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Assessment of Agronomic Traits and Molecular Diversity Using SSR Markers in Soyabean, (*Glycine Max* (L.) Merr. Accessions in Nepal

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ABSTRACT

Soybean, (*Glycine max* (L.) Merr. is an important multipurpose pulse crop that provides edible oil and protein, and improves soil fertility. In spite of being rich in germplasms, the genetic diversity of soybean in Nepal is still underexplored. The purpose of this study is to examine the phenotypic and molecular diversity of soybean by utilizing morphological features in conjunction with simple sequence repeat (SSR) markers. A total of 150 soybean germplasms received from different sources were used for field study at DoAR, Dasharathpur; GLRP, Rampur, and NARC, Khumaltar in consecutive years 2019 and 2020 whereas 141 germplasms were used for molecular study with 30 SSR markers at NBTRC, Khumaltar. Genotypes differed in phenotypic traits, the expression of different agronomic traits and biomass are influenced by genetic differences. The SSR markers supported the presence of genetic diversity showing 124 alleles and 89.11% polymorphism, and an average PIC value of 0.795. Hierarchical clustering and principal coordinate analysis also supported the presence of genetic variation and relationship among the studied genotypes. This study offers insightful information for utilizing these germplasms in future crop improvement in Nepal.

Keywords: Cluster analysis, genetic diversity, phenotypic traits, Simple Sequence Repeats (SSR)

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INTRODUCTION

Soybean, (*Glycine max* (L.) Merr.) is an economically important leguminous crops in the worldwide, and its significance is attributed to its multifaceted utility in various industries, particularly as a source of edible oil, protein-rich fodder, and a nitrogen-fixing crop that enhances soil fertility. Its popularity has surged due to its potential to replace conventional protein sources such as meat and dairy, coupled with its versatility across various industries (Zhang and Chen 2014). Rich in essential amino acids, vitamins, and minerals, soybeans are integral to a well-rounded diet (Wang et al 2019). The adaptability of this crop makes it viable for cultivation in diverse regions worldwide, with production experiencing a substantial increase driven by the escalating demand for plant-based proteins (Sallam et al 2019).

In Nepal, soybean represents a staple crop that has gained prominence over the past few decades due to its adaptability to diverse agro-ecological regions, short growth cycle, and potential to contribute to food and nutritional security. However, despite its growing importance, the genetic diversity of soybean germplasm remains relatively underexplored in the country. However, its popularity among farmers has surged in recent years due to its high yield potential, adaptability to diverse agro-climatic conditions, and market demand. Currently, soybeans are grown in various regions from Terai to the high hill. The major production districts of soybean in Nepal are Kavre, Makawanpur, Kalikot, Salyan, Dadeldhura and Kailali (MOAD 2022). Hill contributes approximately 83% of total soybean area and production, while mountain and Terai occupy approximately 10% and 7% respectively (MOAD 2020).

Soybean cultivation in Nepal is being popular, but there are still issues associated with genotype knowledge and selection criteria, which are fundamental for crop improvement. It provides the basic information for enhancing crop productivity, nutritional value, and resilience against biotic and abiotic challenges through breeding. It is imperative to comprehensively assess the phenotypic and molecular diversity of the available germplasm resources in order to harness the full potential of soybean. Such assessments can aid in the selection of superior genotypes for breeding programs, germplasm conservation, and the development of region-specific cultivars.

Morphological attributes have traditionally been employed for the initial evaluation of genetic diversity. These attributes encompass a wide range of observable traits such as plant height, leaf shape, pod color, and seed characteristics. However, relying solely on morphological characteristics for assessing genetic diversity can have limitations due to environmental influences and the potential for phenotypic plasticity. In recent years, molecular markers, particularly simple sequence repeats (SSRs), have become invaluable tools for the precise quantification of genetic diversity. SSR markers are highly polymorphic, codominant, and distributed throughout the genome, making them ideal for characterizing genetic variation within and among soybean accessions. This molecular marker strategy allows breeders to efficiently select desired features in plant populations, potentially saving both time and money compared to traditional phenotypic selection in the field. This research aims to examine the phenotypic and molecular diversity of soybean germplasms that will address the knowledge gap concerning the genetic diversity of soybean in Nepal. It will ultimately aid in improving knowledge of genetic resources and make it easier to generate improved soybean varieties suited to Nepal's varied agro-climatic conditions.

MATERIALS AND METHODS

Germplasm studied

A total of 150 soybean accessions collected from different sources were used for field study at DoAR, Dasharathpur; GLRP, Rampur, and NARC, Khumaltar in consecutive years 2019 and 2020 (Table 1 and 3). Seeds of 141 germplasm were grown on National Biotechnology Research Centre (NBRC), Nepal Agricultural Research Council (NARC) for molecular characterization.

Table 1. Accessions list with details on plant phenotypic traits

PN	List of accessions	Seed coat color	Hilum color	Flower color	Leaf size and shape
PN-1	NGRC02687	Redish Brown	White	Pink	Narrow Lanceolate
PN-2	Soy Agd-005	Yellowish White	Black	Pink	Intermediate
PN-3	NGRC06811	Black	Black	White	Broad Ovate
PN-4	NGRC02675	Yellowish White	Brown	White	Intermediate
PN-6	TGX1835-10F	Yellowish White	Brown	White	Intermediate
PN-7	NGRC02691	Redish Brown	Brown	Pink	Intermediate
PN-8	TGX1987-62F	Yellowish White	Brown	White	Intermediate
PN-9	Soy Agd-011-2	Yellowish White	Black	White	Intermediate
PN-10	Solu coll#2-2016	Redish Brown	Gray	Pink	Intermediate
PN-11	NGRC06826	Black	Imperfect black	Pink	Intermediate
PN-12	NGRC08244	Yellowish White	Brown	Pink	Intermediate
PN-13	TGX1989-19F	Yellowish White	Brown	White	Intermediate
PN-14	2003KS-KBxTB1-2.1-3	Blackish green	White	White	Narrow Lanceolate
PN-15	Seti	Yellowish White	Brown	Pink	Intermediate
PN-16	2003KS-TB1xKB-5.14-2	Black	Imperfect black	Pink	Narrow Lanceolate
PN-17	Sindhuli Khairo	Brown	Buff	Pink	Narrow Lanceolate
PN-18	NGRC02676	Redish Brown	Brown	Pink	Intermediate
PN-19	Bringi,Pyuthan Bazar-2016	Redish Brown	Brown	Pink	Intermediate
PN-20	Lumle Bhatmas 1	Yellowish White	Brown	White	Intermediate

PN	List of accessions	Seed coat color	Hilum color	Flower color	Leaf size and shape
PN-21	NGRC02716	Black	Black	White	Narrow Lanceolate
PN-22	AGS 377	Pale green	Imperfect black	White	Intermediate
PN-23	Soy Agd-013	Redish Brown	Brown	Pink	Intermediate
PN-24	NGRC02711	Brown	Brown	Pink	Intermediate
PN-25	2003KS-TB1xKB-5.45	Black	Imperfect black	Pink	Narrow Lanceolate
PN-26	NGRC06809	Yellowish White	Gray	White	Intermediate
PN-27	Solu-Small seed	Redish Brown	Brown	Pink	Intermediate
PN-28	Lamjung Local	Yellowish White	Black	Pink	Intermediate
PN-30	NGRC02703	Yellowish White	Brown	White	Narrow Lanceolate
PN-31	NGRC06815	Redish Brown	Gray	Pink	Broad Ovate
PN-32	NGRC02699	Yellowish White	Brown	White	Intermediate
PN-33	NGRC02664	Yellowish White	Brown	Pink	Narrow Lanceolate
PN-34	NGRC02707	Redish Brown	Brown	Pink	Broad Ovate
PN-35	NGRC05101	Black	Black	White	Narrow Lanceolate
PN-36	2003KS-TB1xKB-5.66	Black	Imperfect black	Pink	Intermediate
PN-37	NGRC06830	Yellowish White	Brown	White	Intermediate
PN-38	AGS 377	Yellowish White	Brown	White	Broad Ovate
PN-39	NGRC08243	Brown	Buff	Pink	Narrow Lanceolate
PN-40	NGRC08245	Yellowish White	Brown	White	Intermediate
PN-41	010-10.2	Brown	Brown	Pink	Intermediate
PN-42	NGRC02690	Redish Brown	Gray	White	Narrow Lanceolate
PN-43	IARS-87-1	Yellowish White	Imperfect black	Pink	Intermediate
PN-44	NGRC02686	Redish Brown	White	Pink	Narrow Lanceolate
PN-45	TGX1987-11E	Yellowish White	Black	White	Intermediate
PN-46	TGX 1925-1F	Yellowish White	Black	White	Intermediate
PN-47	AGS-376	Yellow	Brown	Pink	Intermediate
PN-48	NGRC06835	Black	Black	White	Narrow Lanceolate
PN-49	NGRC06822	Black	Imperfect black	White	Broad Ovate
PN-50	Tanahu-Creami	Redish Brown	Brown	Pink	Intermediate
PN-51	GC8234GC-13	Yellow	Brown	White	Intermediate
PN-53	2003KS-TB1xKB-5.4	Black	Imperfect black	Pink	Intermediate
PN-54	Chitwan-9	Yellowish White	Brown	White	Intermediate
PN-55	Baglung Khairo	Redish Brown	Brown	Pink	Narrow Lanceolate
PN-56	NGRC02684	Yellowish White	Brown	White	Intermediate
PN-57	NGRC06813	Yellowish White	Gray	White	Intermediate
PN-58	TGX1987-42F	Yellowish White	Brown	White	Narrow Lanceolate
PN-59	Soy Agd-001	Yellowish White	Brown	Pink	Intermediate
PN-60	Tarkari bhatmas 1	Green	Buff	White	Narrow Lanceolate
PN-61	AGS 371	Green	Buff	White	Intermediate

PN	List of accessions	Seed coat color	Hilum color	Flower color	Leaf size and shape
PN-62	Soy Agd-006	Black	Imperfect black	Pink	Narrow Lanceolate
PN-63	G8754	Yellowish White	Brown	Pink	Intermediate
PN-64	Coll # 6	Redish Brown	White	White	Intermediate
PN-65	NGRC06812	Yellowish White	Gray	White	Intermediate
PN-66	Soy Agd-021	Yellowish White	Brown	White	Narrow Lanceolate
PN-67	NGRC07369	Yellowish White	Imperfect black	White	Intermediate
PN-68	Baglung Seto	Yellowish White	Brown	Pink	Intermediate
PN-69	TGX311-23D	Yellow	Brown	White	Intermediate
PN-70	Baglung Black	Black	White	White	Intermediate
PN-71	NGRC07367	Brown	Buff	Pink	Intermediate
PN-72	Palpa	Green	Buff	Pink	Intermediate
PN-73	NGRC07368	Yellowish White	Imperfect black	White	Intermediate
PN-74	Soy Agd-010	Yellowish White	Black	Pink	Narrow Lanceolate
PN-75	Soy Agd-014	Black	White	White	Intermediate
PN-76	Ransom	Yellow	Black	White	Intermediate
PN-77	Puja	Yellowish White	Brown	Pink	Narrow Lanceolate
PN-78	2003KS-TB1xKB-5.69	Black	Imperfect black	Pink	Intermediate
PN-79	200525(Rampur)	Yellowish White	Brown	White	Intermediate
PN-80	NGRC02679	Redish Brown	White	Pink	Intermediate
PN-81	NGRC02672	Black	Imperfect black	White	Narrow Lanceolate
PN-82	SB0-122	Yellowish White	Gray	Pink	Intermediate
PN-83	Tandi coll # 2	Brown	Buff	White	Narrow Lanceolate
PN-84	F778817	Yellowish White	Brown	White	Intermediate
PN-85	NGRC02693	Redish Brown	Brown	Pink	Intermediate
PN-86	Surkhet#2	Redish Brown	Brown	White	Intermediate
PN-87	2003KS-TB1xKB-5.37	Black	Imperfect black	Pink	Intermediate
PN-89	2003KS-TB1xKB-5.39	Black	Imperfect black	Pink	Intermediate
PN-90	Soy Agd-002	Yellowish White	Brown	White	Broad Ovate
PN-91	Haripur Khairo Bhatmas-1	Brown	Buff	Pink	Intermediate
PN-92	TGX1876-4E	Yellowish White	Brown	White	Narrow Lanceolate
PN-93	Soy Agd-008	Yellowish White	Brown	White	Intermediate
PN-94	2003KS-TB1xKB-5.62	Redish Brown	Brown	Pink	Intermediate
PN-97	NGRC02683	Yellowish White	Brown	White	Broad Ovate
PN-98	P194159	Yellowish White	Brown	Pink	Intermediate
PN-99	TGX1485-ID	Yellow	Imperfect black	Pink	Intermediate
PN-100	Dakshinkali-Creami Bold	Creamy White	Brown	White	Broad Ovate
PN-101	NGRC06834	Brown	Buff	Pink	Narrow Lanceolate
PN-102	NGRC06816	Redish Brown	Brown	Pink	Intermediate
PN-103	Soy Agd-020	Yellowish White	Brown	White	Intermediate
PN-104	Gulmi Kalo	Yellowish White	Brown	Pink	Intermediate
PN-105	NGRC06823	Brown	Brown	White	Intermediate
PN-106	Collection #178	Yellowish White	Gray	Pink	Intermediate

PN	List of accessions	Seed coat color	Hilum color	Flower color	Leaf size and shape
PN-107	2003KS-TB1xKB-5.67	Black	White	Pink	Narrow Lanceolate
PN-108	Collection # 1 mangalpur	Redish Brown	Brown	Pink	Intermediate
PN-109	Brown, Jumla-2016	Redish Brown	Brown	White	Intermediate
PN-110	NGRC02674	Brown	Brown	White	Intermediate
PN-111	272W	Green	Buff	White	Narrow Lanceolate
PN-112	2003KS-TB1xKB-5.61	Redish Brown	Brown	Pink	Intermediate
PN-113	TGX1990-8F	Yellowish White	Brown	White	Intermediate
PN-114	Gulmi Seto	Yellowish White	Brown	Pink	Broad Ovate
PN-115	NGRC02669	Yellowish White	Brown	White	Intermediate
PN-117	NGRC02712	Redish Brown	Buff	Pink	Broad Ovate
PN-118	Tidar	Imperfect black	Imperfect black	White	Intermediate
PN-119	Sathiya	Redish Brown	White	Pink	Intermediate
PN-120	G-8586	Yellowish White	Brown	White	Intermediate
PN-121	Coll # 166	Pale green	Gray	White	Narrow Lanceolate
PN-122	LS-77-16-16	Yellowish White	Brown	White	Narrow Lanceolate
PN-124	NGRC02717	Black	Imperfect black	White	Intermediate
PN-125	NGRC06833	Black	Black	White	Intermediate
PN-126	2003KS-TB1xKB-5.32-2	Redish Brown	Brown	Pink	Intermediate
PN-127	NGRC02710	Redish Brown	Buff	Pink	Intermediate
PN-128	2003KS-KBxTB1-8	Black	Imperfect black	White	Narrow Lanceolate
PN-129	2003KS-TB1xKB-5.34	Black	Imperfect black	Pink	Intermediate
PN-130	2003KS-KBxTB1-2.1-2	Blackish green	White	White	Broad Ovate
PN-131	VI (Bhojpur)	Yellowish White	Imperfect black	Pink	Intermediate
PN-132	NGRC02666	Yellowish White	Brown	Pink	Narrow Lanceolate
PN-133	NGRC02671	Yellowish White	Brown	White	Intermediate
PN-134	2003KS-TB1xKB-5.64	Black	White	Pink	Intermediate
PN-135	Kavre	Yellow	Brown	White	Broad Ovate
PN-136	NGRC06820	Yellowish White	Brown	White	Intermediate
PN-137	NGRC02719	Yellowish White	Brown	White	Narrow Lanceolate
PN-139	NGRC02704	Yellowish White	Brown	White	Intermediate
PN-140	TGX1987-62F	Yellow	Brown	White	Intermediate
PN-141	Ankur	Yellow	Buff	Pink	Narrow Lanceolate
PN-142	Ramechhap Collection	Yellowish White	Brown	Pink	Intermediate
PN-143	NGRC06832	Yellowish White	Black, brown	Pink	Broad Ovate
PN-144	Collection 169	Yellowish White	Brown	Pink	Intermediate
PN-145	NGRC06821	Redish Brown	Brown	Pink	Intermediate
PN-146	TGX1987-14F	Yellowish White	Brown	White	Intermediate
PN-147	Collection 168	Redish Brown	White	Pink	Intermediate
PN-148	2003KS-TB1xKB-5.65	Black	Imperfect black	Pink	Intermediate
PN-149	Collection 175	Brown	Brown	Pink	Narrow lanceolate
PN-150	Soy Agd-017	Yellowish white	Brown	White	Intermediate

PN: Plot number

DNA extraction, quantification, and purity

DNA samples were extracted using Doyle and Doyle method with some optimizations. Leaf samples (1g) were ground in liquid nitrogen and mixed with 700 µL of an extraction buffer (containing 2% CTAB, Tris-HCl, EDTA, NaCl, PVP, and β-mercaptoethanol). The mixture was incubated at 65°C for an hour and centrifuged at 15,000 rpm for 15 minutes. The aqueous phase was transferred to microcentrifuge tubes, followed by the addition of Chloroform isoamyl alcohol (24:1) for phase separation, and centrifuged again. The supernatant was then mixed with isopropanol, chilled at -20°C for 30 minutes, and centrifuged to precipitate the DNA. After washing the pellets with 96% and 70% ethanol, they were air-dried and resuspended in 1× TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 8.0). DNA quantification and quality was assessed by using Nanodrop (Quawell Q-5000). The purity of the DNA was assessed by the absorbance ($A_{260/280}$) ratio and running the DNA samples were run on 0.8% agarose and compared with 1Kb DNA ladder.

PCR reaction and visualization

A total of 30 primers were selected for PCR reactions, conducted using Mygene L series thermo cycler (Table 2). Reactions without DNA were used as negative controls. Each reaction contained 50-60 ng of template DNA, 2X master mix, 0.5 µM of primer, 25 mM $MgCl_2$, 0.2 mM dNTP mix, 0.2 U of Taq polymerase, and 1 mg/mL BSA. The thermo cycler started with an initial denaturation at 94 ℃ for 4 minutes, followed by 35 cycles of 50s annealing, 80 seconds extension at 72°C, a final extension at 72°C for seven minutes, and a hold at 4°C. The PCR products were assessed by using 2.5% agarose gel electrophoresis in 1X TBE stained with ethidium bromide at 80V for two hours. The gel was photographed using Gel Documentation system (VWR®Genosmart 2, UK). Some photographs are given Pic 1, Pic 2 and Pic 3.

Table 2. Details on Simple Sequence Repeat (SSR) markers used in this study

SSR Primer	Forward Primer sequence 5'→ 3'	Reverse Primer sequence 5'→ 3'
Soy satt 173	CCAAGCGAAATCACCTCCTCT	CCGGTCCAATCTTTATTCAAAC
Soy satt 409	CCTTAGACCATGAATGTCTCGAAGATA	CTTAAGGACACGTGGAAGATGACTAC
Soy satt 148	TTAAGGATTAATTGAGACAAAATCA	CTAAAGCATCACAAAACAGAGC
Soy satt 173	CCGGTCCAATCTTTATTCAAAC	CCAAGCGAAATCACCTCCTCT
Soy satt 185	CATATGAATAGGTAAGTTGCACT	TGTCACTATAAATGGTACCTATTA
Soy satt 285	GCGACATATTGCATTAAAAACATACTT	GCGGACTAATTCTATTTTACACCAACAAC
Soy satt 307	GCGCTGGCCTTTAGAAC	GCGTTGTAGGAAATTTGAGTAGTAAG
Satt276	GCGGACGGTAAGGACTATTTATGATA	GCGTCAGATGAAAAAAAATAAGATAC
Satt135	CACGGATTTTAAATCATTATTACAT	TTCCAATACCTCCCAACTAAC
Satt365	TGCTCCCCTCTGCCTTTTTTTCTATTTT	AAGGATGAGTTTGATAAACATGAATGAAGAA
Satt557	GCGGGATCCACCATGTAATATGTG	GCGCACTAACCCTTTATTGAA
Satt316	GTGAGAAACTAGCCAAGAATAGA	CAATTGTTTCCAAATGACACT
Satt319	CAACTCAGTAGGGGTCAATAACAA	TGAAATAGGGAAAATAAGGGAACA
Satt545	CAATGCCATTCCATATTTGTT	CAATTGCCCTAGTTTTGATAG
Satt249	GCGGCAAATTGTTATTGTGAGAC	GGCCAGTGTTGAGGGATTTAGA
Sat_356	GCGCCGGAAAAATGTGAGAAATCATAAAA	GCGTTGCATGACTATCATTCAATCAAAAAT
Satt001	AAAGTCTTTAAAAGTGTGTCTTA	TTAAAAGAAAAATGCAACAT
Satt373	TCCGCGAGATAAATTCGTAAAAT	GGCCAGATACCCAAGTTGTACTTGT
Satt005	TATCCTAGAGAAGAACTAAAAAA	GTCGATTAGGCTTGAAATA
Sat001	GCGGATACGACCAAAAATTGTT	GCGAACTGCGAAGATACTACCC
BarcSatt100	ACCTCATTTTGGCATAAA	TTGGAAAACAAGTAATAATAACA
Satt156	GCGGTGTGGATCCAAAAACTCAAACTT	GCGTGCTAGTTCGATCAGCTTAGTTTC
Satt36	GCGGTGTGGATCCAAAAACTCAAACTT	GCGTGCTAGTTCGATCAGCTTAGTTTC
SAT1	CTGGTGGACTATTGATACGACC	AACTGCGAAGATACTACCCTCC
Satt411	TGGCCATGTCAAACCATAACAACA	GCGTTGAAGCCGCCTACAAATATAAT

SSR Primer	Forward Primer sequence 5’→ 3’	Reverse Primer sequence 5’→ 3’
Sat_076	GCGTAATTAACACCAATATATGACATG	GCGGGGTTAAAAATTCAAAATGT
Sat_417	GCGAATATGGCGTTGAAAATAGTGAT	GCGACCCAGATTCTGTGCTAAGA
Sat_043	GCGGTCCGTCAATGAATATTAAATTAAAA	GCGAAAGCGGCAGAGAGAGAAAGGT
Satt197	CACTGCTTTTTCCCCTCTCT	AAGATACCCCCAACATTATTTGTAA
Sat_342	GCGACTCTGGGGAAAATTAGTTTAG	GCGGAGTCGGGGAGCACTACTTGTC

Sequence published: (Chakraborty 2018, Clever et al 2020, Hwang et al 2020, Koutu et al 2019, Kujane et al 2019, Kumawat et al 2015, Moongkanna et al 2011, Shi et al 2010 and Tantasawat et al 2011)

Data scoring and statistical analysis

SSR markers are codominant markers, and the PCR amplified bands were scored as present (1) or absent (0) on the basis of size compared to the marker (1Kb and 100 bp ladder, Thermo Fisher Scientific, Waltham, USA) and scored to create a single data matrix. The total number of bands and polymorphic bands, average number of bands per primer were calculated. The polymorphic information content (PIC) was calculated as PIC = $(1-\sum p_i^2)$, where p_i is the frequency of the banding pattern (De Riek et al 2001). The pairwise similarity matrix was constructed using Jaccard similarity coefficient. Similarly, the principal coordinate analysis (PCoA) was carried out to visualize multiple dimensional distributions of soybean in scatter plots with Genstat 18th edition.

RESULTS AND DISCUSSION

Phenotypic data and agronomic traits

The phenotypic data (seed coat color, hilum color, flower color, leaf size and shape) of the genotypes provide important insights into the characteristics of soybean plants (Table 1). Different accessions display a range of seed coat colors. For example, yellowish white was mostly observed in sixty accessions including Soy Agd-005, TGX1835-10F, NGRC02675, TGX1987-62F, and others. Reddish brown was found in 28 accessions including NGRC02687, NGRC02691, Lumle Bhatmas 1, Coll # 6, and other accessions. Furthermore, 25 accessions had black seed coats including NGRC06811, NGRC02716, NGRC05101, Baglung Black, and others, but 11 genotypes including NGRC02676, Bringi, Pyuthan Bazar-2016, NGRC06809, NGRC02684, and a few other instances had brown seed coats. Eight genotypes including Tarkari Bhatmas 1, Coll # 166, G8754, and five other were known to have green seed coats, whereas eight accessions including AGS-376, Ransom, Kavre, Ankur and four other have yellow seed coats. Single accession Dakshinkali-Creami Bold, was known to have creamy white seed coat. Moving to the hilum colors, brown was commonly observed in 73 accessions including NGRC02675, NGRC02691, Lumle Bhatmas 1, NGRC06809, and more, followed by imperfect black in 23 accessions including Agd-013, Soy Agd-006, NGRC07369, NGRC06822, and more, black hilum was observed in 13 accessions including Soy Agd-005, NGRC06811, Soy Agd-011-2, NGRC02716. Buff hilum was also detected in 13 accessions including Sindhuli Khairo, NGRC08243, NGRC07367, Dakshinkali-Creami Bold and others, white hilum was detected in 10 accessions including NGRC02687, NGRC02686, Coll # 6, Soy Agd-014, Baglung Black, and gray hilum in nine accessions including NGRC06809, NGRC02690, SB0-122, Collection #178 and others. Breeder can utilize the variablity for selection and may use in hybridization for trait improvement, gene transfer to the other accessions. Chandrawat et al., 2017 reported variation due to genotypes was significant for all the characters under study, similar to our finding.

Flower colors vary with pink commonly found in 71 accessions such as NGRC02687, Soy Agd-005, NGRC02691, Solu coll#2-2016, NGRC02676, and others, while white is present in 70 accessions such as NGRC06811, NGRC02675, NGRC08244, NGRC02716, AGS 377, and more. Leaf size and shape exhibit diversity, including Intermediate in 95 accessions including Soy Agd-005, NGRC02675, Solu coll#2-2016, Lumle Bhatmas 1, NGRC02716, and more; narrow lanceolate observed in 32 accessions including NGRC02687, 2003KS-KBxTB1-2.1-3, NGRC02716, NGRC02664, and others; and broad ovate found in 13 accessions including NGRC06811, NGRC08244, NGRC06815, NGRC02707, and more.

Table 3 presents a comprehensive overview of various soybean accessions, encompassing key agricultural traits such as days to 50% flowering and maturity, plant height, number of primary branches per plant, number of nodes per plant, number of pods per plant, grain yield, hundred seed weight, harvest index, and total biomass. Accessions such as 2003KS-KBxTB1-2.1-3 (103 days) and NGRC06826 (105 days) have extra short maturity periods, which can be advantageous in regions with a shorter growing season. and may be donor for earliness. NGRC06833 and 200525 (Rampur) stand out with exceptionally high grain yields, making them potential candidates for high-yield. Sathiya, 010-10.2-0.18 and Baglung Black had relatively lower harvest index, indicating the need for efficient resource utilization. NGRC06833 and 200525 (Rampur) had a high harvest index and total biomasses, suggesting a balanced resource allocation for substantial grain production. NGRC02687 exhibited an expedited flowering and maturity process, requiring 52 and 108 days, respectively. The plant attains a moderate height of 71.9 cm, with a considerable low grain yield of 769 kg ha^{-1}, showcasing its potential for early cultivation and substantial output. Conversely, NGRC06835 demonstrated an extended maturity period of 118 days, coupled with a taller stature (87.2 cm) and a notable 14.3 nodes per plant, contributing to a robust grain yield of 2041 kg ha^{-1}. Similar result was reported by Baraskar et al (2014) the genotypes were differences for all the morphological characters studied. Aditya et al. (2011) reported similar findings in soybean as all studied agro-morphological traits were different in genotypes.

The presentation of diverse features is significantly influenced by genotypic diversity, which calls for a careful selection procedure for cultivars that are suited to certain growing circumstances and agricultural goals. A characteristic like the number of pods on a plant, which directly affects grain yield, must be quantitatively evaluated. A crucial factor in determining seed quality and yield estimation is the hundred seed weight. Furthermore, the thorough assessment of total biomass which includes both above-ground and below-ground biomass components is essential for determining overall plant production. Plant height, branching pattern, and node count vary significantly, providing options for different farming practices and preferences (Duc et al 2023), (Deivasigamani and Swaminathan 2018) and (Qi et al 2020) supported above findings.

Table 3. Average of Agronomic traits of 150 Soybean accessions across three locations of year 2019 and 2020

List of genotypes	Days to 50% flowering	Days to Maturity	Plant height	No of primary Branch	No of node per plant	No of pod per plant	Grain yield	Hundred seed weight	Harvest index	Total bio mass
NGRC02687	52	108	71.9	4.9	10.9	43.3	769.2	15.4	0.36	257
Soy Agd-005	57	114	41.0	4.5	9.5	57.6	999.0	14.8	0.42	271
NGRC06811	61	121	61.8	5.0	10.9	58.5	1098.6	9.5	0.41	249
NGRC02675	59	121	55.7	5.9	12.5	86.5	1754.6	8.5	0.58	249
TGX1835-10F	63	120	74.0	5.4	13.3	67.1	1309.3	9.2	0.53	218
NGRC02691	55	111	43.7	4.5	9.5	49.6	926.8	15.1	0.40	257
TGX1987-62F	62	122	54.3	5.7	12.5	102.5	1988.3	9.0	0.62	238
Soy Agd-011-2	58	119	44.2	4.1	9.5	47.9	889.1	14.1	0.41	244
Solu coll#2-2016	58	116	58.2	5.1	10.7	61.9	1318.0	16.6	0.52	238
NGRC06826	52	105	38.7	5.0	9.8	52.6	1008.6	17.9	0.41	283
NGRC08244	57	120	59.9	4.8	12.2	50.0	994.5	12.6	0.40	292
TGX1989-19F	63	122	63.9	5.0	12.4	54.6	1124.9	8.3	0.42	274
2003KS-KBxTB1-2.1-3	50	103	33.0	3.9	8.8	31.7	570.8	18.1	0.31	249
Seti	60	118	49.4	4.3	9.2	65.2	1080.5	15.9	0.40	283
2003KS-TB1xKB-5.14-2	52	108	35.5	4.3	9.2	41.0	795.2	21.5	0.37	248
Sindhuli Khairo	57	115	62.0	4.6	10.8	40.8	690.6	8.9	0.32	279
NGRC02676	54	116	59.5	5.4	12.2	72.4	1299.0	14.0	0.51	242
Bringi,Pyuthan Bazar-2016	57	111	57.0	4.9	11.2	59.0	1080.2	16.7	0.47	230
Lumle Bhatmas 1	60	120	81.9	6.1	15.1	75.3	1437.6	17.5	0.57	214
NGRC02716	55	117	57.4	5.5	11.3	50.4	926.2	4.6	0.40	228
AGS 377	52	110	46.2	5.0	11.1	72.7	1415.4	24.5	0.51	254
Soy Agd-013	57	113	52.9	4.1	9.0	38.5	794.3	16.8	0.34	292
NGRC02711	52	107	50.9	5.3	9.9	51.5	927.4	13.9	0.41	258
2003KS-TB1xKB-5.45	52	107	36.3	4.1	8.8	35.7	701.3	18.3	0.35	251
NGRC06809	51	110	33.9	5.2	10.7	84.8	1624.3	9.2	0.55	253
Solu-Small seed	54	112	60.8	5.9	13.0	74.9	1357.5	17.2	0.52	244
Lamjung local	53	107	37.1	4.3	8.6	44.1	810.6	14.6	0.35	285
NGRC02703	54	116	64.4	4.9	10.6	36.8	657.5	14.8	0.30	304

List of genotypes	Days to 50% flowering	Days to Maturity	Plant height	No of primary Branch	No of node per plant	No of pod per plant	Grain yield	Hundred seed weight	Harvest index	Total bio mass
NGRC06815	54	115	49.0	4.7	10.0	41.3	749.1	15.7	0.33	297
NGRC02699	55	117	67.9	6.2	13.9	74.9	1395.9	16.2	0.53	243
NGRC02664	54	117	49.7	4.5	10.5	42.4	711.0	13.5	0.35	253
NGRC02707	53	110	48.2	4.8	10.1	41.1	791.8	15.3	0.36	281
NGRC05101	55	116	54.2	4.1	9.2	31.1	415.3	5.6	0.22	269
2003KS-TB1xKB-5.66	55	110	31.2	3.7	7.6	28.5	534.3	18.8	0.28	278
NGRC06830	55	118	70.1	4.8	11.4	59.4	1090.	18.1	0.45	243
AGS 377	53	115	55.3	5.3	11.3	44.6	868.9	23.7	0.41	243
NGRC08243	54	113	59.3	5.0	11.3	49.4	859.4	14.2	0.36	245
NGRC08245	53	116	73.5	5.0	11.7	44.1	820.6	14.8	0.38	264
010-10.2	52	111	55.0	3.4	9.9	29.5	356.4	17.2	0.18	317
NGRC02690	55	119	90.4	6.2	14.6	73.5	1422.3	14.8	0.52	255
IARS-87-1	56	118	56.2	5.1	11.2	58.3	1114.9	12.7	0.44	278
NGRC02686	53	109	56.5	4.6	11.7	53.1	1022.1	14.4	0.44	260
TGX1987-11E	58	120	80.3	5.7	12.8	83.2	1579.2	14.6	0.54	267
TGX 1925-1F	57	116	69.9	5.4	13.3	58.3	1083.0	15.2	0.45	265
AGS-376	58	119	57.4	5.3	11.0	50.6	935.3	12.6	0.42	258
NGRC06835	61	118	87.2	5.8	14.3	108.5	2041.0	8.9	0.62	249
NGRC06822	56	121	107.9	4.6	9.6	59.9	838.7	10.8	0.35	283
Tanahu-Creami	51	111	29.9	3.8	9.7	33.3	604.6	19.2	0.29	291
GC8234GC-13	61	117	66.9	6.3	14.2	93.7	1952.2	9.0	0.61	246
2003KS-TB1xKB-5.4	53	111	34.2	4.3	8.3	33.2	566.3	19.0	0.30	262
Chitwan-9	55	109	58.6	4.8	11.5	49.9	884.7	12.2	0.35	305
Baglung Khairo	53	119	67.8	4.3	8.6	42.2	736.4	13.6	0.33	298
NGRC02684	53	115	58.8	5.0	11.6	53.2	952.3	13.1	0.40	272
NGRC06813	53	106	42.6	5.3	9.8	56.2	1138.1	13.2	0.50	225
TGX1987-42F	59	116	67.8	5.9	14.4	86.7	1575.5	9.7	0.57	231
Soy Agd-001	60	119	50.1	4.2	9.1	46.1	850.2	17.9	0.35	287
Tarkari bhatmas 1	51	108	29.0	3.7	8.9	49.5	972.0	18.4	0.41	284
AGS 371	58	117	39.8	5.0	11.6	55.1	1108.0	18.8	0.50	224
Soy Agd-006	54	123	86.7	4.7	10.7	44.5	790.6	14.9	0.36	286
G8754	56	118	77.7	6.0	14.2	81.2	1456.7	18.3	0.54	232
Coll # 6	54	120	63.6	5.3	11.0	46.7	936.2	17.5	0.41	246
NGRC06812	56	117	73.9	5.0	12.1	61.5	1032.0	17.6	0.42	261
Soy Agd-021	60	117	61.6	6.3	13.4	80.1	1475.8	9.7	0.56	227
NGRC07369	55	111	47.5	4.5	9.6	28.8	521.8	16.0	0.27	281
Baglung Seto	55	121	88.0	6.0	13.5	71.1	1237.0	13.0	0.50	229
TGX311-23D	55	111	87.1	6.0	13.6	78.7	1479.7	13.1	0.56	228
Banglung black	54	120	45.0	3.6	8.6	25.8	348.0	13.8	0.19	286
NGRC07367	56	111	54.9	4.9	11.4	45.9	922.7	15.7	0.39	287
Palpa	51	113	39.4	4.0	9.9	35.6	585.0	11.2	0.29	263
NGRC07368	57	118	48.5	6.1	13.5	75.7	1569.1	15.4	0.57	228
Soy Agd-010	58	115	69.0	4.1	9.5	39.0	760.4	12.6	0.35	258
Soy Agd-014	59	119	65.3	4.8	10.8	47.2	868.7	9.1	0.39	265

List of genotypes	**Days to 50% flowering**	**Days to Maturity**	**Plant height**	**No of primary Branch**	**No of node per plant**	**No of pod per plant**	**Grain yield**	**Hundred seed weight**	**Harvest index**	**Total bio mass**
Ransom	60	120	59.8	5.4	11.7	65.1	1303.5	14.3	0.51	252
Puja	55	118	59.1	6.1	12.5	83.6	1604.7	14.4	0.57	239
2003KS-TB1xKB-5.69	50	107	39.2	3.1	8.6	29.0	425.3	18.7	0.23	282
200525(Rampur)	55	115	74.3	6.4	14.3	106.3	2074.9	9.8	0.62	252
NGRC02679	54	115	49.0	4.8	10.5	55.1	945.1	11.7	0.42	250
NGRC02672	57	114	61.1	5.6	12.1	55.0	1000.6	9.5	0.45	237
SB0-122	56	114	48.2	4.9	11.0	64.1	1304.1	10.3	0.48	274
Tandi coll # 2	62	128	72.4	4.3	10.7	41.4	628.3	12.7	0.31	269
F778817	62	118	52.0	5.4	11.2	63.9	1306.7	10.7	0.50	255
NGRC02693	54	110	55.0	4.3	10.3	36.1	608.3	15.5	0.27	307
Surkhet#2	59	118	82.4	5.0	12.3	54.0	1076.3	18.2	0.43	273
2003KS-TB1xKB-5.37	53	112	29.9	3.6	8.0	32.4	608.9	19.1	0.31	255
2003KS-TB1xKB-5.39	51	107	32.8	4.0	9.4	43.9	731.3	18.5	0.33	269
Soy Agd-002	60	117	75.6	6.2	13.5	64.6	1220.1	10.0	0.50	240
Haripur Khairo Bhatmas-1	55	123	68.0	3.9	9.3	37.5	616.9	18.5	0.30	277
TGX1876-4E	54	109	63.9	4.8	10.3	53.2	898.0	9.9	0.38	273
Soy Agd-008	60	116	62.4	6.3	12.9	77.8	1384.2	12.4	0.52	243
2003KS-TB1xKB-5.62	51	112	46.1	3.5	9.5	29.6	445.0	19.2	0.24	278
NGRC02683	56	117	52.5	5.9	12.2	80.9	1565.9	12.7	0.59	215
P194159	56	116	56.2	6.0	13.4	86.6	1663.4	8.6	0.58	237
TGX1485-ID	58	120	72.6	6.0	12.9	70.7	1210.4	11.6	0.48	266
Dakshinkali-Creami Bold	59	118	42.0	4.5	10.4	48.8	888.0	22.6	0.40	266
NGRC06834	52	107	40.0	4.7	10.3	45.2	929.2	16.9	0.37	290
NGRC06816	57	119	61.5	4.8	11.0	57.0	962.1	15.8	0.41	272
Soy Agd-020	56	116	46.2	5.6	11.6	54.1	1047.3	13.6	0.43	269
Gulmi Kalo	38	110	35.3	5.3	12.8	41.1	786.1	13.1	0.35	272
NGRC06823	55	117	60.6	5.2	12.6	57.4	973.5	16.6	0.43	251
Collection #178	59	125	56.9	4.3	8.5	48.2	378.6	14.7	0.22	277
2003KS-TB1xKB-5.67	51	109	56.0	3.2	8.7	28.2	447.7	19.2	0.26	248
Collection # 1 Mangalpur	53	108	51.3	4.8	10.5	48.5	859.9	13.8	0.40	259
Brown, Jumla-2016	52	109	85.6	4.4	10.7	39.5	753.4	16.6	0.34	289
NGRC02674	53	117	66.0	4.7	10.7	33.7	588.2	6.1	0.30	261
272W	56	116	65.6	5.9	13.4	82.4	1480.7	8.8	0.53	256
2003KS-TB1xKB-5.61	52	107	43.0	4.0	9.1	35.7	627.8	18.7	0.30	294
TGX1990-8F	61	121	59.2	5.0	12.5	69.4	1247.4	14.3	0.49	258
Gulmi Seto	55	127	107.7	4.6	11.4	48.9	739.6	14.9	0.35	244
NGRC02669	56	118	57.6	4.4	10.7	45.2	752.4	11.5	0.33	288
NGRC02712	53	111	44.8	4.9	9.6	52.3	1005.8	18.4	0.39	288
Tidar	52	106	46.8	4.0	10.2	33.7	578.3	7.9	0.29	290
Sathiya	50	106	44.0	3.6	8.5	25.1	481.8	18.1	0.25	293
G-8586	52	108	34.3	4.5	10.9	64.2	1209.4	9.6	0.48	254

List of genotypes	Days to 50% flowering	Days to Maturity	Plant height	No of primary Branch	No of node per plant	No of pod per plant	Grain yield	Hundred seed weight	Harvest index	Total bio mass
Coll # 166	50	116	72.2	4.7	12.1	46.6	855.7	19.3	0.39	272
LS-77-16-16	50	107	42.1	5.2	10.7	73.6	1656.9	10.9	0.57	243
NGRC02717	58	119	67.2	4.8	11.4	40.3	716.2	5.4	0.35	241
NGRC06833	59	120	82.2	5.9	12.9	110.1	2074.3	16.2	0.63	245
2003KS-TB1xKB-5.32-2	53	110	53.9	3.5	9.8	26.4	470.5	19.2	0.24	295
NGRC02710	53	116	33.5	5.0	8.6	46.1	866.7	18.5	0.36	290
2003KS-KBxTB1-8	52	108	32.3	4.3	9.0	35.0	727.7	17.2	0.33	289
2003KS-TB1xKB-5.34	51	105	44.7	4.2	9.2	28.0	522.7	22.7	0.27	278
2003KS-KBxTB1-2.1-2	55	118	44.9	4.1	10.3	36.6	682.6	21.7	0.35	253
VI (Bhojpur)	54	122	88.3	5.6	11.6	51.3	880.4	17.6	0.37	275
NGRC02666	54	114	43.2	4.5	9.9	57.7	883.2	13.3	0.39	266
NGRC02671	56	118	58.7	4.9	11.7	68.6	936.5	9.2	0.39	274
2003KS-TB1xKB-5.64	51	107	46.4	3.6	8.6	32.3	486.3	17.6	0.25	287
Kavre	56	115	73.2	5.4	12.7	94.2	1816.1	8.2	0.58	254
NGRC06820	52	106	35.1	4.7	8.8	46.3	882.4	15.4	0.39	262
NGRC02719	56	119	66.6	5.4	12.3	71.6	1208.2	13.3	0.51	230
NGRC02704	56	118	46.3	5.2	11.5	60.6	1107.4	14.3	0.48	233
TGX1987-62F	63	118	55.8	5.3	12.2	65.9	1310.5	9.1	0.49	260
Ankur	55	117	64.6	6.1	13.5	73.0	1376.9	7.9	0.50	251
Ramechhap Collection	58	115	45.0	3.9	8.8	44.5	916.3	17.2	0.37	296
NGRC06832	54	114	49.5	5.2	10.4	55.1	1104.8	14.3	0.44	247
Collection 169	53	109	65.7	4.5	10.6	52.1	759.9	15.9	0.36	269
NGRC06821	53	109	55.1	6.0	11.7	61.6	1162.0	14.0	0.49	227
TGX1987-14F	51	110	50.2	5.4	11.5	59.4	1197.0	13.8	0.48	256
Collection 168	55	111	51.1	4.7	9.4	41.3	723.4	16.0	0.35	270
2003KS-TB1xKB-5.65	53	109	47.6	4.3	9.4	38.3	694.2	18.2	0.32	281
Collection 175	52	112	89.2	6.5	14.1	69.9	1226.0	12.4	0.53	212
Soy Agd-017	54	111	39.9	4.6	9.9	45.3	1024.2	18.0	0.44	256

Diversity analysis

Reliable amplification and reproducible patterns were observed for the markers that were used further for data analysis. Table 4 presents an exhaustive overview of various primers used in genetic analysis, highlighting key parameters such as the number of total alleles, size range in base pairs (BP), number of polymorphic alleles, percentage of polymorphisms, and the polymorphism information content (PIC). The primer for soy-satt-173 revealed six alleles spanning 150-1000 BP, indicating complete polymorphism (100%) with a high PIC value of 0.892. Similarly, satt 409 displayed five alleles ranging from 100 to 700 BP, with a full polymorphic profile (100%) and a PIC value of 0.793. The primer sat 342 revealed three alleles with the lowest PIC of 0.62 among all markers. In the overall analysis, a total of 124 alleles were identified across all primers, yielding an average of 4.13 alleles per primer. The cumulative number of polymorphic alleles was 114, contributing to an impressive overall polymorphism percentage of 89.11%. The mean PIC value for all primers was 0.795.

The dendrogram based on UPGMA with Jaccard similarity across 141 soybean accessions revealed five major clusters, as shown in Figure 1. Most of the accessions were clustered II, with 84 accessions, and the fewest were clustered I and V, with 5 and 3 accessions, respectively. Clusters III and IV included a large number of accessions after Cluster II, with 39 and 10 accessions, respectively. Three accessions of clusture V were AGS-377, NGRC02717 and F778817, they were with narrow to intermediate leaf size, white flower colour, black to imperfect black hilum colour, and normally medium for most of the agronomic traits. Clusture one carries five cultivars they were TGX1989-19F, AGS-371, Coll#6, Baglung black and Tandi coll#2, main traits of this group were comparatively late for maturity, white flower colour and with narrow to intermediate leaf size. Altogether ten accessions were grouped in clusture IV they were LS-77-16-16 (Khajura Bhatmas-1), VI (Bhojpur), NGRC06820, Collection 169, NGRC06821, TGX1987-14F, Collection 168, 2003KS-TB1xKB-5.65, Collection 175 and Soy-017. These genotypes were comparatively earlier for flowering and maturity, narrow to intermediate leaf size, black to imperfect black hilum colour (Table 1 and 3). When several released varieties are grouped together in one cluster, it is likely that most of the released varieties will become unstable and less resistant to changes in abiotic stressors, diseases, and pests.

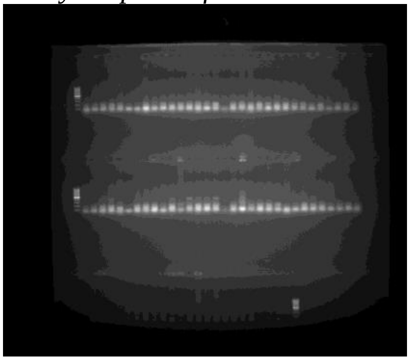
PCoA analysis scattering (Figure 2) also revealed variation in the accessions that formed five major clusters. The reproducibility and reliability of the data produced by SSR markers have made them one of the preferred systems for accessing genetic diversity studies. The high percentage of polymorphic loci observed in this study was consistent with the findings of previous studies (Hwang et al 2020 and Koutu et al 2019). Assessing the genetic relatedness and divergence of breeding materials has a large impact on how agricultural plants are improved. Genetic variety in soybeans has the potential to broaden the genetic base of breeding material for selection, aid in the understanding of germplasm structure by geneticists and breeders and assist them forecasting which pairings will provide the greatest number of offspring. In order to create selectable genetic variation utilizing genotypes that are genetically distinct, information on genetic distances based on microsatellite markers must be preferred (Hwang et al 2020 and Koutu et al 2019). Using 40 SSR markers, (Wang et al 2006) examined 17 wild types, 23 cultivated soybean varieties, and native varieties. They found that the great genetic diversity of the wild types resulted from the loss of several alleles during the evolutionary process. Similarly, Song et al. (2013) using 72 markers, analyzed 185 accessions of genetic resources collected from Korea, China, Japan, India, Myanmar, the Philippines, and the United States. The study's high PIC value on the SSR marker demonstrated the strong informativeness of the genotype discrimination and separation, similar result was also reported by Clever et al. (2020), with 20 markers having high PIC values than 0.4 indicating molecular polymorphism across the regions of genome. SSR markers are highly polymorphic and have the potential to create highly significant allelic variants, according to Jeffreys et al. (1994). Park et al (2009) examined the use of SSRs and other molecular markers in diversity studies and reported that SSRs had the highest levels of genetic variation and heterozygosity.

Table 4. Detail on genetic information generated by SSR markers in 141 soybean accessions

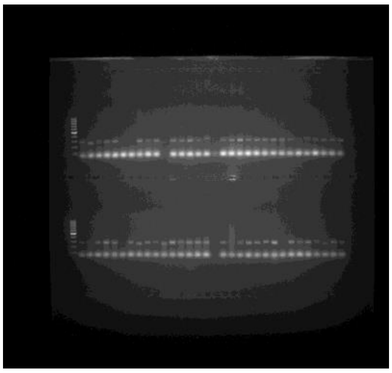
Primers	Number of total Alleles	Range (BP)	Number of bands in Each species	No of polymorphic Alleles	Percentage of Polymorphism	*PIC
soy-satt-173	6	150-1000	1-6	6	100	0.89
satt 409	5	100-700	1-5	5	100	0.79
satt 005	2	100-200	2	1	50	0.63
satt 148	4	200-650	1-4	4	100	0.78
satt 173	6	180-1000	1-6	6	100	0.96
satt 185	2	100-200	2	1	50	0.76
satt 285	3	200-400	1-3	2	66.67	0.79
satt 307	8	100-600	1-8	7	87.5	0.67
satt 276	5	180-380	1-5	5	100	0.91
satt 135	2	180-200	2	2	100	0.76
satt 365	3	100-300	1-3	2	66.67	0.72
satt 557	2	180-200	2	2	100	0.73
satt 316	6	180-800	1-6	6	100	0.75
satt 319	7	100-750	1-7	7	100	0.94
satt 545	5	100-350	1-5	5	100	0.84
satt 249	7	115-500	1-7	6	85.72	0.84
satt 256	3	180-300	1-3	3	100	0.80
satt 001	3	100-450	1-3	2	66.67	0.79
satt 373	5	110-450	1-5	5	100	0.79
sat 001	3	100-800	1-3	2	66.67	0.67
Barcsatt_100	4	100-450	1-4	4	100	0.67
satt 156	3	150-450	1-3	3	100	0.75
satt 36	3	100-400	1-3	2	66.67	0.81

SAT 1	3	100-300	1-3	3	100	0.87
satt 411	2	100-180	2	2	100	0.84
sat 076	3	150-300	1-3	3	100	0.87
sat 417	5	150-900	1-5	5	100	0.95
sat 043	5	110-400	1-5	5	100	0.83
satt 197	6	150-1000	1-6	6	100	0.84
sat 342	3	100-250	1-3	2	66.67	0.63
Total	124			114	2673.24	23.86
Mean	4.13			3.8	89.108	0.80

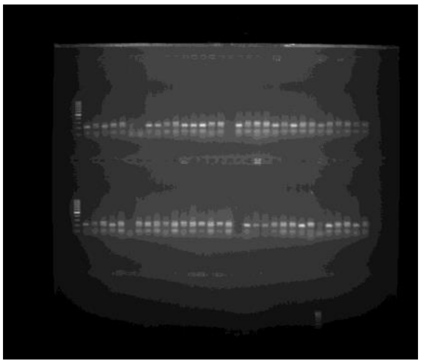
**Polymorphic Information Content*



PN 24 a Picture-1



PN 12-a Picture-2



PN 17-a Picture-3

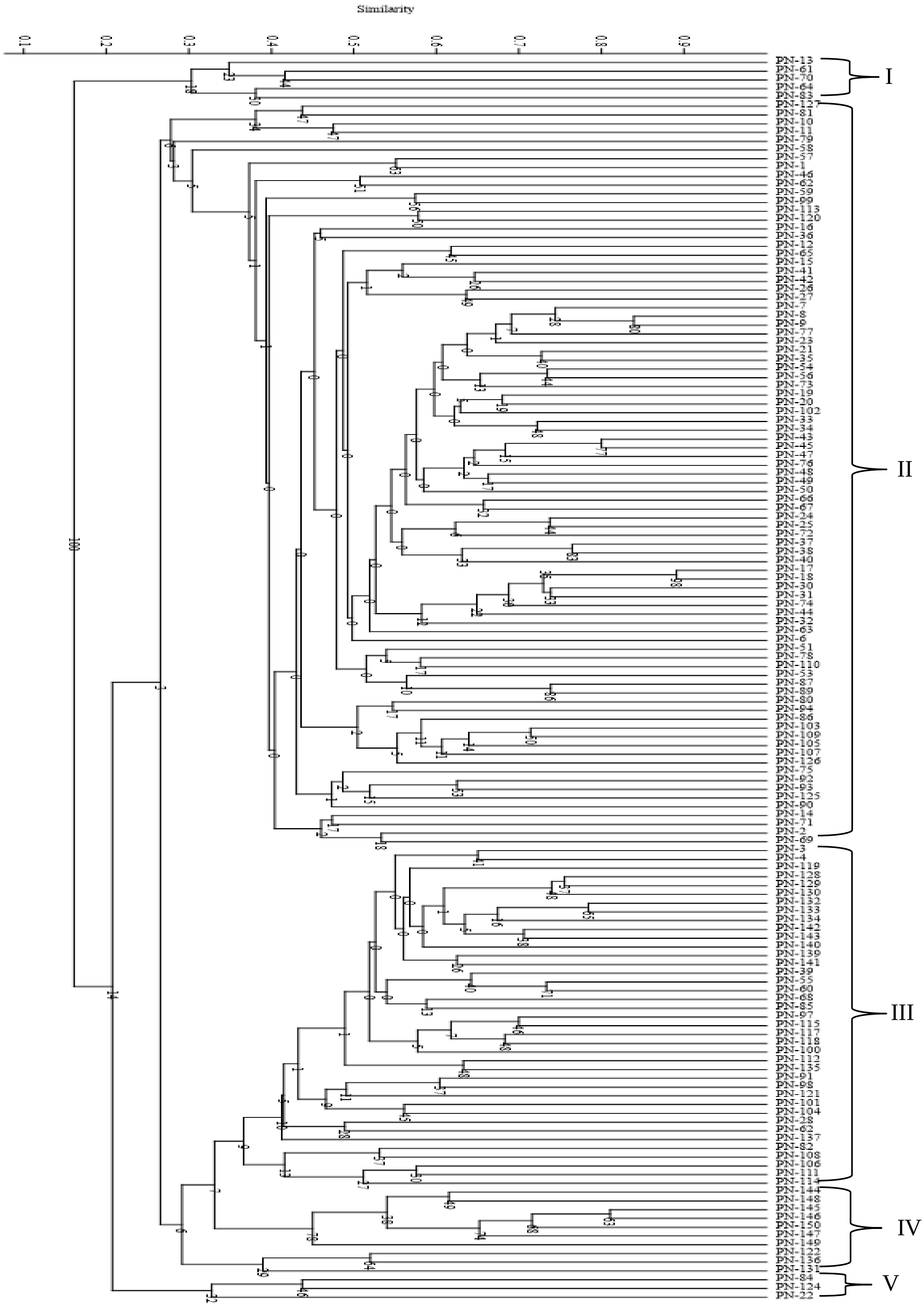


Figure 1.UPGMA dendogram showing genetic similarity among 141 soybean accessions based on SSR markers forming five main clusters

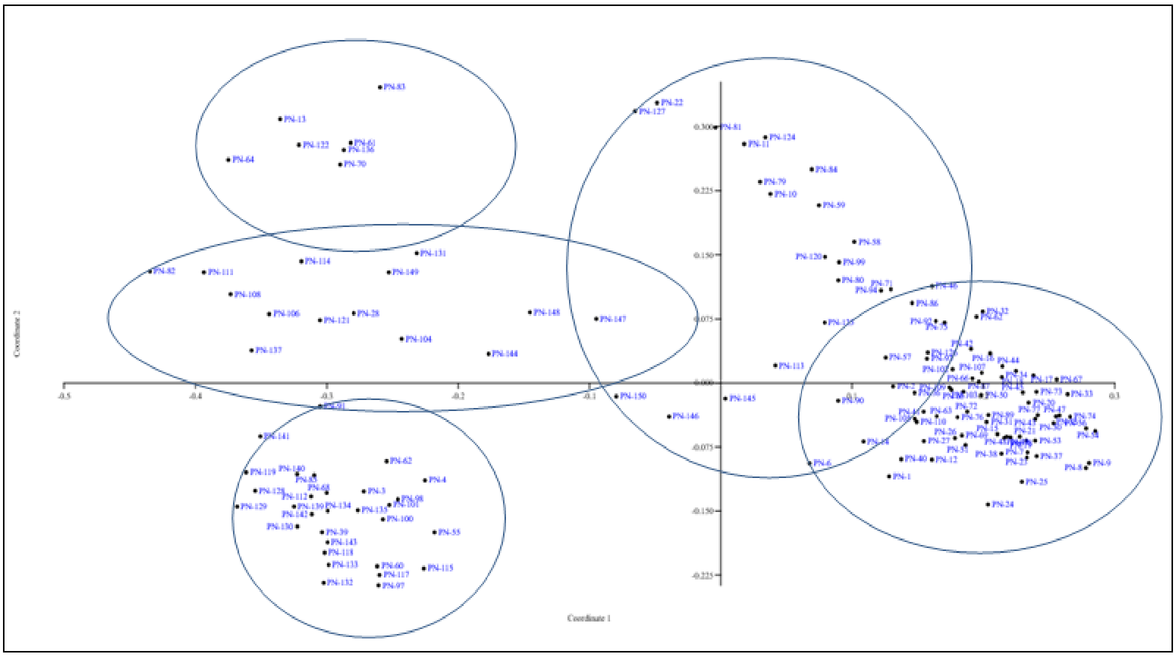


Figure 2. PCoA analysis on the basis of SSR marker showing five main clusters from the analysis

CONCLUSION

In conclusion, agronomic traits provide insights into various aspects of soybean accessions, such as flowering and maturity dynamics, plant architecture, and yield potential. Analyzed 141 soybean genotypes using 30 SSR markers that have been previously confirmed to be highly polymorphic. Five clustures were formed with the use of PCoA and UPGMA analytical tools. Observed wide range of variability among the studied cultivars may be due to their different genetic constitution. The information on genetic diversity will help to identify genotypes and build a core collection for soybean conservation and breeding. It also aids in the analysis of genetic resources, including recently developed soybeans. Finally, this study will aid in the development of future varieties with beneficial traits such as disease and drought resistance.

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AUTHORS' CONTRIBUTION

Netra Hari Ghimire in consultation with respected members of advisory committee formulated the research proposal, carried out the field and laboratory experiment, collected the data, and prepared ANOVA and manuscript.

CONFLICTS OF INTEREST

The authors have no any conflict of interest to disclose.

REFERENCES

Aditya JP, P Bhartiya and A Bhartiya. 2011. Genetic variability, heritability and character association for yield and component characters in soybean (G. max (L.) Merrill). Journal of Central European Agriculture **12**(1):27-34 DOI: 10.5513/JCEA01/12.1.877

Baraskar VV, VH Kachhadia, JH VachhanI, HR Barad, MB Patel and MS Darwankar. 2014. Genetic variability, heritability and genetic advance in soybean [Glycine max (L.) Merrill]. Electronic Journal of Plant Breeding **5(**4): 802-806. ISSN 0975-928X.

Chakraborty S. 2018. Genetic diversity analysis in soybean (Glycine max (L .) Merrill) . using SSR markers. 7(June), 2380-2384.

Chandrawat KS, KS Baig, S Hashmi, DH Sarang, A Kumar and PK Dumai. 2017. Study on Genetic Variability, Heritability and Genetic Advance in Soybean, Int. J. Pure App. Biosci. **5**(1): 57-63. doi: http://dx.doi.org/10.18782/2320-7051.2592.

Clever M, T Phinehas, M Mcebisi, D Shorai, OD Isaac, O Tonny, K Hellen and R Patrick. 2020. Genetic diversity analysis among soybean genotypes using SSR markers in Uganda. African Journal of Biotechnology **19**(7): 439-448. https://doi.org/10.5897/ajb2020.17152

De Riek J, E Calsyn, I Everaert, E Bockstaele, E Van, and De M Loose. 2001. AFLP based alternatives for the assessment of distinctness, uniformity and stability of sugar beet varieties. Theor Appl Genet.**103**:1254-1265.

Deivasigamani S and C Swaminathan. 2018. Evaluation of Seed Test Weight on Major Field Crops. International Journal of Research Studies in Agricultural Sciences **4**(1): 08-11. http://dx.doi.org/10.20431/2454-6224.0401002I

Duc NT, A Ramlal, A Rajendran, D Raju, SK Lal, S Kumar, RN Sahoo, V Chinnusamy. 2023. Image-based phenotyping of seed architectural traits and prediction of seed weight using machine learning models in soybean. Front Plant Sci. 2023 Sep 12;**14**:1206357. doi: 10.3389/fpls.2023.1206357. PMID: 37771485; PMCID: PMC10523016.

Hwang TY, BS Gwak, J Sung, and KS Kim. 2020. Genetic diversity patterns and discrimination of 172 Korean soybean (Glycine max (L.) merrill) varieties based on SSR analysis. Agriculture (Switzerland) **10**(3). https://doi.org/10.3390/agriculture10030077

Jeffreys AJ, K Tamaki, A MacLeod, DG Monckton, DL Neil, and JAL Armour. 1994. Complex gene conversion events in germline mutation at human minisatellites. Nature Genetics **6**(2): 136–145. https://doi.org/10.1038/ng0294-136

Koutu GK, A Shrivastava, Y Singh, and S Tiwari. 2019. Molecular characterization and genetic diversity assessment of soybean varieties using SSR markers. International Journal of Current Microbiology and Applied Sciences **8**(04): 173–182. https://doi.org/10.20546/ijcmas.2019.804.018

Kujane K, MM Sedibe and A Mofokeng. 2019. Genetic diversity analysis of soybean (Glycine max (L.) Merr.) genotypes making use of SSR markers. Australian Journal of Crop Science **13**(7): 1113–1119. https://doi.org/10.21475/ajcs.19.13.07.p1638

Kumawat G, G Singh, C Gireesh, M Shivakumar, M Arya, DK Agarwal and SM Husain. 2015. Molecular characterization and genetic diversity analysis of soybean (Glycine max (L.) Merr.) germplasm accessions in India. Physiology and Molecular Biology of Plants **21**(1): 101–107. https://doi.org/10.1007/s12298-014-0266-y

MOAD. 2020. Statistical information on Nepalese agriculture, (2018/19). Government of Nepal. Ministry of Agricultural Development, Monitoring, evaluation and statistics division, Agri-statistics section: Singh durbar, Kathmandu Nepal.

MOAD. 2022. Statistical information on Nepalese agriculture, (2020/21). Government of Nepal. Ministry of Agricultural Development, Monitoring, evaluation and statistics division, Agri-statistics section: Singh durbar, Kathmandu Nepal.

Moongkanna J, S Nakasathien, WP Novitzky, P Kwanyuen, P Sinchaisri and P Srinives. 2011. SSR markers linking to seed traits and total oil content in soybean. Thai Journal of Agricultural Science **44**(4): 233-241.

Park YJ, JK Lee and NS Kim. 2009. Simple sequence repeat polymorphisms (SSRPs) for evaluation of molecular diversity and germplasm classification of minor crops. Molecules **14**(11): 4546–4569. https://doi.org/10.3390/molecules14114546

Qi Z, J Song, K Zhang, S Liu, X Tian, Y Wang, Y Fang, X Li, J Wang J, C Yang, S Jiang, X Sun, Z Tian, W Li and H Ning. 2020. Identification of QTNs Controlling 100-Seed Weight in Soybean Using Multi locus Genome-Wide Association Studies. Front. Genet. **11**:689. doi: 10.3389/fgene.2020.00689

Sallam A, A Amro, A Elakhdar, MFA Dawood, YS Moursi and PS Baenziger. 2019. Marker–trait association for grain weight of spring barley in well-watered and drought environments. Molecular Biology Reports **46**(3): 2907–2918. https://doi.org/10.1007/s11033-019-04750-6

Shi A, P Chen, B Zhang and A Hou. 2010. Genetic diversity and association analysis of protein and oil content in food-grade soybeans from Asia and the United States. Plant Breeding **129**(3): 250–256. https://doi.org/10.1111/j.1439-0523.2010.01766.x

Song JY, X Piao, YM Choi, GA Lee, JW Chung, JR Lee, Y Jung, HJ Park and MC Lee. 2013. Evaluation of genetic diversity and comparison of biochemical traits of soybean (Glycine max L.) germplasm collections. Plant Breeding and Biotechnology **1**(4): 374-384. https://doi.org/10.9787/PBB.2013.1.4.374

Tantasawat P, J Trongchuen, T Prajongjai, S Jenweerawat and W Chaowiset. 2011. SSR analysis of soybean (Glycine max (L.) Merr.) Genetic relationship and variety identification in Thailand. Australian Journal of Crop Science **5**(3): 283-290.

Wang J, P Zhou, X Shi, N Yang, L Yan, Q Zhao, C Yang and Y Guan. 2019. Primary metabolite contents are correlated with seed protein and oil traits in near-isogenic lines of soybean. The Crop Journal **7**(5): 651–659. https://doi.org/10.1016/j.cj.2019.04.002

Wang L, R Guan, L Zhangxiong, R Chang and L Qiu. 2006. Genetic diversity of chinese cultivated soybean revealed by SSR markers. Crop Science **46**(3): 1032–1038. https://doi.org/10.2135/cropsci2005.0051

Zhang BBDJ and PSS Chen. 2014. Planting date, irrigation, and row spacing effects on agronomic traits of food-grade soybean. Advances in Crop Science and Technology **02**(04). https://doi.org/10.4172/2329-8863.1000149