Ethiopia (Campbell et al. 1994, Kumar et al. 2018). Its nutritious benefits render it incredibly versatile, making it suitable as food for people, and as feed and fodder for animals (Lambein et al. 2019).

In addition, grass pea offers various environmental benefits. It demonstrated remarkable tolerance to abiotic threats, including drought, water scarcity, excess precipitation and flooding, soil fertility constraints, and climate change (Girma and Korbu 2012). Its hardy and penetrating root system allows it to be grown on a wide range of soils, including impoverished, saline, nutrient-deficient, and heavy clays (Abd-El-Moneim et al. 2001, Girma and Korbu 2012). Through symbiosis, it biologically fixes atmospheric nitrogen (108-125 kg/ha per season), which enriches the soil health and reduces the need for synthetic fertilizers (Campbell et al. 1994, Lambein et al. 2019). Grass pea is an excellent source of protein, starch, lysine, calcium, and vitamins for both human food and animal feed (Kouris-Blazos and Belski 2016). The immature seeds, foliage, and young plants are the main parts used of this plant (Chowdhury et al. 2005).

Barishal, the southern region of Bangladesh, located between the coastal zones and the deltaic plains, faces agricultural challenges such as cyclones, tidal surges, and high levels of soil salinity

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and waterlogging (Rahman and Uddin 2021, Al-Imran et al. 2025). These extreme conditions severely constrain the cultivation of traditional food crops. Grass pea is an indispensable resource for rainfed agriculture (Lambein et al. 2019). The continued success of the crop in this high-risk area, therefore, depends heavily on maintaining and utilizing its genetic diversity (Gonçalves et al. 2022, Dwivedi et al. 2023).

Despite its importance, the widespread adoption and genetic improvement of the grass pea have been considerably hindered by the presence of a neurotoxin, β -N-oxalyl-L- α , β -diamino propionic acid (ODAP) (Bekele-Alemu et al. 2024, Kuma and Shiferaw 2024). Long-term consumption of high-ODAP-containing varieties can lead to lathyrism (Kumar and Dubey 2001, Vaz Patto and Rubiales 2014). Consequently, the primary goal of modern breeding is to develop genotypes that combine high yield with low or zero ODAP content (Hanbury et al. 2000). Achieving these breeding objectives depends on a thorough understanding of the crop's genetic variability, which is essential for plant improvement (Kumar et al. 2012, Cobb et al. 2013).

The molecular characterization of *Lathyrus sativus* in Bangladesh appears to be in its early stages. Current research is primarily limited to a population structure analysis of 100 accessions using the EST-SSR marker and a separate diversity study using the ISSR marker (Asadova et al. 2020). A cytogenetic analysis has been conducted on only two released varieties, BARI Khesari-2 and BARI Khesari-3 (Rahman et al. 2023). This limited research demonstrates that the extensive genetic pool of local landraces, particularly those adapted to specific agro-ecological zones, remains largely underexplored.

To address this critical knowledge gap, the genetic resources of *Lathyrus* in key cultivation zones, such as the southern coast of Bangladesh, need to be systematically characterized. Under these circumstances molecular markers can be utilized as an essential tool in characterizing these genetic resources (Wani et al. 2012). The RAPD (Random Amplified Polymorphic DNA) is a PCR (Polymerase Chain Reaction) based technique that is a valuable tool for analyzing genetic polymorphism, constructing a genetic fingerprint, and selecting hybrids (Kumari and Thakur 2014, Babu et al. 2020). It is a very simple, fast, and effective method that doesn't require prior DNA sequence information (Sony et al. 2013, Mitra et al. 2017, Dutta et al. 2025). Further, it scans larger parts of the genome to reveal even small genetic differences (Ren et al. 2003). While the genetic diversity of *Lathyrus* has been studied globally, research in Bangladesh remains limited.

Therefore, this study was undertaken to molecularly characterize 10 grass pea varieties collected from Bangladesh Agricultural Research Institute (BARI) and different regions of southern Bangladesh to assess genetic diversity and identify unique, resilient genotypes for immediate use in breeding and conservation programs.

Materials and Methods

The current study involved ten different varieties of grass peas, of which five high-yielding varieties were released by BARI: V1 (BARI Khesari-1), V2 (BARI Khesari-2), V3 (BARI Khesari-3), V4 (BARI Khesari-5), and V5 (BARI Khesari-6). In addition, five local varieties were sourced from farmers, labeled as V6 (local variety: Faridpur), V7 (local variety: Madaripur), V8 (local variety: Barishal), V9 (local variety: Jhalokathi), and V10 (local variety: Bagerhat), and were collected from the local farmers and named after their source of collection areas. Seeds were sown and maintained in the net house of the University of Barishal.

Tender leaves of selected varieties were harvested, and total genomic DNA was extracted by using a modified CTAB method (Attitalla 2011). DNA concentration was quantified through a spectrophotometer (T60 UV-visible spectrophotometer). The PCR reaction mixture (25 μ l)

included 2 µl of template DNA (25 ng/µl), 15.3 µl of deionized distilled water, 5 µl of 5× Taq buffer, 1 µl of MgCl₂, 1 µl of primer, 1 µl of dNTPs (10 mM), and 0.2 µl of Taq DNA polymerase. PCR amplification was performed in an oil-free Mini-Amp thermal cycler (Applied Biosystems, Singapore) for 35 cycles after initial denaturation at 94°C for 5 min, denaturation at 94°C for 45 sec, annealing at 32-34°C for 30 sec, extension at 72°C for 3 min, and final extension at 72°C for 7 min. Out of twenty primers from Operon Technologies, USA, twelve produced clear banding patterns. The primers used in this RAPD analysis are listed in Table 1.

Table 1. The features of twelve arbitrary RAPD primers used in the present study.

Sl. No.	Primer	Annealing temperature (°C)
1	OPA01 (5'- CAG GCC CTT C -3')	34
2	OPA03 (5'- AGT CAG CCA C -3')	32
3	OPA04 (5'- AAT CGG GCT G -3')	32
4	OPA06 (5'- GGT CCC TGA C -3')	34
5	OPA07 (5'- GAA ACG GGT G -3')	32
6	OPA09 (5'- GGG TAA CGC C -3')	32
7	OPA10 (5'- GTG ATC GCA G -3')	32
8	OPB01 (5'- GTT TCG CTC C -3')	32
9	OPB02 (5'- TGA TCC CTG G -3')	32
10	OPB04 (5'- GGA CTG GAG T -3')	32
11	OPB05 (5'-TGC GCC CTT C -3')	34
12	OPB06 (5'-TGC TCT GCC C -3')	34

The amplified products were separated using electrophoresis on a 1% agarose gel, prepared with 1.0 g of agarose powder, 8 µl of ethidium bromide (10 mg/ml), and 100 ml of 1 × TAE buffer. Electrophoresis was conducted at 100V for 30 min, with a 1 kb plus DNA ladder run alongside the RAPD reactions as a marker. DNA bands were visualized on a UV transilluminator and photographed with a gel documentation system (FAS-Digi, Germany). After electrophoresis, the sizes of the amplification products were estimated by comparing them to the 1-kb plus molecular weight marker. Distinct bands, or RAPD markers, were assigned identification numbers and scored for presence (1) or absence (0) using “NTSYSpc V.2.10” (Sharifi et al. 2018). The data were then analyzed and combined into a single matrix, enabling the estimation of polymorphism levels, genetic distances, and the construction of a UPGMA (Unweighted Pair Group Method of Arithmetic Means) dendrogram for the ten grass pea varieties.

Results and Discussion

The utilization of RAPD markers has proven to be effective in demonstrating significant polymorphism and genetic variation, making it a suitable approach for *Lathyrus sativus* L. diversity studies (Bhadragoudar and Patil 2011, Thul et al. 2012). In this study, 20 RAPD primers were screened across 10 grass pea varieties (V1-V10), and 12 were selected for their ability to produce reproducible, scorable multiband fingerprints (Fig. 1). These 12 primers generated 549 distinct amplified bands, with product sizes ranging from 300 to 5000 bp.

The analysis of the scorable loci, as shown in Table 2, revealed an average of 9.17 loci per primer, resulting in 99 polymorphic loci out of a total of 110 loci. This resulted in a high overall average polymorphism rate of 90% across the markers. The observed polymorphism rate is higher

than other pulse crops, such as mung bean, green gram, and chickpea. This reinforces the concept that- grass pea germplasm maintains a broad genetic base for molecular analysis (Datta et al. 2010). The efficiency of the primers varied: five primers—OPA04, OPA06, OPA09, OPA10, and OPB04 exhibited maximum polymorphism at 100%, while the OPA01 primer recorded the minimum polymorphism rate at 81.82%.

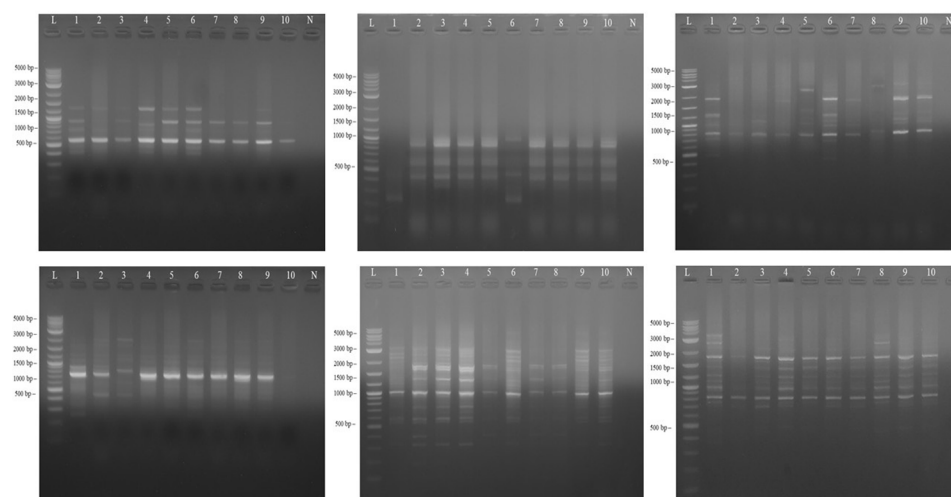


Fig. 1. RAPD analysis of ten grass pea (*Lathyrus sativus* L.) varieties. From left to right- 1 Kb plus DNA ladder (L), varieties BARI Khesari-1 (1), BARI Khesari-2 (2), BARI Khesari-3 (3), BARI Khesari-5 (4), BARI Khesari-6 (5), local variety: Faridpur (6), local variety: Madaripur (7), local variety: Barishal (8), local variety: Jhalokathi (9), local variety: Bagerhat (10), and Negative control (N).

Table 2. Levels of polymorphism within ten grass pea (*Lathyrus sativus* L.) varieties according to the markers.

Marker	ID Sequence (5'—3')	Total no. of RAPD loci	No. of polymorphic RAPD loci	No. of monomorphic RAPD loci	Percentage (%) of polymorphism
OPA01	CAG GCC CTT C	11	9	2	81.82
OPA03	AGT CAG CCA C	6	5	1	83.33
OPA04	AAT CGG GCT G	10	10	-	100
OPA06	GGT CCC TGA C	9	9	-	100
OPA07	GAA ACG GGT G	9	8	1	88.89
OPA09	GGG TAA CGC C	12	12	-	100
OPA10	GTG ATC GCA G	6	6	-	100
OPB01	GTT TCG CTC C	7	6	1	87.14
OPB02	TGA TCC CTG G	10	9	1	90
OPB04	GGA CTG GAG T	8	8	-	100
OPB05	TGC GCC CTT C	6	5	1	83.33
OPB06	TGC TCT GCC C	16	15	1	93.75

Polymorphism was also measured across the individual varieties (Table 3). The highest percentage of polymorphism was found in V6 (local variety: Faridpur) at 89.47%, while the lowest was observed in V8 (local variety: Barishal) at 77.14%. However, a past study reported a lower level of 59.09% polymorphism in three BARI-released grass pea varieties (Akter et al. 2015). The observed high polymorphism is likely due to interspecific variation within the

germplasm and the use of primers with high GC content (60-70%), which has been correlated with increased band amplification and polymorphism in molecular fingerprinting (Costa et al. 2016, Ongom et al. 2021). The 80 monomorphic bands (which were consistently present across all 10 varieties) indicate conserved genomic regions, likely essential for species survival and shared evolutionary history (Table 2).

Table 3. Levels of polymorphism within ten grass pea (*Lathyrus sativus* L.) varieties.

Varieties	Total no of bands	Total no of polymorphic bands	Total number of monomorphic bands	% of polymorphism	
				Exists in varieties	Average in varieties
V1 (BARI Khesari-1)	75	67		89.33	
V2 (BARI Khesari-2)	43	35		81.39	
V3 (BARI Khesari-3)	58	50		86.20	
V4 (BARI Khesari-5)	70	62		88.57	
V5 (BARI Khesari-6)	41	33	8	80.49	84.35
V6 (local variety: Faridpur)	76	68		89.47	
V7 (local variety: Madaripur)	39	31		79.49	
V8 (local variety: Barishal)	35	27		77.14	
V9 (local variety: Jhalokathi)	57	49		85.96	
V10 (local variety: Bagerhat)	55	47		85.45	

Genetic variability was quantified using Nei's (1972) genetic distance matrix (Table 4). The matrix showed a considerable range of genetic distance from 0.1371 to 0.6292. The observed genetic distance indicates substantial genotypic diversity, which is crucial for future breeding efforts. The highest divergence (0.6292) was observed between V2 and V10, suggesting that these accessions carry the most distinct genomic information and should be prioritized for hybridization to maximize heterosis and produce high-yielding, low-neurotoxin varieties (Kumar et al. 2011). Conversely, the low distance (0.1371) between V7 and V8 suggests these local varieties are closely related due to shared ancestry or common localized seed dispersal. The divergence level 0.069-1.003 is reported in a large study of *Lathyrus* populations using ISSR markers; also, the same type of phenomenon was observed in regional grass pea studies (Belaïd et al. 2006). This distance range aligns with the variability reported in *Lathyrus* populations using Nei's (1972) formula (Belaïd et al. 2006).

Table 4. Summary of Nei's (1972) genetic distances of ten grass pea (*Lathyrus sativus* L.) varieties.

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
V1	0.0000									
V2	0.4284	0.0000								
V3	0.4048	0.2999	0.0000							
V4	0.3127	0.3673	0.2424	0.0000						
V5	0.5008	0.5531	0.4354	0.3486	0.0000					
V6	0.2783	0.4834	0.4078	0.3290	0.4757	0.0000				
V7	0.5075	0.4447	0.2812	0.3526	0.3927	0.4507	0.0000			
V8	0.6068	0.5519	0.4211	0.4153	0.3603	0.5030	0.1371	0.0000		
V9	0.3917	0.4892	0.3542	0.2474	0.4905	0.2825	0.3320	0.2997	0.0000	
V10	0.4533	0.6292	0.4428	0.3297	0.5885	0.2808	0.4575	0.5224	0.2661	0.0000

The genetic relationship was visualized through a dendrogram constructed using the neighbour-joining method based on Nei's distance (Fig. 2). The cluster analysis corroborated the results of the distance matrix, leading to a dendrogram divided into two clusters (C1 and C2), which were further stratified into sub-clusters and sub-sub-clusters. Major cluster 1 (C1) included five varieties (V1, V4, V6, V9, and V10), while major cluster 2 (C2) included the remaining five. This main cluster highlights a significant genetic split in the studied germplasm. Within cluster-1 (C1), the close grouping of V1 and V6, as well as V9 and V10, indicated high genetic similarity even though these pairs have been collected from two distant districts. Moreover, the unique position of V4, which showed 88.57% polymorphism, indicated a genetic relationship with BARI Khesari- 1 and, interestingly, also with the local varieties from Faridpur, Jhalokathi, and Bagerhat, highlighting its potential as a valuable genetic bridge in breeding programs.

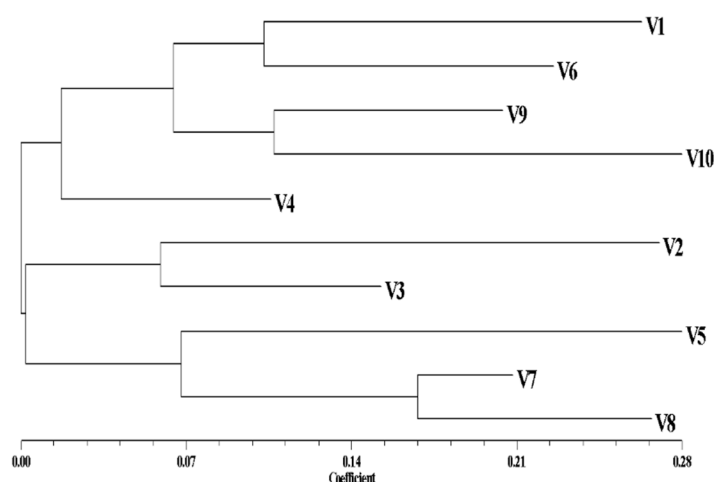


Fig. 2. A dendrogram was constructed based on Nei's (1972) genetic distance summarising the data on differentiation among ten grass pea (*Lathyrus sativus* L.) varieties by neighbour-joining methods on RAPD analysis. Here, V1 (BARI Kharsari-1), V2 (BARI Kharsari-2), V3 (BARI Kharsari-3), V4 (BARI Kharsari-5), V5 (BARI Kharsari-6), V6 (local variety: Faridpur), V7 (local variety: Madaripur), V8 (local variety: Barishal), V9 (local variety: Jhalokathi), and V10 (local variety: Bagerhat).

Within cluster-2 (C2), the groupings offer informative insights. The BARI-released varieties V2 and V3 clustered closely, confirming their expected shared genetic distance. Notably, V5 formed its own distinct branch, indicating it is genetically separated from the other BARI varieties in this cluster. A distinct sub-cluster formed by V7 and V8 confirmed the lowest genetic distance, placing them as the most genetically similar group, which is separated from the others.

These results, where genetic relatedness overshadows defined breeding history, are common in legume diversity studies (Smýkal et al. 2015). This categorization provides a framework for breeders, similar to mapping efforts in tomato (Foolad 2007). The clear separation of the two major clusters confirms that crossing parents selected from C1 and C2 would likely yield the most genetically diverse and vigorous progeny. The molecular characterization using RAPD analysis proved effective, and this successfully generated DNA fingerprints for grass pea varieties of Bangladesh. The results of the present study further indicated that RAPD markers represent efficient tools for estimating the genetic variability and the genetic relationships among the ten grass pea varieties, which can help researchers for immediate use in breeding and conservation programs.

This study successfully generated DNA fingerprints, and quantified the polymorphism and genetic variations among ten grass pea varieties using RAPD markers. The high polymorphism rate of 90.0% and the wide range of genetic distance (0.1371 to 0.6292) confirm the existence of a diverse genetic base. The identification of genetically divergent pairs (V2 and V10) and closely related pairs (V7 and V8) is valuable for germplasm management. This genetic classification offers a valuable framework for the strategic selection of parent plants for hybridization and facilitates the application of marker-assisted selection (MAS) tools to enhance the genetics of grass pea.

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