J. Agr. Sci. Tech. (2022) Vol. 24(6): 1429-1442

Deciphering Genetic Diversity in Grass Pea (*Lathyrus sativus* L.) Collections Using Agronomic and Forage Quality Traits and SSR Markers

V. Kumar Yadav[1*], A. Radhakrishna[1], M. Mohan Das[1], T. Singh[1], S. Yadav[1], P. Sharma[1], A. Kumar Mishra[1], and A. Sarker[2]

ABSTRACT

Grass pea (*Lathyrus sativus* L.) is an important dual-purpose crop in drought and famine prone areas as it is used as human food as well as livestock feed and fodder. However, the variation for forage quality traits of grass pea remains largely unexplored. This study aimed to characterize the genetic diversity of grass pea collections from Africa, Asia, and Europe, and identify genotypes for superior agronomic and forage nutritional quality traits. The principal component analysis revealed that the first three principal components from nutritional quality parameters *viz*., NDF, ADF, cellulose, lignin and ash percent, and from agronomic traits *viz*., plant height, nodes per plant, leaf area, green and dry biomass accounted for the majority of the total variation. In addition, a total of 59 polymorphic alleles were detected at 11 SSR loci with an average of 5.36 alleles per locus and the polymorphic information content ranged from 0.49 to 0.76. Three accessions (IF1872, IF2177 and IF2156) with higher biomass than the check and four accessions (IF1327, IF1312, IL-10-76 and IF1307) with excellent nutritive value in both green forage as well as straw were identified. The present study revealed high genetic variation for biomass and nutritional quality traits in grass pea collections that could be useful for development of high-yielding, nutritionally rich, and dual-purpose varieties.

Keywords: Dual-purpose crop, IVDMD, SSR markers, Straw quality, Trait-specific germplasm.

INTRODUCTION

Grass pea (*Lathyrus sativus* L.) is an economically important annual legume commonly grown for food, feed, and fodder purposes. Grass pea is cultivated in many countries of Europe, Northern Africa, parts of Mediterranean region and South Asian countries like India, Bangladesh, Nepal and Pakistan. It is a popular crop in abiotic stress-prone areas due to its remarkable ability to withstand extreme environments like drought, waterlogging, capability to grow in poor and marginal soils, and inherent ability to fix atmospheric nitrogen to increase the fertility of soils (Campbell *et al.*, 1994; Croft *et al.*, 1999). Grass pea is a viable resource in the entire Semi-Arid Tropics (SAT) region where scarcity of water and fodder are the most important constraints faced by the livestock husbandry.

At present, India faces a net deficit of 31% green fodder and 12% dry fodder resulting in nutritional imbalance and reduced livestock productivity. As the land area for forage crop cultivation cannot be increased due to high pressure for cultivation of food and commercial crops, optimization of fodder production, value addition of crop residues, utilization of unexplored feed resources and breeding forage varieties to withstand the environmental constraints are essentially required. In India, grass pea is

[1] ICAR- Indian Grassland and Fodder Research Institute, Jhansi- 284003, UP, India.
[2]International Center for Agriculture Research in the Dry Areas, New Delhi, India.
*Corresponding author; e-mail: vijayyadav777@gmail.com

[DOI: 10.52547/jast.24.6.1429]

cultivated on 521,100 ha mainly in the states of Chhattisgarh, Bihar, Jharkhand, Maharashtra, Orissa, Assam, West Bengal parts of Uttar Pradesh and Madhya Pradesh (Sarker *et al.,* 2015). Further, every year, approximately 2 MT of dry crop residue produced goes unutilized, even though studies have shown that grass pea hay can be safely incorporated into ruminant's diet without any adverse effects on animal health (Das *et al.,* 2015). In the past, grass pea improvement programs in India were successful in developing varieties like Ratan, Mahateora, Prateek, Pusa24 and Nirmal with low ODAP content (Singh *et al.,* 2013). Several studies have been conducted to assess genetic diversity using morphological and molecular markers in range legumes (Zarabiyan and Majidi, 2015, Irani *et al*., 2016). However, such efforts in grass pea were mainly related with ODAP levels and seed yield using agro-morphological traits, biochemical and molecular markers (Wang *et al.,* 2015; Gupta *et al.,* 2018; Kumar *et al.,* 2011; Lioi *et al.,* 2011; Basaran *et al.,* 2013, Arslan *et al*., 2020). However, only few studies investigated the forage nutritional aspects of grass pea (Basaran *et al.,* 2011).

Thus, the present study was carried out to estimate the genetic variability based on the agronomical, molecular and forage nutritional quality traits with emphasis for identification of germplasm for future dual purpose breeding programs.

MATERIALS AND METHODS

Plant Material and Location of the Experiment

A total of 44 accessions with low ODAP content in seeds including five released varieties *viz*., Ratan, Nirmal, Pusa 24, Mahateora, and Prateek were selected for this study. All the accessions, except released varieties and eight landraces, were procured from ICARDA, Lebanon, originally collected from 8 countries (Table 1). The experiment was conducted during winter season of 2016-17 at ICAR-Indian Grassland Fodder Research Institute (25^{o} 4' N, 78^{o} 6' E; 285 m above sea level) Jhansi, India. The climate of the location was typically semi-arid with yearly average minimum and maximum temperatures of 18^{o}C and 32.6^{o}C, respectively. The experiment was conducted in Randomized Complete Block Design (RCBD), with three replications for each accession represented by a plot size of 3.2×4 m dimensions with 8 rows in each plot with 40 cm row to row and 10 cm plant to plant distance.

Trait Evaluation

Grass pea accessions were evaluated for agronomic and forage quality traits. Agronomic traits *viz*., plant height (cm), nodes

Table 1. List of grass pea (*Lathyrus sativus*) accessions and their source.

Accession ID	Origin
IF3	Turkey
IFS 463, IF 471, IF 478	Ethiopia
IF 587	Syria
IL-10-55, IL-10-65, IL-10-58, IL-10-75, IL-10-76, IL-10-54, IL-10-57, IL-10-61, Ratan, Nirmal, Pusa 24, Mahateora and Prateek	India
IF225	Slovakia
IF1928	Nepal
IF1304, IF1306, IF1307, IF1309, IF1312, IF1316, IF1322, IF1327, IF1332, IF1341, IF1310, IF1311, IF1313, IF1314, IF1337, IF1344, IF1346. IF1347, IF1348	ICARDA
IF1351	Bolivia
IF1872, IF2156, IF2177, IF2329	Bangladesh

[DOI: 10.52547/jast.24.6.1429]

per plant, internode length (cm), days to 50% flowering, green forage yield (kg ha^{-1}) and dry matter yield (kg ha^{-1}) were recorded at 50% flowering stage while straw yield (kg ha^{-1}), 100-seed weight (g) and seed yield were recorded at maturity after harvesting and threshing of crop. Green forage yield was obtained by harvesting 4 rows/plot, and dry matter yield was recorded by oven drying the samples at 65ºC for 24 hours. To estimate fodder quality-related attributes, 50 g samples of each accession was drawn from green dried samples and straw, and analyzed for Crude Protein (CP), organic matter, and ash content as per the standard procedures of AOAC (2005), Neutral Detergent Fibre (NDF) as described by Van Soest *et al.* (1991), while Acid Detergent Fiber (ADF) and Acid Detergent Lignin (ADL) were determined according to the method described by Goering and Van Soest (1970) and *In Vitro* Dry Matter Digestibility (IVDMD) using the 2-stage technique of Tilley and Terry (1963).

Data Analysis

To describe the variability among the accessions, several simple univariate statistics including mean, range and variation were used. The Coefficient of Variation (CV) was also calculated from the variance components and overall mean for all the investigated traits. Clustering of accessions was carried out based on the morphological data using hierarchical clustering. A dendrogram was constructed on the basis of fusion level to examine similarities in pattern of performance among accessions. Correlations amongst traits were computed utilizing agronomic and forage quality traits. Data of all traits were standardized to a mean of zero and variance of one. Principal Component Analysis (PCA) was estimated utilizing the matrix of correlation coefficient derived from the standardized data to investigate the importance of different traits in explaining multivariate polymorphism. Dissimilarity matrices were constructed using cluster R packages. To assess the resemblance between the genotypic and phenotypic matrices, the correlations and their significances were tested with the Mantel Z test (Mantel, 1967) with 9,999 permutations using the vegan R package.

DNA Isolation and PCR Amplification

Total genomic DNA was extracted from fresh tender leaves based on a modified Cetyltrimethyl Ammonium Bromide (CTAB) method described by Stein *et al.* (2001). The quantity and quality of the DNA was confirmed on 0.8% agarose gel stained with ethidium bromide as well as spectrophotometrically. A total of 27 cross-transferable *Trifolium* SSR markers were used to screen the 44 grass pea accessions in this study as shown in Table 2. Amplification of samples through Polymerase Chain Reaction (PCR) was performed in a 20 µL final volume containing 20 ng of template DNA, 1X PCR buffer with 15 mM $MgCl_2$,1µL each primer (forward and reverse), 200 µM each dNTP, 1U Taq DNA polymerase. The reaction was performed in a BIOER thermocycler programmed as 94°C for 3 minutes; followed by 34 cycles at 94°C for 30 seconds; annealing at 54-56°C for 45 seconds; extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. Amplified products were separated on 2% (w/v) agarose gels and visualized by ethidium bromide staining.

SSR Analysis

SSR fragments were scored using a binary system for the presence or absence of each fragment for all markers. The total number of monomorphic and polymorphic bands were scored into a binominal matrix and Polymorphism Information Content (PIC) of each marker was calculated (Powell *et al.*, 1996). The SIMQUAL program of NTSYS-

[DOI: 10.52547/jast.24.6.1429]

Table 2. Primer sequences and Polymorphic Information Content (PIC) of 11 SSRs in 44 accessions of grass pea.

Primer ID	Forward primer sequence (5' – 3')	Reverse primer sequence (5' – 3')	Polymorphic alleles	PIC
GpSSR5	ACCACTGCACCATACAACCA	CCGAAAACAAACCATCAGC	6	0.55
GpSSR102	ACCACCATCAACCAACCCTA	AATTCTATGGAGCACGGGA	5	0.76
GpSSR118	TTTGGTGAACGGAACGAGT	AGTACCTGGGAGTGGTCACG	7	0.49
GpSSR124	AGGGAGTGGTGAAGGAGAGG	CAGAGGGCACATCTTACCC	3	0.67
GpSSR125	TAGATTGAGCCCATTGGAGG	GAGCCTACCGCAGCAATAA	6	0.69
GpSSR150	TCAGCAATGTTTGCGAACTC	CCTGACACTGGACACGACA	6	0.62
GpSSR156	TGCTCCCAAAGGTCACAAA	GAGCATCGACGAGAAGAAG	6	0.61
GpSSR165	CCATCCAAAAACCCTTCTCA	GAACTTCATCCCCTCAACCA	6	0.66
GpSSR172	ATGGGGTTGTTGGAAATGA	TCACCACCACCAATTCACTC	3	0.75
GpSSR179	TAGATGCACCGATCAACCAA	TGACAGGCAGAAGAAGAGCA	6	0.55
GpSSR180	ACGTCTGAATCGGATTTTCC	GCTGCAGGAATCTTCAAAA	5	0.71

pc (Ver 2.1) software (Rohlf, 2000) was used to calculate Jaccard's similarity coefficient and a dendrogram was constructed by Unweighted Pair-Group Method with Arithmetic average (UPGMA) method.

RESULTS

Trait Evaluation

Univariate evaluation based on agronomic and nutritional quality traits of 44 grass pea accessions including five commercial cultivars showed wide variation for most of the evaluated traits. Descriptive statistical analysis for agronomic traits showed that green forage yield (3,900-25,350 kg ha^{-1}), dry matter yield (996-5,744 kg ha^{-1}) and straw yield (1,200-8,395 kg ha^{-1}) were highly variable (CV> 38%), whereas plant height (34.74-62.91 cm), nodes/plant (9.84-17.69), internode length (1.21-2.74 cm), 100 seed weight (6.43-15.46 g), and days to 50% flowering (57-92 days) were moderately variable (CV 10-20%) and leaf size was the least variable (Table 3). Among nutritional attributes, green fodder crude protein (13.97-21.32%) showed maximum variation.

Correlation Analysis

The Pearson's correlation coefficients were computed for 28 traits. Among the possible correlation combinations, 19 character pairs showed significant correlation either in positive or negative direction (P≥ 0.01). Among the agronomic traits, DMY showed significant positive associations with GFY, straw yield and days to 50% flowering. Straw yield had significant positive associations with GFY and days to 50% flowering. Seed yield showed significant negative (P≥ 0.01) association with days to 50% flowering, GFY, DMY and straw yield. Crude protein of green forage showed negative significant associations with plant height, nodes/plant and seed yield (Table 4).

Cluster Analysis

Cluster analysis of grass pea accessions was performed to reveal complex relationships among the evaluated accessions. Similar accessions were clustered according to minimal distance analysis based on the mean values of 28 agro-nutritional traits. The 44 genotypes were classified into five clusters and a number of sub-clusters (Figure 1). The cluster analysis showed significant inter-cluster and intra-cluster diversity. The clusters comprised 5 to 17 accessions that were similar for specific traits. The distribution pattern indicated that maximum number of genotypes (17) were included in cluster II followed by cluster III (09), cluster V (7), cluster I (6) and least in cluster IV (5). Difference in cluster means existed for almost all the characters studied. Clusters mean value of important

[DOI: 10.52547/jast.24.6.1429]

Table 3. Descriptive statistics of agronomic traits and nutritional traits in grass pea accessions. [a]

Traits	Mean±SE	Range	CV%
Plant height (cm)	48.06±0.86	34.74-62.91	11.85
Nodes/Plant (No.)	13.77±0.23	9.84-17.69	11.31
Internode length (cm)	1.93±0.06	1.21-2.74	19.48
Leaf size (cm^2)	8.58±0.11	7.18-10.58	8.77
100 Seed weight (g)	8.74±0.26	6.43-15.46	19.89
Days to 50% flowering	75.48±1.44	57.00-92.00	12.66
GFY (kg ha^{-1})	14480±937.02	3900-25350	42.92
DMY (kg ha^{-1})	3735±216	996-5744	38.38
Straw yield (kg ha^{-1})	5198±327	1200-8395	41.78
Seed yield (kg ha^{-1})	1281±39.09	671-1696	20.23
aCP	17.27±0.30	13.97-21.32	11.48
bCP	10.66±0.12	8.85-12.58	7.58
aIVDMD	63.41±0.21	61.35-66.50	2.17
bIVDMD	57.58±0.18	55.08-60.34	2.07
aNDF	44.93±0.66	35.72-52.45	9.67
bNDF	52.99±0.45	45.66-59.93	5.68
aADF	35.21±0.62	27.18-41.45	11.65
bADF	38.21±0.38	33.33-43.41	6.53
aHemicellulose	9.77±0.29	6.50-14.43	19.91
bHemicellulose	14.98±0.47	7.69-21.42	20.8
aCellulose	27.62±0.49	20.17-32.45	11.83
bCellulose	28.47±0.33	24.19-32.65	7.59
aLignin	7.80±0.18	4.70-9.39	14.9
bLignin	9.52±0.18	7.33-12.72	12.64
aAsh	8.26±0.09	6.80-9.76	7.46
bAsh	8.54±0.11	6.23-10.96	8.92
aOM	91.74±0.09	90.24-93.20	0.67
bOM	91.41±0.13	88.77-93.77	0.94

[a] GFY= Green Forage Yield, DMY= Dry Matter Yield, CP= Crude Protein, ADF= Acid Detergent Fiber; NDF= Neutral Detergent Fiber; IVDMD= *In-Vitro* Dry Matter Digestibility, OM= Organic Matter, CV= Coefficient of Variation, SE= Standard Error. Green forage nutritional quality traits, Straw nutritional quality traits.

traits like green biomass, seed yield and forage quality characters are shown in Table 5. The higher mean value for GFY, seed yield, forage quality traits, delayed maturity was recorded in the first cluster; indicating the genetic potentiality to contribute for better biomass and seed yield. Cluster IV with lowest value for days to 50% flowering (62 days) indicated the presence of early maturing lines. Variation for straw quality traits was not found among the clusters.

SSR Markers and Clustering

In this study, 70 SSR markers of *Trifolium* were screened, of which 27 SSR markers (38.5%) were found to be cross-transferable and were found suitable to assess the genetic diversity in grass pea. Of the 27 markers screened, 11 markers (40.7%) that displayed clear and reproducible bands were included in the analysis as shown in Table 2 and Figure 2 (a-b). The level of polymorphism among the 44 accessions was evaluated by calculating the polymorphic alleles and PIC values of 11 markers. A total of 59 polymorphic alleles were detected at 11 SSR loci across the 44 accessions with the number of alleles per locus ranging from 3 to 7, with an average of 5.36 alleles per locus. The highest number of alleles (7) was recorded in the primer GpSSR118, followed by 6 alleles in six primers (GpSSR5, 125, 150, 156, 165 and 179) 5 alleles in two primers (GpSSR102 and GpSSR180).

[DOI: 10.52547/jast.24.6.1429]

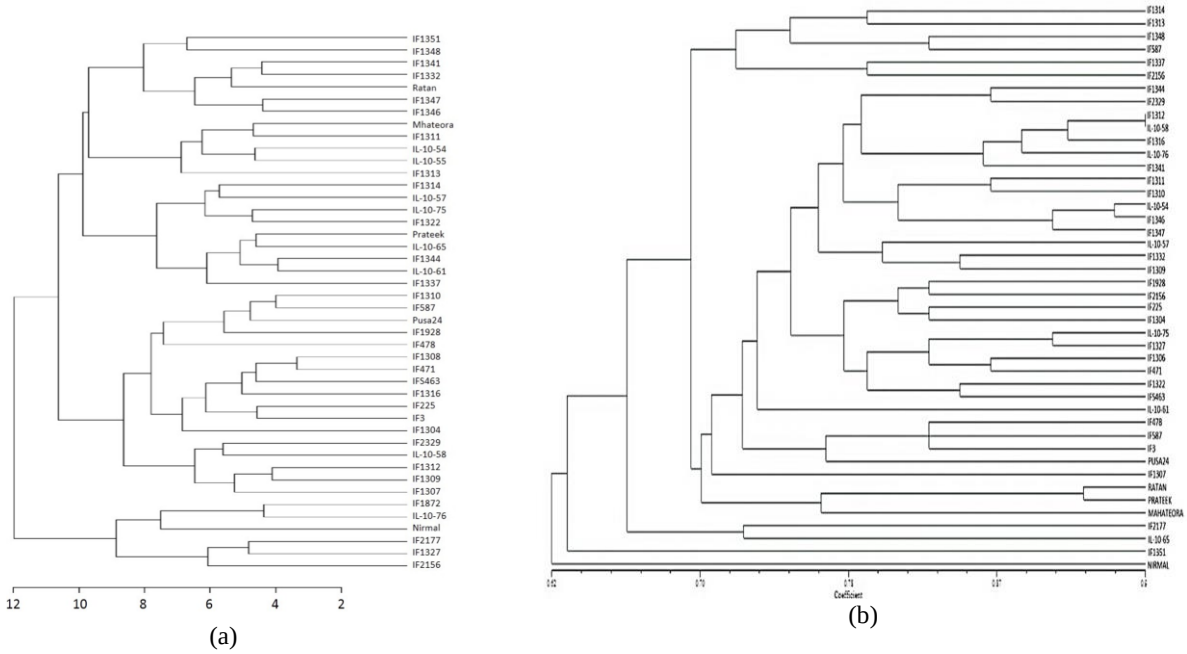

Figure1. Dendogram showing the genetic relationship among 44 grass pea accessions based on: (A) Agro-nutritional traits and (B) SSR based markers.

Table 4. Correlation between agronomic and forage and straw quality traits in grass pea accessions. [a]

Traits	Pht	NPP	INL	LS	HSW	DTFF	GFY	DMY	STY	SY
NPP	0.79**									
INL	0.28	0.11								
LS	0.61**	0.40**	0.19							
HSW	0.13	-0.01	-0.2	0.2						
DTFF	-0.1	-0.06	-0.04	0.(	-0.26					
GFY	0.01	-0.03	0.08	-0.	-0.22	0.61**				
DMY	0.1	0.01	0.13	0.(	-0.21	0.58**	0.97**			
STY	0.1	0.01	0.11	0.(	-0.21	0.60**	0.97**	0.97**		
SY	0.2	0.25	-0.11	0.(	0.16	-0.57**	-0.51**	-0.44**	-0.43**	
aNDF	0.16	0.23	-0.18	-0.	0.09	-0.06	-0.02	-0.03	-0.01	0.25
aADF	0.15	0.21	-0.2	-0.	0.06	0.08	0.05	0.04	0.06	0.23
aHemicellulose	0.19	0.2	0.09	-0.	0.08	-0.17	-0.07	-0.03	-0.04	0.04
aCellulose	-0.02	0.07	-0.2	-0.	-0.08	0.18	0.17	0.16	0.18	0.07
aLignin	0.09	0.07	-0.05	-0.	0.10	-0.09	0.1	0.14	0.15	0.15
asAsh	-0.22	-0.21	0.02	-0.	0.13	-0.19	-0.01	-0.02	-0.04	0.14
aOM	0.22	0.21	-0.02	0.1	-0.13	0.19	0.01	0.02	0.04	-0.14
aCP	-0.48**	-0.53**	0.07	-0.	-0.25	0.17	0.05	-0.01	-0.02	-0.42**
aIVDMD	-0.02	-0.06	0.21	0.1	-0.1	-0.06	0.05	0.08	0.06	-0.17
aNDF	-0.08	0.04	-0.24	-0.	0	-0.06	0.22	0.14	0.15	0.04
bADF	0.16	0.3	-0.31	0	0.16	-0.24	-0.12	-0.15	-0.14	0.36
bHemicellulose	-0.25	-0.16	0.02	-0.	-0.18	0.17	0.23	0.18	0.19	-0.2
bCellulose	0.41**	0.42**	-0.29	0.1	0.14	-0.11	-0.09	-0.04	-0.03	0.34
bLignin	-0.28	-0.23	-0.11	-0.	0.06	-0.07	-0.18	-0.31	-0.3	-0.13
bAsh	-0.12	-0.06	-0.01	0.(	-0.19	0.39	0.13	0.11	0.12	-0.38
bOM	0.05	0.04	0.07	-0.	0.17	-0.44**	-0.16	-0.15	-0.17	0.35
bCP	0.04	-0.19	0.34	0.2	0.06	-0.06	-0.16	-0.13	-0.15	-0.04
bIVDMD	-0.02	-0.1	0.3	0.1	-0.02	-0.13	-0.01	0.01	-0.01	0.07

[a] CP= Crude Protein, ADF= Acid Detergent Fiber, NDF= Neutral Detergent Fiber, IVDMD= *In-Vitro* Dry Matter Digestibility, OM= Organic Matter, Pht= Plant Height, NPP= Nodes/Plant, INL= Internode Length, LS= Leaf Size, HSW= 100 Seed Weight, DTFF= Days To 50% Flowering, GFY= Green Forage Yield, DMY= Dry Matter Yield, STY= Straw Yield, SY= Seed Yield. [a]Green forage nutritional quality traits, [b]Straw nutritional quality traits. ** Significant at P< 0.01 respectively.

[DOI: 10.52547/jast.24.6.1429]

Table 5. Cluster-wise mean performance of yield and forage quality attributes in 44 grass pea accessions.

Cluster no		DTFF	GFY	SY	STY	aCP	aIVDMD	bCP	bIVDMD
	IF1327	82	18571	6500	925	21.32	61.35	10.82	56.23
	IL-10-76	80	19500	7190	1220	19.86	63.60	12.58	57.21
1	IF1872	88	25100	7985	737	18.43	63.80	11.86	57.58
	IF2156	92	25350	8155	763	20.05	62.81	9.73	57.01
	IF2177	88	25230	7980	903	19.74	62.15	9.21	57.05
	Nirmal	70	20100	7995	1360	14.32	65.23	10.56	58.26
	Cluster Mean	83	22309	7634	985	18.95	63.16	10.79	57.22
	IF3	66	7800	3020	1109	19.89	65.58	10.64	58.11
	IF225	78	16250	5600	1083	19.11	66.08	11.31	59.37
	IFS 463	80	7800	2855	1472	16.62	64.56	10.75	58.58
	IF 471	79	9750	3330	1336	17.93	64.26	11.09	58.25
	IF 478	70	3900	1200	1629	19.53	63.25	11.84	58.64
2	IF 587	69	9230	3260	1697	19.27	62.23	11.11	57.45
	IF1304	78	9230	3670	1139	18.09	65.11	11.79	57.03
	IF1306	80	12188	3200	996	18.74	65.50	10.98	59.70
	IF1307	71	13000	5560	1278	17.74	65.62	11.48	60.34
	IF1309	68	16250	6160	1385	20.50	64.40	10.78	60.05
	IF1312	62	22939	7540	1400	18.94	66.50	10.93	59.79
	IF1316	83	10953	4120	980	17.54	63.49	10.62	57.23
	IL-10-58	73	19500	7990	1125	17.03	65.13	9.94	58.10
	IF1928	81	6500	2510	1291	20.62	62.52	10.76	56.34
	IF2329	91	24200	7990	671	19.46	64.98	9.91	59.09
	IF1310	73	13000	4390	1483	16.03	62.65	10.98	58.35
	Pusa 24	72	13000	4950	1472	16.17	63.73	10.65	57.20
	Cluster Mean	75	12676	4550	1267	18.42	64.45	10.92	58.45
	IF1322	84	13923	5168	1088	16.34	62.56	12.01	57.42
	IL-10-75	84	16250	6470	1027	18.12	64.26	10.57	56.12
	IL-10-57	86	21645	7130	909	14.81	61.56	10.40	55.08
3	IF1314	84	16250	6560	1330	15.02	63.25	10.63	57.52
	IL-10-61	85	14996	5695	1671	16.02	63.45	10.05	56.85
	IL-10-65	87	19500	7290	1393	14.95	62.89	9.54	57.51
	IF1337	88	21645	8395	1125	15.31	61.56	10.80	57.00
	IF1344	82	19500	7390	1430	13.97	62.56	10.42	56.26
	Prateek	73	24375	7950	1503	14.95	62.89	10.46	56.63
	Cluster Mean	84	18676	6894	1275	15.50	62.78	10.54	56.71
	IL-10-55	59	5571	1930	1443	17.22	62.15	10.41	57.32
	IL-10-54	68	7800	2510	1443	15.72	61.56	9.78	56.64
4	IF1311	64	7215	2220	1420	17.46	62.56	10.59	57.83
	IF1313	57	5571	2100	1310	15.90	63.78	10.27	55.18
	Mahateora	64	8450	2710	1390	15.50	63.45	10.29	56.12
	Cluster Mean	62	6921	2294	1401	16.36	62.70	10.27	56.62
	IF1332	77	17726	6950	1420	17.88	62.14	11.30	57.23
	IF1341	63	12000	4450	1343	16.25	61.42	10.11	56.34
5	IF1346	78	13923	5320	1608	15.90	62.56	9.07	57.60
	IF1347	76	14996	5510	1593	16.07	63.45	8.85	58.07
	IF1348	68	10790	3895	1534	14.25	62.12	12.32	57.80
	IF1351	57	5909	2110	1495	15.55	63.55	10.75	58.45
	Ratan	63	9750	3820	1452	15.63	61.56	10.05	57.52
	Cluster Mean	69	12156	4579	1492	15.93	62.40	10.35	57.57

[a] CP= Crude Protein, ADF= Acid Detergent Fiber, NDF= Neutral Detergent Fiber, IVDMD= *In-Vitro* Dry Matter Digestibility, OM= Organic Matter, Pht= Plant Height, NPP= Nodes/Plant, INL= Internode Length, LS= Leaf Size, HSW= 100 Seed Weight, DTFF= Days To 50% Flowering, GFY= Green Forage Yield, DMY= Dry Matter Yield, STY= Straw Yield, SY= Seed Yield. a a Green forage nutritional quality traits, b Straw nutritional quality traits. ** Significant at P< 0.01 respectively.

[DOI: 10.52547/jast.24.6.1429]

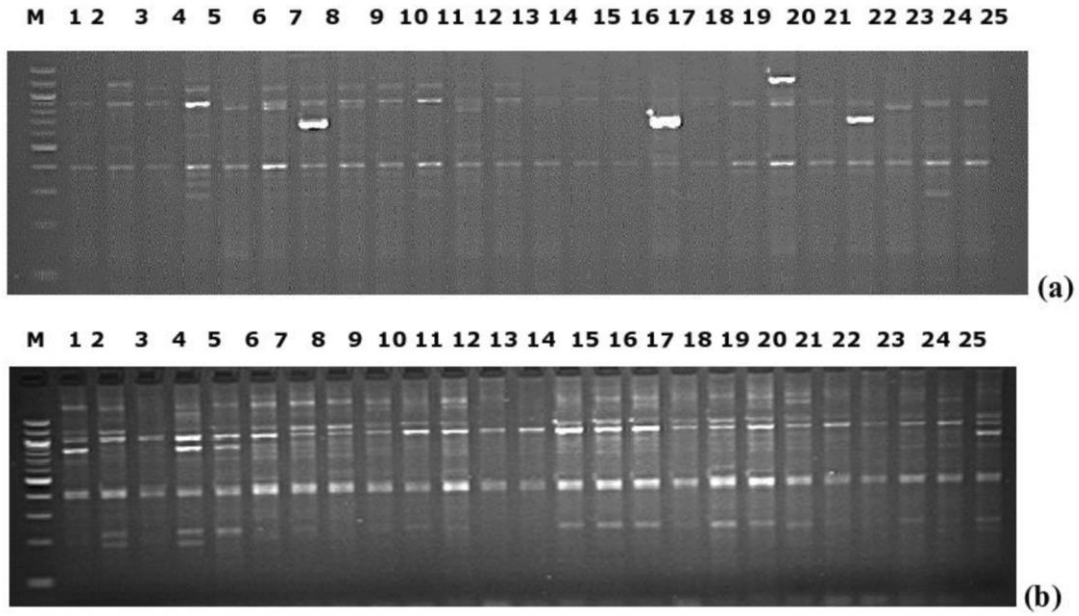

Figure 2. Polymorphism among 24 accessions of grass pea as revealed using primer: (a) GpSSR 156, M ladder (100 bp) and (b) GpSSR 150.

The lowest number of alleles (3) was found in GpSSR172.The PIC values of the SSR markers ranged between 0.49 and 0.76, with an average of 0.64.

The cluster analysis was carried out based on the similarity coefficients generated from the binary data of SSR markers. Significant genetic variation was observed among the grass pea genotypes with the similarity coefficient value ranging from 0.62 to 0.95. At 0.66 similarity coefficient, all the grass pea genotypes were classified into 4 clusters. Cluster I comprised 40 accessions that were further grouped into 3 sub-clusters at a similarity coefficient of 0.7 (Figure 1). Cluster Ia included 6 accessions (IF1314, IF1313, IF1348, IF587, IF1337 and IF2156). Cluster Ib included 30 accessions. Cluster II included two accessions: IF2156 and IL-10-65. Clusters III and IV included one accession each of IF1351 and Nirmal, respectively.

Principal Component Analysis

The PCA of 44 grass pea accessions for 28 traits revealed that the first eight principal components exhibited more than one eigenvalue and accounted for 80.2% of the total variation (Table 6). The grass pea lines and characters were super imposed on the biplot derived from first and second PC contributed 36.47% of the total variation (Figure 3). Green forage quality traits NDF, ADF, cellulose, and biomass contributing traits GFY, straw yield, DMY, days to 50% flowering, and seed yield were well represented with high amount of variability, while internode length, leaf size, seed weight, OM, ash content and hemicellulose showed the lowest variability. The grass pea lines IF1307, IF1351, IF 478, IF1872, IF2156 IF3, IF225, Prateek distant from origin showed more variation and less similarity with other varieties. The variation in the remaining six principal components is originated by plant height, nodes/plant, internode length, leaf size; green forage quality traits like NDF, ADF, hemicellulose, cellulose and lignin; and straw quality traits like ash and hemicellulose.

Mantel correlation assay between the agronomic, forage quality, genotypic and combined dissimilarity matrices showed that molecular markers and green forage quality markers had significant correlation.

[DOI: 10.52547/jast.24.6.1429]

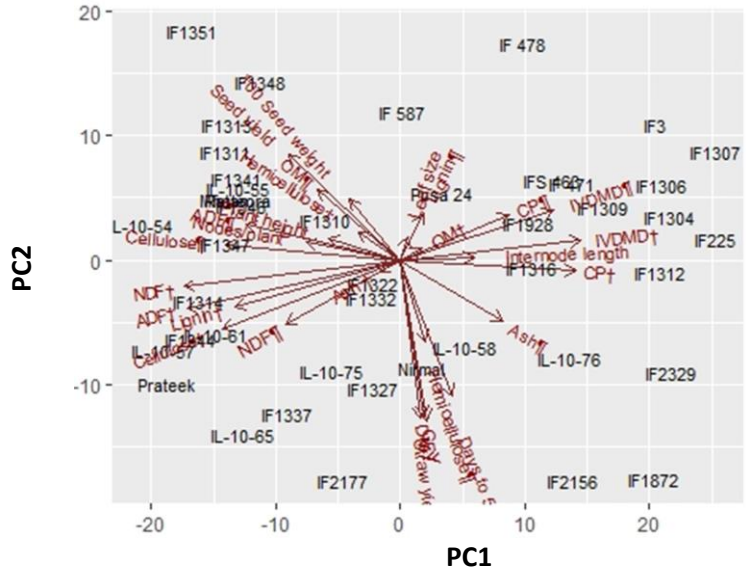

Figure 3. PCA-Biplot of biomass, forage quality, and straw quality traits; and grass pea genotypes.

Table 6. Principal component analysis of agronomic and forage quality traits in 44 grass pea accessions.

Characters	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
Plant height	-0.11	0.04	-0.45	0.26	-0.01	0.02	-0.13	0.08
Node/Plant	-0.16	0.03	-0.38	0.18	-0.12	-0.02	-0.20	0.26
Internode length	0.14	0.01	-0.15	0.22	0.34	0.13	-0.02	0.23
Leaf size	0.03	0.06	-0.36	0.23	-0.08	0.15	-0.16	-0.36
100 Seed weight	-0.09	0.15	0.01	0.11	0.01	-0.13	0.10	-0.41
Days to 50% flowering	0.12	-0.34	-0.10	-0.11	-0.17	0.17	0.12	-0.08
GFY	0.07	-0.42	-0.12	-0.02	0.14	-0.24	0.07	-0.10
DMY	0.07	-0.41	-0.17	0.04	0.16	-0.23	0.08	-0.10
Straw yield	0.06	-0.41	-0.17	0.03	0.15	-0.21	0.08	-0.11
Seed yield	-0.21	0.26	-0.01	0.15	0.08	-0.08	-0.19	-0.04
aNDF	-0.38	-0.09	0.04	0.01	0.11	0.16	0.14	0.19
aADF	-0.37	-0.15	0.03	0.00	0.06	0.28	-0.01	-0.06
aHemicellulose	-0.08	0.07	-0.08	0.07	0.10	-0.20	0.45	0.57
aCellulose	-0.31	-0.19	0.06	-0.08	0.04	0.27	0.12	-0.11
aLignin	-0.28	-0.14	0.05	0.12	0.15	0.25	0.14	-0.05
aAsh	-0.05	-0.03	0.35	0.46	0.05	-0.17	0.09	-0.07
aOrganic matter	0.05	0.03	-0.35	-0.46	-0.05	0.17	-0.09	0.07
bNDF	-0.21	-0.18	0.19	-0.05	-0.05	-0.25	-0.43	0.06
ADF	-0.28	0.07	-0.06	-0.03	-0.30	-0.36	0.11	-0.12
bHemicellulose	0.05	-0.21	0.24	0.01	0.12	0.07	-0.55	0.18
bCellulose	-0.31	0.02	-0.17	0.05	-0.23	-0.19	0.03	-0.08
bLignin	0.02	0.13	0.11	-0.37	-0.08	-0.04	0.17	-0.08
bAsh	0.19	-0.15	0.09	0.23	-0.49	0.12	0.06	0.14
bOrganic matter	-0.16	0.18	-0.07	-0.22	0.50	-0.14	-0.05	-0.12
bCP	0.20	0.13	0.00	0.23	0.17	0.29	0.16	-0.21
bIVDMD	0.26	0.14	-0.09	0.03	0.16	-0.25	-0.03	-0.02
Eigen value	2.25	2.09	1.76	1.48	1.37	1.22	1.18	1.12
Proportion of variance (%) explained	19.54	16.93	11.97	8.48	7.3	5.74	5.39	4.85
cumulative variance (%) explained	19.54	36.47	48.43	56.91	64.28	69.96	75.34	80.2

[a] GFY= Green Forage Yield, DMY= Dry Matter Yield, CP= Crude Protein; ADF= Acid Detergent Fiber; NDF= Neutral Detergent Fiber; IVDMD= *In-Vitro* Dry Matter Digestibility, OM= Organic Matter. a Green forage nutritional quality traits, b Straw nutritional quality traits.

[DOI: 10.52547/jast.24.6.1429]

DISCUSSION

Grass pea is a popular legume crop among resource-poor farmers as it requires minimal external inputs and can successfully establish in adverse agro-climatic conditions (Kumar *et al.,* 2011). The rising deficit of green and dry fodder has emphasized the need to increase the efficiency of the feed or fodder to further improve the productivity of livestock. Although extensive studies have been conducted to minimize the levels of ODAP in grass pea seed to make it fit for human and animal consumption (Rizvi *et al.,* 2016; Denekew and Tsega, 2009; Aksu *et al*., 2021), genetic diversity assessment for nutritional aspects of the grass pea hay and straw has not gained much importance. Genetic diversity among the grass pea accessions was assessed using agronomic, molecular, and forage quality parameters to identify genetically distant accessions for fodder yield and nutritional quality traits. The accessions from central India were distributed within the same cluster III, while the varieties were distributed one each in all clusters, indicating the presence of genetic variability among them. Further, the lines from ICARDA were distributed in different clusters due to their varied genetic background. Study indicated that the geographical distribution and genetic divergence follow the same trend with few exceptions. Murty and Arunachalam (1966) stated that genetic drift and selection in different environments could cause greater diversity than geographical distance, thus cluster analysis could be utilized for the selection of parents in transgressive breeding.

The principal component analysis reflects the importance of the largest contributor to the total variation at each axis for differentiation (Sharma, 1997). Accordingly, important agronomic and forage quality traits in the first eight principal components contributed to more than 80% variation. Important traits viz., GFY, straw yield, DMY, days to 50% flowering, seed yield; and forage quality traits NDF, ADF, cellulose in different principal components have grouped together and contributed towards the variability and tend to remain together and could be utilized in the breeding program. Tadesse and Bekele (2011) also reported biomass yield, seed yield, and flowering time as important traits in contribution to total variation.

The evaluation of diverse grass pea collections resulted in the identification of trait-specific accessions for agronomic traits *viz*., high biomass yield and plant height; and for forage nutritional qualities (green and straw) *viz*., CP, IVDMD and lignin. Grass pea accessions *viz*., IF1872 (25,100 kg ha^{-1}), IF2177 (25,230 kg ha^{-1}) and IF2156 (25,350 kg ha^{-1}) recorded higher biomass yield than check variety Prateek (24,375 kg ha^{-1}). Grass pea accessions IF1327 with high CP content (CP> 21%) and IF1312 (IVDMD> 66%) were selected for nutritional quality traits in green forage, while IL-10-76 (CP>12%) and IF1307 (IVDMD> 60.34) for nutritional quality traits in straw. The diverse and trait specific accessions selected from geographically diverse germplasm set will be useful in designing a grass pea breeding program.

In this study, EST-derived Simple Sequence Repeat (eSSR) markers developed from *Trifolium alexandrinum* were evaluated and utilized to assess the genetic diversity among grass pea collections. About 38.5% of *T. alexandrinum* markers were cross transferable to grass pea that was slightly lower than the transferability rate of 50% reported by Lioi *et al.* (2011). Out of the 27 primer pairs screened, 11 (40.7%) were polymorphic compared to 33% polymorphic primers reported in *Medicago* (Chandra, 2011). The average number of alleles obtained in our collections (5.36) was higher than 4.0 and 3.2 reported by previous studies (Gupta *et al.,* 2018) and lower than alleles reported in a diverse collection of grass pea (Wang *et al.,* 2015; Arslan *et al.,* 2020). These differences in the alleles might be due to genotypes collected from diverse geographical regions. The PIC values ranged between 0.49 and 0.76, with an average of 0.64 indicating these markers

[DOI: 10.52547/jast.24.6.1429]

were highly informative and could be utilized in genetic diversity studies of grass pea, as locus with PIC greater than 0.5 is considered to be highly diverse in nature (Botstein *et al.*, 1980). This study showed that cross-transferable markers developed from *Trifolium* were useful to study genetic diversity in grass pea. Accessions selected for superior agronomic and forage quality traits may be utilized as donors in the future breeding program in grass pea for higher biomass and forage nutritional quality.

CONCLUSIONS

This study showed that ample genetic variation exists among the germplasm lines that could be utilized in grass pea breeding. Although grass pea is an insurance crop for adverse agricultural conditions, forage quality of hay needs to be studied, as hay-based diets are reported to be safe for ruminant's consumption. Results of the study also indicated the possibility of utilizing and improving grass pea as a dual-purpose crop due to the existence of wide variability in fodder quality determining traits in straw. This investigation resulted in the identification of genetically distant accessions for forage quality, which may be crossed with high yielding lines to produce low ODAP and high forage quality cultivars in near future. As most of the accessions evaluated are part of global collections of ICARDA, Lebanon, thus, grass pea germplasm can be accessible to all researchers for future genetic improvement programs.

ACKNOWLEDGEMENTS

Authors are grateful to ICARDA, Lebanon for sharing the germplasm of grass pea and to the Department of Agriculture and Co-operation, Ministry of Agriculture and Farmers Welfare, Govt. of India for providing financial assistance under National Food Security Mission.

REFERENCES

1. Aksu, E., Dogan, E. and Arslan, M. 2021. Agro-Morphological Performance of Grasspea (*Lathyrus sativus* L.) Genotypes with Low β-ODAP Content Grown Under Mediterranean Environmental Conditions. *Fresenius Environ. Bull.*, **30**: 638-644.
2. AOAC International. 2005. *Official Methods of Analysis.* 18th Edition, AOAC Inc., Gaithersburg, MD, USA.
3. Arslan, M., Basak, M., Aksu, E., Uzun, B. and Yol, E. 2020. Genotyping of Low β-ODAP Grass Pea (Lathyrus sativus L.) Germplasm with EST-SSR Markers. *Braz. Arch. Bio. Tech.*, **63**: e20190150.
4. Basaran, U., Acar, Z., Karacan, M. and Onar, A. N. 2013. Variation and Correlation of Morpho- Agronomic Traits and Biochemical Contents (Protein and β - ODAP) in Turkish Grass Pea (*Lathyrus sativus* L.) Landraces. *Turk. J. Field Crops*, **18:** 166-173.
5. Basaran, U., Mut, H., Asci, O. O., AcarZ. and Ayan, I. 2011. Variability in Forage Quality of Turkish Grass Pea (*Lathyrus sativus* L.) Landraces. *Turk. J. Field Crops*, **16:** 9-14.
6. Botstein, D., White, R. L., Skolnick, M. and Davis, R. W. 1980. Construction of a Genetic Linkage Map in Man Using Restriction Fragment Length Polymorphisms. *Am. J. Hum. Genet*., **32:** 314–331.
7. Campbell, C. G., Mehra, R. B., Agrawal, S. K., Chen, Y. Z., Abd El Moneim, A. M., Khawaja, H. I. T., Yadov, C. R., Tay, J. U. and Araya, W. A. 1994. Current Status and Future Research Strategy in Breeding Grasspea (*Lathyrus sativus*). *Euphytica*, **73:** 167–175.
8. Chandra, A. 2011. Use of EST Database Markers from *M. truncatula* in the Transferability to Other Forage Legumes. *J. Environ. Biol*., **32**: 347–354.
9. Croft, A. M., Pang, E. C. K. and Taylor, P. W. J. 1999. Molecular Analysis of *Lathyrus*

[DOI: 10.52547/jast.24.6.1429]

sativus L. (grasspea) and Related Lathyrus Species. *Euphytica*, **107:** 167–176.

10. Das, M. M., Yadav, V. K., Singh, A., Sharma, P. and Ghosh, P. K. 2015. Nutrient Utilization and Growth Performance of Jalauni Lambs Fed Grass Pea (*Lathyrus sativus*) Hay Based Diet. *Sci. Papers Ser. D. Anim. Sci*., **58:** 111-114.
11. Denekew, Y. and Tsega, W. 2009. Evaluation of β -ODAP Content in Forage, Grain and Straw of *Lathyrus sativus* in North West Ethiopia. Livestock Research for Rural Development. Available in http://www.lrrd.org/lrrd21/12/dene21212.htm (Accessed September 2020).
12. Gana, A. S., Shaba, S. Z. and Tsado, E. K. 2013. Principal Component Analysis of Morphological Traits in Thirty-Nine Accessions of Rice (*Oryza sativa*) Grown in a Rainfed Lowland Ecology of Nigeria. *J. Plant .Breed. Crop Sci*., **5:** 120-126.
13. Goering, H. K and Van Soest, P. J. 1970. Forage Fiber Analysis. In: "*Agricultural Handbook No. 379*". Agricultural Research Service, US Department of Agriculture, Washington DC.
14. Gupta, P., Udupa, S. M., Gupta, D. S., Kumar, J. and Kumar, S. 2018. Population Structure Analysis and Determination of Neurotoxin Content in a Set of Grass Pea (*Lathyrus sativus* L.) Accessions of Bangladesh Origin. *Crop J*., **6:** 435-442.
15. Irani, S., Majid, M. M. and Mirlohi, A. 2016. Genetic Variation for Clonal Propagation and Trait Association with Field Performance in Sainfoin. *Trop. Grassl*., **4:** 38−46.
16. Kumar, S., Bejiga, G., Ahmed, S., Nakkoul, H. and Sarker, A. 2011. Genetic Improvement of Grass Pea for Low Neurotoxin (ODAP) Content. *Food Chem. Toxicol*., **49:** 589-600.
17. Lioi, L., Sparvoli, F., Sonnante, G., Laghetti, G., Lupo, F. and Zaccardelli, M. 2011. Characterization of Italian Grass Pea (*Lathyrus sativus* L.) Germplasm Using Agronomic Traits, Biochemical and Molecular Markers. *Genet. Resour. Crop Evol*., **58:** 425-437.
18. Murty, B. R. and Arunachalam, V. 1966. The Nature of Genetic Divergence in Relation to Breeding System in Crop Plants. *Indian J. Genet. Plant Breed*., **26:** 188-198.
19. Powell, W., Machray, G. C. and Provan, J. 1996 Polymorphism Revealed by Simple Sequence Repeats. *Trends Plant Sci*., **1:** 215-222.
20. Rizvi, A. H., Sarker A. and Dogra, A. 2016. Enhancing Grass Pea (*Lathyrus sativus* L.) Production in Problematic Soils of South Asia for Nutritional Security. *Indian J. Genet. Plant Breed*., **76:** 583-592.
21. Rohlf, F. J. 2000. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System Version 2.1. Exeter Publishing Setauket, New York.
22. Sarker, A., Sah, P., Yadav, V. K. and Das, M. M. 2015. Grasspea: A Potential Fodder and Feed Resources. In Proceedings of the 23rd International Grassland Congress (Sustainable Use of Grassland Resources for Forage Production, Biodiversity and Environmental Protection)", New Delhi, 1529 PP.
23. Sharma, R. N., Kashyap, O. P., Chitale, M. W. and Pandey, R. L. 1997. Genetic Analysis for Seed Attributes over the Years in Grasspea (*Lathyrus sativus* L). *Indian J. Genet. Plant Breed*., **57:** 154-157.
24. Singh, M., Upadhyaya, H. D. and Bisht, I. S. 2013. *Genetic and Genomic Resources of Grain Legume Improvement.* Elsevier, Oxford, United Kingdom.
25. Stein, N., Herren, G. and Keller, B. 2001. A New DNA Extraction Method for High-Throughput Marker Analysis in a Large Genome Species such as *Triticum aestivum*. *Plant Breed*., **120:** 354-356.
26. Tadesse, W. and Bekele, E. 2011. Factor Analysis of Components of Yield in Grass Pea (Lathyrus sativus L.). *Lathyrus Lathyrism Newslet.*, **2**: 43-46
27. Tilley, J. M. A. and Terry, R. A. 1963. A Two-Stage Technique for the *In Vitro*

[DOI: 10.52547/jast.24.6.1429]

Digestion of Forage Crops. *J. Br. Grassl. Soc*., **18:** 104-111.
28. Van Soest, P. J., Robertson, J. B. and Lewis, B. A. 1991. Method for Dietary Fiber, Neutral Detergent Fiber and Non-starch Polysaccharides in Relation to Animal Nutrition. *J. Dairy Sci*., **74:** 3588–3597.
29. Wang, F., Yang, T., Burlyaeva, M., Li, L., Jiang, J., Fang, L., Redden, R. and Zong, X. 2015. Genetic Diversity of Grass Pea and its Relative Species Revealed by SSR markers. *PLoS One*. **10:** e0118542.
30. Zarabiyan, M. and Majidi, M. M. 2015. Genetic Diversity and Relationships Within and Among Onobrychis Species Using Molecular Markers. *Turk. J. Bot.*, **39:** 681-692.
31. Zarabiyan, M., Majidi, M. M. and Ehtemam, M. H. 2013. Genetic Diversity in a Worldwide Collection of Sainfoin using Morphological, Anatomical and Molecular Markers. *Crop Sci*., **53**: 2483-2496.
32. Mantel, N. 1967. The Detection of Disease Clustering and a Generalized Regression Approach. *Cancer Res*., **27:** 209–220.

آشکارسازی تنوع ژنتیکی در کلکسیون نخودعلوفه ای (*Lathyrus sativus* L.) با استفاده از صفات زراعی و کیفی علوفه و نشانگرهای SSR

او. کومار یاداو، ا. رادهاکریشنا، م. موهان داس، ت. سینک، س. یاداو، پ. شارما، ا. کومار میشرا، و ا. سارکر

چکیده

نخود علوفه ای (*Lathyrus sativus* L.) گیاهی دو منظوره و مهم در مناطق مستعد خشکسالی و قحطی است زیرا به عنوان غذای انسان و همچنین خوراک دام و علوفه استفاده می‌شود. با این حال، تغییرات صفات کیفیت علوفه نخود علوفه ای تا حد زیادی بررسی نشده است. این مطالعه با هدف شناسایی تنوع ژنتیکی کلکسیون نخود علوفه ای از آفریقا، آسیا و اروپا و شناسایی ژنوتیپ‌ها برای صفات برتر زراعی و کیفیت تغذیه‌ای علوفه انجام شد. تجزیه و تحلیل مؤلفه های اصلی(principal component analysis) نشان داد که اکثریت کل تغییرات مربوط است به سه مولفه اصلی اول از پارامترهای کیفیت تغذیه ای یعنی NDF، ADF، سلولز، لیگنین و درصد خاکستر و از صفات زراعی یعنی ارتفاع بوته، گره در بوته، سطح برگ، زیست توده سبز و خشک. افزون بر این، در مجموع ۵۹ آلل چندشکلی در ۱۱ جایگاه SSR با میانگین ۵.۳۶ آلل در هر مکان شناسایی شد و محتوای اطلاعات چندشکلی بین ۰.۴۹ تا ۰.۷۶ بود. سه نمونه (IF1872، IF2177 و IF2156) با زیست‌توده بیشتر از تیمار شاهد و چهار نمونه (IF1327، IF1312، IL-10-76 و IF1307) با ارزش غذایی عالی در علوفه سبز و همچنین کاه شناسایی شد. نتایج این پژوهش تنوع ژنتیکی زیادی را برای

[DOI: 10.52547/jast.24.6.1429]

صفات زیست توده و کیفیت غذایی در کلکسیون‌های نخود علوفه‌ای نشان داد که می‌تواند برای ایجاد واریته‌های پرمحصول و غنی از نظر تغذیه و دو منظوره مفید باشد.

[DOI: 10.52547/jast.24.6.1429]