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Study on Morpho-nutritional Diversity in Grasspea (*Lathyrus sativus* L.)

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ICAR-INDIAN AGRICULTURAL RESEARCH INSTITUTE

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Study on Morpho-nutritional Diversity in Grasspea (*Lathyrus sativus* L.)

A Thesis

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K R RAMYA

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CERTIFICATE

This is to certify that the thesis entitled, **"Study on Morpho-nutritional Diversity in Grasspea (*Lathyrus sativus* L.)"** submitted to the **Faculty of the Graduate School, ICAR- Indian Agricultural Research Institute, New Delhi,** in partial fulfillment of the requirements for the award of **DOCTOR OF PHILOSOPHY (AG.) IN PLANT GENETIC RESOURCES,** embodies the results of *bona fide* research work carried out by **Ms. K R Ramya, Roll No: 11564,** under my guidance and supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help availed during the course of investigation as well as sources of information have all been duly acknowledged.

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(Dr. Kailash Chandra Bhatt)
Chairperson
Advisory Committee

DEDICATED TO:

My Beloved Parents, Chairperson,

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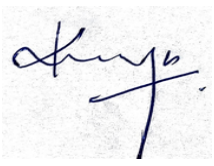

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(K R RAMYA)

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CHAPTER I

INTRODUCTION

Grain legumes hold a unique position in modern agriculture on account of being a rich protein source both as food and feed. They occupy a vital place in the crop rotation system by virtue of their nitrogen fixation ability. “Dahl” made from dehusked splits form 75% of the consumption in India. Moreover, sprouted grain legumes are rich in vitamin C. Consequently, they form an important part in the diet of vegetarians. Besides, pulses possess a tremendous potential to uplift the economic status of farmers especially small and marginal. Despite India being the largest producer and consumer of pulses in the world, high price elasticity of demand has led to significant downfall in pulse production which has resulted in widespread protein malnutrition. However, due to concerted efforts by Government of India, the total area under pulses was increased to 29.36 million hectares (production: 24.5 million tonnes; yield: 835 kg/ha]. The Recommended Dietary Allowances (RDA) for adult males and females is 60 g and 55 g per day. The per capita availability of pulses is @ 52 g per day (Pulses Retrospect, 2017).

Lathyrus sativus L. (2n=14) commonly called as Grasspea, Khesari, Kalai, Chickling vetch or Sabberi is an annual pulse crop belongs to the family Fabaceae. The word "*Lathyrus*" is derived from the ancient Greek word “λάθυρος (láthuros)” which means “exciting”, and refers to the aphrodisiac properties of grass pea (Loudon, 1855). *L. sativus, L. cicera,* and *L. hirsutus* are the most extensively cultivated species for food and feed whereas *L. latifolius* and *L. odoratus* are gorgeous looking ornamental plants produced commercially in Europe (Tyagi et al. 2017).

The genus *Lathyrus* consists of more than 160 annual and perennial species (Chtourou-Ghorbel et al. 2001; Plitmann et al. 1995) and 187 species and subspecies (Allkin et al. 1986) belonging to 15 divisions based on morphological features (Smartt et al. 1994). However, only one species is widely cultivated as a food crop (*L. sativus*), while other species are cultivated to a lesser extent for both food and forage (Jackson and Yunus, 1984). Grass pea is an herbaceous perennial with a well-developed taproot system that is greatly branched, straggling, or ascending and have deep root system that enables them in better absorption of soil water and hence, this also contributes to the drought tolerance.

Stem is quadrangular, slender and flowers are axillary solitary. Flowers are of different color and blue violet is the most common color. The average of three to five seeds of the pods is present. Seeds are hardy and are varied shape and color. It is primarily a self-pollinated species, but has a high rate of out-crossing, ranging from 9.8 - 27.8%. Insects like honey bees are the main pollinators and also twisted keels with a slight opening in flowers aid in cross pollination (Rahman et al. 1995; Emmrich et al. 2020).

Grasspea is grown as a pulse and fodder crop in Southeast Asian countries since time immemorial. The earliest archaeological evidences date back from the 8th millennium BCE to the Neolithic age from the village of Jamro lying at the foothills of the Zagros Mountains in Northern Iraq (close to the Turkish and Iranian borders) at an altitude of 800msl (Jackson and Yunus, 1984; Lambein et al. 2019). The important archaeological evidences of grasspea are noted in adjacent areas of Tepe Sadz (7500-5700 BCE) and Ali Kosh (9500-7600 BC) in Iran (Jackson and Yunus, 1984) and in the Gangetic plains, India (2000-1500 BCE) (Saraswat, 1980) with presumption of its introduction from West Asia. Probable remains of *L. cicera* have also been reported at Azmaska Moghila, in Bulgaria during 7000 BCE (Renfrew, 1969). Several archaeobotanical and phytogeographical evidence prove that grasspea was initially domesticated in the Balkan Peninsula during the early neolithic era, around the 6th millennium BCE (Kislev, 1989). It is widely distributed in Asia (Bangladesh, China, India, Nepal and Pakistan), in the Middle East (Iraq, Iran, Afghanistan, Syria and Lebanon), in Northern Africa (Ethiopia, Egypt, Morocco, Algeria and Libya) and in Southern Europe (France, Spain and Italy) (Campbell, 1997; Patto et al. 2006; Piergiovanni et al. 2011; Dixit et al. 2016). Grasspea has the immense potential to grow as a rice-fallow pulse crop in northern India. A study has shown that out of 11.6 million hectares of fallow land in India ~0.5 million hectares could be easily brought under grass pea cultivation to improve land productivity and raise revenue for farmers as a second crop (Kumar et al. 2020). In India, the major grasspea cultivating states are Chhattisgarh, Bihar, Jharkhand, Maharashtra, Odisha, Assam, West Bengal and Eastern Uttar Pradesh and the other parts of northern India. At present in India, about 3.62 lakh ha area of land is under grasspea cultivation (Department of Agriculture and Farmers Welfare, 2022). It is grown as a relay crop in the Rice-Based Cropping system, utilizing the available moisture. It is also taken as mixed crop and intercrop during rabi season and as sole crop under *Utera* conditions.

Grasspea is considered as the climate smart crop species since cultivation of this crop requires minimal inputs and cost; thereby, it can be successfully incorporated in the conservation agriculture and breeding programmes for development of climate resilient varieties. It is a hardy crop and has many advantageous traits that make it an attractive crop such as deep penetrating root system which help in drought tolerance and at the same time it is not affected by excessive rainfall and can be cultivated in areas subject to flooding when lentil and chick pea are not expected to give good yields and also it can be cultivated on diverse types of soils, including low fertility soils to heavy clays (Sidorova et al. 2013). As an efficient nitrogen fixer grasspea is a good green manure that improves the soil fertility by supplementing 67 kg/ha of nitrogen in a single season and benefits the subsequent non leguminous crop (Singh et al. 2013). It can serve a variety of purpose apart from human food as feed and fodder with 18-36% and 17% protein content in seeds and leaves respectively (Rizvi et al. 2016, Barpete et al. 2020). The seeds are high level source of polyunsaturated fatty acids (Grela et al. 2010) and it is the only known dietary source of aminoacid L- homoarginine which benefits cardio vascular disease treatments (Singh and Rao, 2013; van Wyk et al. 2016) and in overcoming the consequences of hypoxia (Jammulamadaka et al. 2011). Therefore, as neutraceutical, grasspea is an excellent source and act as potential functional food (Llorent-Martinez et al. 2017). Nevertheless, in common with other grain legumes, grasspea seeds contain a variety of anti-nutritional factors, among that the major one which disrupts its usage as food legume is β-ODAP (β-N-oxalyl-l-α, β diaminopropionic acid). Some Chinese toothpaste brands also use its herbal extracts to avoid bleeding gums (Lambein et al. 2019 and Sharma et al. 2018).

Different species in genus *Lathyrus* including cultivated grasspea have greater potential for nutritional and functional use in the industry compared to other legumes. Therefore, Indian government and some international organizations are paying more attention and importance to the conservation and utilization of grasspea genetic resources due to their versatile uses under rapidly changing environmental conditions (Kumar et al. 2011 and Ramya et al 2022). Several efforts in breeding low β-ODAP grasspea varieties and identification of improved morpho-nutritional germplasm were undertaken by breeders in various countries recognizing its multifaceted use in several areas of research. Global Crop Diversity Trust project entitled “Adapting Agriculture to Climate Change” in collaboration with International Centre for Agricultural Research in the Dry Areas

(ICARDA) considers grasspea as one among the priority crops which improves the subsistence livelihood of marginal farmers (Dempewolf et al. 2014). Studies on the characterization of diverse grasspea germplasm for agro-morphological and nutritional aspects are need of hour to support their possible production and marketing and for enhanced utilization of grasspea in climate changing scenario. The characterization, evaluation and utilization of grasspea germplasm for low β-ODAP and other improved nutritional and agronomic traits make this orphan crop find its way back to farming and bring it as the part of sustainable agriculture.

Keeping in view the importance of genetic diversity assessment of diverse germplasm of grasspea germplasm, the present study is planned to conduct with the following objectives:

- Agro-morphological characterization of diverse germplasm
- Molecular characterization of the germplasm accessions.
- Biochemical characterization (seeds and leaves) for nutritional and anti-nutritional parameters.

CHAPTER II
REVIEW OF LITERATURE

Grasspea (*Lathyrus sativus* L., Leguminosae) is an ancient legume has over the past decade received increased interest as a plant that is adapted to arid conditions and contains high levels of protein, a component that is increasingly becoming hard to acquire in many developing areas. Because of the importance of the species as a survival food for some of the poorest people in the world, yet recognizing the dangers that excessive consumption can cause, in 1991 grasspea was listed among the crops included in the multilateral system of access and benefit sharing under the International Treaty on Plant Genetic Resources for food and Agriculture (ITPGRFA). Lathyrus is considered as drought-tolerant hardy crop, and is grown in low-rainfall regions under rainfed conditions, during winter when lentil and chickpea are not expected to give good yields. The crop has unique tolerance ability against stress environmental conditions not only drought but also for water logging. In addition to use as dal and chapatti, it is usually grown as fodder crop. Lathyrus leaves about 36-48 kg/ha nitrogen economy for the succeeding cereal. In India, the grains are sometimes boiled whole, but are most often processed through a dal mill to obtain split dal. Dal, a soup-like dish, is the most common method of retailing the crop in the Indian subcontinent. The flour, made from grinding either the whole or split seed, is sold as basan. In many parts of Bangladesh, roti (unleavened bread) made out of grasspea flour is a staple for the landless labourers. More recently the dal or basan has been used to adulterate pigeon pea dal and chickpea basan as these crops demand a higher price on the market.

An attempt has been made to review the available literatures on agro-morphological characterization, molecular diversity and biochemical characterization of grasspea under the following headings

2.1. Origin, Evolution and Distribution

2.2. Taxonomy

2.3. Agro-morphological characterization

2.4. Molecular characterization using SSR marker

2.5. Biochemical characterization

2.1. Origin, Evolution and Distribution

Based on archaeobotanical and phytogeographical evidence, the origin of cultivation of *L. sativus*

has been suggested to be the Balkan Peninsula (Kislev, 1989). No truly wild *Lathyrus sativus* persists today, although the plant occasionally appears as a weed among other crops and growing in the wild as escapees from cultivation (Jackson and Yunus, 1984). The species *L. sativus* is likely derived from its closest wild relative *L. cicera* (red pea) (Belaid et al. 2006). Fossilized seeds of *L. sativus* and allied species (*L. cicera* and *L. ochrus*) have been found in several Neolithic sites in the Fertile Crescent, Eastern Europe and the Mediterranean, dated to earlier than 6000 BCE (Marinova, 2007; Coward et al. 2008; Kislev, 1989; Peña-Chocarro et al. 2013). Despite this ancient origin of the crop, grasspea is not currently grown as a major crop globally and the acreage under cultivation with grasspea has been decreasing. The distributions of Grasspea are between the old and new world viz, mostly throughout the temperate regions of northern hemisphere and extend into Tropical East Africa and South America and the main centers of diversity includes Mediterranean and Irano Turanian regions (Kupicha, 1983).

2.2. Taxonomy

The word "Lathyrus" comes from the Greek word "lathyros" that hints at something exciting, referring to the aphrodisiacal qualities ascribed to grasspea (Loudon, 1855). Grasspea is a food, feed and fodder crop belonging to the family Leguminosae (= Fabaceae), subfamily Papilionoidea, tribe Vicieae. The genus *Lathyrus* consists of about 160 annual and perennial species (Plitmann et al. 1995; Chtourou-Ghorbel et al. 2001), also reported as 187 species and subspecies by Allkin and coauthors (1986). The species are separated into 15 sections based on their morphological traits (Smartt et al. 1994). Species are found in the Old World and the New World. There are centres of diversity for Old World species in Asia Minor and the Mediterranean region (Zeven and de Wet, 1982). The different common names of Grasspea in different countries are as follows Bangladesh –Khesari; China -San lee do, Cyprus Fovetta, pharetta, dog-toothed pea; Ethiopia- Sabberi, guaya; India - Kesare, khesari, karas, karil, kasar, khesari dhal, khesra, lang, chural, latri, lakhori, Lakhodi, chattra matur, santal, teora, tiuri, batura, chickling vetch, chickling pea; Nepal–Kheshari;Pakistan-Matri,mattra;Venezula Frijol, gallinazo, garbanzo, Almorta (Spain),Gilban (Sudan), Gisette (France),Pisello brettone (Italy) (Campbell,1997).

2.3. Agro-morphological Characterization

Characterization and evaluation of the assembled germplasm is essential to facilitate its utilization. In order to utilize effectively the available genetic diversity, the material must be properly characterized and catalogued. Grasspea accessions were morphologically characterized for

qualitative, quantitative and nutritional parameters (leaf protein) to identify promising genotypes for desired traits for future utilization in grasspea improvement programmes. The earlier studies on characterization of grasspea germplasm using morphological descriptors are presented here under:

In Nepal Yadav (1996), reported that pods per plant varied from 13 to 59 with a mean of 36 when 72 local germplasm accessions were evaluated. Thus, this character shows a large range of variation. General and specific combining abilities have been estimated in grasspea for pods per plant, 100-seed weight, seeds per pod, grain yield per plant and β-ODAP content (Dahiya and Jeswani 1974). It was generally found that non-additive gene action was predominant in both the F1 and the F2.

Evaluations by Mehra et al. 1996 indicated that pod lengths of 2.92 to 3.05 cm from 223 accessions that were evaluated. They also report that 48 accessions from Syria had pod lengths ranging from 3.6 to 4.0 cm while 12 accessions from Canada ranged from 3.08 to 4.0 cm in length.

Poligano et al. 2005 had done the characterization for the seventy-six grasspea entries of different geographical origin and found that there is a wide range of variation observed for the traits seed yield, biomass, leaf width and seeds/pod; and the lowest values of variation were estimated for time to flowering and time of emergence; all the other traits showed intermediate values.

The several characterization and other breeding efforts in grasspea are Tavolette et al. 2005 characterized 16 accessions of grasspea (*L. sativus*) collected from central Italy for agro-morphological traits and for β-ODAP content observed great variation for seeds per pod, seed weight, plant height and for number of primary branches.

Grela et al. 2010 studied Thirty-one European accessions of grasspea originating from Italy, Spain, Northern France, Germany and Poland. Substantial morphological variation exists in *L. sativus*. The genepool can be divided into two groups—one comprising the accessions from the Mediterranean basin (Italy and Spain) and the other group accessions from West-central Europe (Northern France, Germany and Poland). plants produced from the seeds obtained from West-central Europe appeared to be about 10 cm taller than the Mediterranean accessions and had predominantly white flowers. Their seeds were more than two times smaller than seeds of the accessions of Italian or Spanish origin. There were not noted any considerable differences in nutrient contents or antinutritional factors in seeds between these two groups of Lathyrus

accessions.

Grela et al. 2012 studied 54 grasspea accessions originating from Czech Republic, Hungary, Slovak Republic, Poland, Ukraine and Russia together with 18 red pea (*L. cicera*) accessions from Greece, Spain and Italy. They observed the wide range of variation plant height, days to flowering, pods per plant, seed size and test weight. Average value of this trait for grasspea was 203 g (variation 86–366 g) with considerably higher (P B 0.05) weight of *L. sativus* accessions originating from south-central Europe in comparison to those from east-central European countries (214 vs. 157 g).

Barpete (2015) reported genetic variation in seed morphological characteristics (shape, coat color, coat texture, size and 100-seeds weight) and biochemical characteristics (total seed protein content) in 53 Indian accession of grasspea. A comparison of results showed 100 seeds weight and protein content range from 4.03 to 8.82 g and 21.9 to 33.7% with the mean value 5.95 g and 25.6% respectively.

Correlation studies were done on the mutagenic populations revealed that all the yield contributing traits were positively correlated with each other and the experiment was conducted in 2012-15 winter seasons at District Seed Farm, BCKV, West Bengal, India. Correlation study was computed between yield and nine different yield components at different chemical concentration and radiation level in M2 and M3 generations. The correlation coefficient between seed yield per plant and its components were estimated at phenotypic level and the result revealed that the 100-seed weight was vital yield improving character in all three varieties of lathyrus under study (Singh et al. 2017)

Jeberson et al. 2018 carried out variability and association studies in seven parental lines with 21 F1 combination of grasspea observed that the higher PCV, GCV, heritability along with high GAM were observed for the traits plant height, number of pods per plant, number of primary branches and by association analysis the characters like plant height, number of pods per plant and biological yield can be given the importance.

Kosev and Vasileva (2019) had done the morphological characterization of six grasspea varieties in Bulgaria and found out nodule weight was positive correlated with the plant weight (r = 0.517). High coefficient of inheritance in a broad sense was found for the root weight (99.24%) and root length (92.65%) at the technical maturity stage, for the seed weight (77.94%) and number of branches per plant (72.32%), root weight (59.67%) and root length (48.09%) in the flowering

stage.

Mahapatra et al. 2020 investigated 20 different genotypes of grasspea was during winter season of 2018-19 at the Regional Research Sub-Station, Chakdah Nadia, West Bengal in Randomized Complete Block Design (RCBD) with three replications. They observed high to moderate heritability (%) coupled with high to moderate genetic advance was observed for plant height (cm), days to 50 % flowering, number of pods plant. Genotypic path co-efficient analysis revealed that during selection greater emphasis should be given on number of pods plant, number of seeds pod and 100 seed weight (g) for improvement of seed yield and with D^2 analysis resulted in five clusters among which maximum inter cluster distance was found between Cluster V and I (14.755).

2.4. Molecular characterization using SSR marker

SSR markers, also known as microsatellites, have been widely used in genetic studies and molecular breeding of various crop species, including Lathyrus. These markers are based on simple sequence repeats, consisting of short tandem repeats of nucleotides. They are valuable tools for assessing genetic diversity, population structure, and marker-assisted selection (MAS) in crop improvement programs. These studies have provided valuable insights into the level of genetic variation present in various populations, identifying potential sources of diversity for breeding programs. For instance, SSR marker studies have been conducted on grasspea (*L. sativus*) accessions collected from different geographical regions, revealing distinct genetic clusters and identifying accessions with unique allelic profiles. Characterizing accessions using SSR markers helps in the identification of duplicate accessions, ensuring the accuracy and integrity of germplasm collections. Furthermore, assessing the genetic diversity within collections aids in the establishment of core collections, which represent the broader genetic diversity of the entire collection and serve as a valuable resource for breeders and researchers. Such information is crucial for identifying potential parental lines for hybridization and introgression of desirable traits. The development of SSR markers by *In silico* mining of nucleotide sequences (Soren et al. 2020) and the use of SSR (Kumar et al. 2019; Lioi et al. 2011; Wang et al. 2015) and RFLP, RAPD (Croft et al. 1999); Chtourou-Ghorbel et al. 2000; Durga et al. 2014) markers for the genetic diversity analysis their application in marker–trait association for plant phenology and yield-related traits will play a significant role in understanding the association of novel alleles in trait expression in future lathyrus crop improvement programmes (Soren et al. 2020). The genetic

diversity studies using SSR markers are briefed as follows

Shiferaw et al. 2012 developed sequence-based markers for understudied species like grasspea using expressed sequence tags (ESTs) in public databases and cross-species transferable markers and estimated the genetic diversity of the grasspea populations in Ethiopia using cross-transferable EST-SSRs derived from *Medicago truncatula* L. A total of 45 alleles were found utilizing eleven EST-SSRs were identified and the gene diversity was ranged from 0.205 for marker Ls942 to 0.804 for MtBA32F05, with an average of 0.477. FST estimates by analysis of molecular variance were observed to be 0.01 for among regions, 0.15 for among accessions, and 0.84 for within accessions, respectively.

Using seven newly created Lathyrus expressed sequence tag-derived simple sequence repeat (EST-SSR) markers and four cross-transferable EST-SSRs obtained from *Medicago truncatula* L., Ponnaiah et al. 2011 investigated 20 grasspea accessions collected from diverse locations of Ethiopia. The number of alleles per locus varied from two to seven, with four alleles per locus was observed and it was contrast to the expected heterozygosity (He), which ranged from 0.354 to 0.470, the observed heterozygosity (Ho) ranged from 0.320 to 0.504. For comparisons between regions, accessions, and within accessions, the FST values determined by analysis of molecular variance were 0.01, 0.15, and 0.84, respectively.

SSR markers were created using an enriched genomic library of grasspea by Lioi et al. 2013. A total of 400 randomly chosen clones were sequenced, and approximately 30% of them had SSRs. Simple, interrupted, and compound SSRs were present in 75%, 9%, and 16% of the clones, respectively. Seven primer pairs produced distinguishable DNA banding patterns for the 10 SSRs that were examined. Successively, four different grasspea germplasm accessions were successfully evaluated using SSR primer pairs to identify polymorphism. Three closely related Lathyrus species, *L. cicera*, *L. ochrus*, and *L. tingitanus*, as well as a legume crop *Pisum sativum*, showed high SSR marker transferability.

Yang et al. 2014 used a set of 23 grasspea accessions and one red pea (*L. cicera*) accession in SSR marker testing and genetic diversity analysis. 651,827 simple sequence repeat (SSR) loci were identified using the 454 FLX Titanium pyrosequencing technology, and 50,144 nonredundant primer pairs were generated. Of these, 288 were randomly chosen for validation amongst the panel. 144 samples had no PCR result, 74 were polymorphic, 70 were monomorphic. The observed heterozygosity ranged from 0 to 0.9545, the observed allele count ranged from 2 to 5, and the

Shannon information index varied from 0.1013 to 1.0980. The dendrogram, which was developed using the unweighted pair group method with arithmetic mean (UPGMA) based on Nei's genetic distance, clearly distinguished the 24 accessions and revealed their relationships.

Wang et al. 2015 used 30 SSR markers to examine the genetic diversity between the grasspea and its related species. The study included 283 individuals from wild and domesticated populations in Africa, Europe, Asia, and ICARDA. The allele number per loci ranged from 3 to 14. They observed the average polymorphism information content (PIC) of 0.4817 and the average gene diversity index was 0.5340.

Arslan et al. 2020 genotyped about 22 accessions of Low β-ODAP Grasspea (*L. sativus* L.) germplasm with 31 EST-SSR markers. The molecular investigations found a total of 133 alleles with a range of 142 to 330bp. The value of the mean polymorphic information content (PIC) was determined to be 0.49 and observed the highest genetic distances between the GP6/GP11, GP4/GP11, and GP5/GP8 accessions.

Soren et al. 2020 emphasized the development of genomic resources and their application in marker–trait association for plant phenology and yield-related traits in lathyrus. The association mapping panel consisting of fifty geographically diverse accessions of *Lathyrus* spp. from India (30), Syria (10), Ethiopia (5), France (2), Italy (1), Bangladesh (1), and Korea (1) was framed. These accessions include 41 of *L. sativus*, six of *L. cicera* L., and three of the wild relatives of *L. aphaca* L. Among the 203 simple sequence repeat (SSR) motifs identified, trimer repeats (62%) having the highest frequency followed by tetramer (19%), hexamer (10%), pentamer (6%) and dimer (3%). Out of 150 SSR markers examined, 60 were amplified using 75 alleles from 50 germplasm lines, averaging 2-3 alleles per locus, and a polymorphic information content of 0.45 was found.

Using SSR markers, Mekonen et al. 2022 assessed the genetic diversity and population structure of 25 grasspea accessions obtained from North-Western Ethiopia using ten SSR. With an average of 5.13, a total of 41 alleles were identified and gene diversity and polymorphism information content values ranged from 0.074 to 0.944, with respective means of 0.474 and 0.536. Between the North Gondar and East Gojjam groups, the largest pairwise genetic distance (0.365) was observed. According to AMOVA, the PT (analogue of the FST test) estimates for population within regions, between regions, and between accessions were 0.24, 0.20, and 0.05, respectively. In terms of genetic differentiation, accessions have the highest value (76%) whereas among

regions have the lowest value (20%).

Yadav et al. 2022 deciphered the genetic diversity in grasspea using agronomic and forage quality traits and SSR markers. A total of 44 accessions with low β-ODAP content in seeds including five released varieties viz., Ratan, Nirmal, Pusa 24, Mahateora, and Prateek were studied. According to the results of the principal component analysis, the first three principal components from nutritional quality parameters, such as NDF, ADF, cellulose, lignin, and ash percent, as well as from agronomic traits, such as plant height, nodes per plant, leaf area, and green and dry biomass, explained the majority of the variation. Additionally, 11 SSR loci yielded a total of 59 polymorphic alleles, with an average of 5.36 alleles per locus, with a polymorphism information content ranging from 0.49 to 0.76. Four accessions (IF1327, IF1312, IL-10-76, and IF1307) with outstanding nutritive value in both green forage and straw as well as three accessions (IF1872, IF2177, and IF2156) with higher biomass than the check were identified.

2.5. Biochemical constituents of *L. sativus* germplasm

For biochemical evaluation, 168 accessions of grasspea and four checks (Ratan, Prateek, Mahateora and Narayangon) both seed and leaves were powdered and the flour was subjected to NIR scanning. With the spectra obtained and 20 clusters were obtained through the dendrogram with the Unscrambler software with K value 20. From each cluster two representative sample were selected and forwarded for the further biochemical analysis. Accessions were analyzed for eight biochemical parameters *i.e.*, ash, moisture, protein, sugars, phenols, antioxidant, starch, minerals, β-ODAP and amino acids

2.5.1. NIRS Modelling

Near infra-red spectroscopy method that uses the near infra-red region of the electromagnetic spectrum (780nm-2500nm) is widely used in agriculture for determining the quality of forages, grains, grain products, oilseeds, coffee, tea, spices, fruits, vegetables, sugarcane, beverages, fats, and oils, dairy products, eggs, meat, and other agricultural products. As this is a reliable, rapid, non-destructive and inexpensive technique, it is widely used to quantify the composition of agricultural products Burns (2007). For predicting the nutritive value of forages with R^2 more than 0.90 which have been reported where NIRS has been successfully applied in a variety of forages, feed stuffs, young leaves and grasses (Lenne et al. 2003; Stuth et al. 2003; Cozzolino and Moron 2004; Garcıa and Cozzolino, 2006; Tefera, 2006). Malidadi (2006) had taken 20 cowpea leaf

samples and calculated an R^2 value of 0.94 for crude protein content % and Magesa (2006) obtained an R^2 value of 0.84 for crude protein in the samples grown under greenhouse condition. Towett et al. 2013 analyzed crude protein in a variety of cowpea accession and predicted protein content with NIRS (R^2=0.93) with standard error of cross validation=0.74. Food and feed safety and quality control can be greatly improved by the combination of NIR hyperspectral imaging spectroscopic technique with chemometrics Pierna et al. 2012. Okonya and Mass (2014) successfully evaluated protein content of leaves of 6 cowpea varieties using NIRS to determine the crude protein content ranged from 28.02% to 31.84%. The literature review for the biochemical analysis is given as follows

Rao (1978) studied the sensitive color reaction of o-phthalaldehyde (OPT) with α, β-diaminopropionic acid (DAP) and determined the DAP content in tissues of the rat injected with this amino acid. The method indicated the presence in the liver and kidney of a alkali labile metabolite of DAP. A procedure is also described to determine the *Lathyrus sativus* neurotoxin β-N-oxalyl-L-α, β-diaminopropionic acid (β-ODAP) in tissues of the rat injected with the neurotoxin and also its content in the seeds. The procedure is rapid and would be of use in studies with DAP and the *L. sativus* neurotoxin.

Aletor et al. 1994 studied the trypsin inhibitor activity (TIA), the neurotoxic -N-oxalylamino-L-alanine (BOAA), the catechin equivalents (CE), and the crude protein (CP) of 68 lines of *L. sativus*, 16 lines of *L. cicera*, and 16 lines of *L. ochrus*. *L. cicera* (CP = 295.39 ± 18.69 g kg−1 DM) and *L. sativus* (CP = 324.99 ± 13.40 g kg−1 DM) were the species with the lowest and highest CP, respectively. In terms of BOAA concentration, *L cicera* had the lowest level (1.26 ± 0.18 g kg−1 DM) while *L sativus* had the highest level (5.63 ± 0.43 g kg−1 DM). *L. sativus* and *L. cicera* genotypes showed greater intraspecies variability in BOAA levels than did *L. ochrus*. BOAA concentrations were typically four to five times greater in *L. ochrus* and *L. sativus* than in *L. cicera*. *L. sativus* had a PPT level that was comparable to that of the other species (4.54 ± 0.31 g kg−1 DM). The large coefficients of variation indicate that there were significant differences in CE values between and within species. The average TIA across all *L. sativus* lines was 18.16 ± 2.38 g kg−1 DM, which was comparable to *L. ochrus* and almost twice as high as *L. cicera*. The coefficients of variation were between 10.93% and 17.13% for *L. ochrus* and *L. sativus*, respectively.

Khan et al. 1994 conducted a simple procedure for the precolumn derivatization of toxic and

non-toxic non-protein amino acids occurring in the legume crop *Lathyrus sativus* and other Lathyrus species with phenyl isothiocyanate and an HPLC method for the separation of the derivatives in the nano- and picomole range are reported. The results are compared with those of conventional ion-exchange chromatography for the separation of non-protein amino acids with post column ninhydrin reaction. The relative standard deviations for the two methods are compared.

In Ethiopia, Urga et al. 1995 examined a collection of 25 high yielding cultivars of grasspea samples for the presence of certain nutrients and antinutrients. A significant variation in the contents of NPN (0.4-0.5%), NPN as percentage of total N (9.3-11.4%), total protein (22.6-28.1%), true protein (20.4-25.5%), fat (1.0-1.6%), ash (1.0-2.1%), phosphorous (380.4-511.6 mg/100g), starch (32.0-43.9%), iron (6.6-18.4 mg/100g), calcium (131.6-200.1 mg/100g), phytate (525-1028 mg/100g), tannin (500-856 mg/100g), trypsin inhibitor (16783-26183 IU/g) and β-ODAP (172-353 md/100g) in the grasspea varieties were observed and concluded that Crude fiber (7.2-8.3%), total carbohydrate (51.8-58.5%), total sugars (5.1-6.2%) and reducing sugars (1.5-1.9%) contents of the grasspea varieties did not vary significantly.

Akalu et al. 1998 in their study on the starch and fiber's physical, chemical, and functional characteristics in unprocessed and processed grasspea seeds reported that on a dry matter basis, it was discovered that whole grasspea seeds contained 41% starch and 17% total dietary fiber (2% soluble and 15% insoluble).

Wang et al. 2000 studied rapid and simple method is presented for determining neuro-excitatory nonprotein amino acid 3-N-oxalyl-2,3-diaminopropionic acid (β-ODAP) and non-protein amino acids in *Lathyrus sativus*. Seed and foliage extracts of *Lathyrus sativus* were treated with 1-fluoro-2,4-dinitrobenzene (FDNB) and a reversed-phase high-performance liquid chromatography method (RP HPLC) for the separation of the derivatives in the pmol range is reported. The RP HPLC method and a colorimetric method were compared for measuring β-ODAP.

Fikre et al. 2008 studied the free and protein amino acids of nine different genotypes of grasspea (*Lathyrus sativus* L.) seeds were analyzed by HPLC with pre-column PITC (phenyl isothiocyanate) derivatization. Among the free amino acids, homoarginine was quantitatively the most important (up to 0.8% seed weight) and stable while the neuro-excitatory amino acid β-ODAP (β-N-oxalyl-l-α, β-diaminopropionic acid) showed highest variation (0.02–0.54%) in the nine genotypes examined. Among protein amino acids, glutamic acid was quantitatively most

significant, followed by aspartic acid, arginine, leucine, lysine and proline. The Sulphur amino acid, methionine, showed the lowest concentration in all the *L. sativus* genotypes, and also in lentil (*Lens culinaris*) and in soybean (*Glycine max*) seeds analyzed at the same time.

Pastor-cavada et al. 2011 studied the nutritional characteristics of seed proteins of 15 Spanish Lathyrus species have been analyzed. Protein contents in studied Lathyrus ranged from 17.7% in *L. sativus* to 25.6% in *L. tingitanus* with a 22.4% average protein content in studied Lathyrus. Among essential amino acids the most abundant were Leu, Lys, Phe, Thr and Val.

Tamburino et al. 2012 studied the nutritional and metabolic properties of *L. sativus* grown in Valle Agricola, Italy. According to their findings, these grasspea seeds have significant concentrations of both essential amino acids (7.92 g/100 g) and proteins (25.6 0.20 g/100 g). The important polyunsaturated fatty acids -linolenic, linoleic, and -linolenic acids are the most prevalent among them, making up 1.67–0.18 g/100 g of total lipids. Folic acid (206.708.30 g/100 g) accounts for 50% of the recommended daily allowance (RDA) for this vitamin, together with ascorbic acid (13.500.30 mg/100 g) and glutathione (15.900.10 mg/100 g). Estimates have been made for the total phenolic content (174.918.39/100 g) and the radical scavenging activity against. DPPH radical and ABTS radical cation.

In 2014, Fratianni et al. studied the phenolic content and antioxidant properties of cultivars of chickpea (*Cicer arietinum*), lentil (*L. culinaris*), and grasspea (*L. sativus*) from the Campania area of Southern Italy. In their study, in comparison to other grasspea cultivars, the tested grasspeas (cv San Gerardo and san Rufo) showed lower levels of total polyphenols, as well as a negligible amount of hydrolysable and condensed tannins. These low levels of the two types of tannins imply that the tested grasspeas are more nutritive and should not be harmful to people or monogastric animals.

Arslan et al. 2017 was conducted experiment to determine the β-N-oxalyl- L-2,3-diaminopropionic acid (β-ODAP), L-homoarginine, and asparagine content of a total of 173 *L. sativus* L. genotypes, of which 93 were collected under natural conditions in Antalya. The β-ODAP, L-homoarginine, and asparagine content of *L. sativus* L. genotypes ranged from 1.55 to 20.8 mg/g seeds, 1.35 to 11.64 mg/g seeds, and 0.59 to 5.22 mg/g seeds, respectively.

Arslan et al. 2017 conducted to determine vitamin A, B, C, p-carotene and amino acid profile in 18 genotypes and four grasspea (*L. sativus* L.) varieties which have low β-ODAP. Present results indicated that retinol, p-carotene, thiamine, riboflavin, niacin, pantothene, pyridoxine, folic

acid and ascorbic acid ranged from 25.6 to 44.1 μg/kg; 240.8 to 410.1 μg/kg, 3.74 to 5.44; 1.86 to 2.76; 12.37 to 20.25; 14.43 to 22.41; 4.92 to 6.62; 4.04 to 6.77 and 33.4 to 58.2 mg/kg, respectively in seeds. In addition to, the amino acid profile of the genotypes differed significantly and total amino acid amounts were found to be 19.69 to 23.48 g/100 g seeds. A large and significant variation was observed among these genotypes with low β-ODAP content in respect to the quality of the nutrient content. This variability will be useful to breeders for utilization in grasspea improvement.

Kartika et al. in a study conducted in 2018 reported that, 702 germplasm accessions from the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria, were examined for seed β-ODAP concentration and seed protein content. They were divided into seven sets of trials based on their provenance. The ICARDA germplasm set had the biggest range of seed protein content (28.82-30.72%), whereas the ETH2 (0.32-0.47%) and PAK (0.38-0.53%) germplasm sets had the highest range of seed β-ODAP content. For future grasspea breeding, new prospective sources with low seed β-ODAP content, like ILG468, ILG1934, ILG1950, and ILG1951, and for high protein content, such ILG311, ILG670, ILG688, ILG691, and ILG708, were found based on the best linear unbiased predictor values.

Soni et al. in 2018 in their study on the examination of protein content variance in the grasspea collection, the protein estimate using Lowry method was used to 37 local genotypes that were gathered from various districts in Bihar and neighboring districts in Jharkhand, as well as three national controls. The protein content of the genotype (IC127616) is highest, whereas that of the LKH L-15 genotype is lowest.

CHAPTER III

MATERIALS AND METHODS

The present study was carried out to characterize 168 germplasm for its agro-morphological traits, biochemical traits and genetic diversity study through SSR markers. A brief account on experiments conducted, details of the genotypes studied and the experimental design used and statistical procedures followed in the present study are outlined as under.

3.1. Location

The experiment was conducted at Pusa Research Farm of ICAR (Indian Council of Agricultural Research) - NBPGR (National Bureau of Plant Genetic Resources) at IARI (Indian Agricultural Research Institute), New Delhi for two consecutive seasons during *Rabi* 2019-2020 and 2020-2021.

3.2. Materials

The experimental material consists a total of 168 multi-panel germplasm (117 exotic and 51 indigenous accessions) with four check varieties - Ratan, Prateek, Mahateora and Narayangon from Indian National Genebank located at ICAR - NBPGR. The list of germplasm used in this study were given in the Table 3.1.

Table 3.1. List of grasspea germplasm

S.no	Accessions	S.no	Accessions	S.no	Accessions	S.no	Accessions
1	IFLA 143	43	IFLA1715	85	IC148364	127	BANG13
2	IFLA276	44	IFLA1193	86	IC148352	128	BANG22
3	IFLA1795	45	IFLA144	87	IC574469	129	BANG32
4	IFLA1870	46	IFLA127	88	IC148356	130	BANG33
5	IFLA2026	47	IFLA2129	89	IC489623	131	BANG38
6	IFLA151	48	IFLA1849	90	IC574470	132	BANG41
7	IFLA2282	49	BANG208	91	IC208429	133	BANG43
8	IFLA2475	50	BANG206	92	IC208430	134	BANG44
9	IFLA274	51	BANG257	93	IC208433	135	BANG45

10	IFLA2765	52	BANG268	94	IC208434	136	IC424511
11	IFLA2998	53	BANG263	95	IC208436	137	BANG209
12	IFLA2736	54	BANG286	96	IC208437	138	IC574468
13	IFLA2968	55	BANG284	97	IC212672	139	IC525179
14	IFLA2924	56	BK551	98	IC212649	140	BANG270
15	IFLA172	57	BK401	99	IC415656	141	BANG285
16	IFLA1720	58	BK3111	100	IC415658	142	BANG198
17	IFLA128	59	BK101	101	IC415678	143	BANG219
18	IFLA2636	60	BK391	102	IC395836	144	BANG204
19	BANG211	61	BK541	103	IC396712	145	BANG149
20	BANG184	62	BK301	104	IC470982	146	IC525185
21	BANG291	63	IFLA479	105	IC427679	147	IC525181
22	BANG297	64	IFLA220	106	IC427710	148	IC0634654
23	BANG308	65	IFLA190	107	IC421914	149	IC0634655
24	BANG218	66	IFLA2861	108	IC349809	150	IC0634656
25	BANG198	67	IFLA2460	109	IC347378	151	IC0634657
26	BANG224	68	BIO520	110	IC18879	152	IC0634658
27	BANG292	69	IFLA123	111	IC567350	153	IC0634659
28	BANG299	70	IFLA118	112	BANG27	154	IC0634660
29	BANG227	71	IFLA1826	113	BANG31	155	IC0634661
30	BK154	72	IFLA2441	114	BANG15	156	IC0634662
31	BK148	73	IFLA3001	115	BANG20	157	IC0634663
32	IFLA1522	74	SeI190	116	BANG21	158	IC0634664
33	IFLA2750	75	IFLA2990	117	BANG26	159	IC0634665
34	IFLA1926	76	IFLA1439	118	BANG37	160	IC0634666
35	IFLA1857	77	IFLA1913	119	BANG9	161	IC0634667
36	IFLA432	78	BANG309	120	BANG18	162	IC0634668
37	IFLA2341	79	BANG310	121	BANG28	163	IC0634669
38	IFLA2025	80	BANG186	122	BANG29	164	IC0634670
39	IFLA1864	81	BANG249	123	BANG30	165	IC0634671
40	IFLA2687	82	BANG182	124	BANG3	166	IC0634672
41	IFLA126	83	BANG267	125	BANG4	167	IC0634673
42	IFLA1813	84	IC525182	126	BANG5	168	IC0634674

3.2.1. Experimental layout

The experiment was carried out in *Rabi* 2019-2020 and 2020-2021. The entire germplasm accessions of 168 numbers along with four checks Ratan, Prateek, Mahateora and Narayangon were raised in an Augmented Block Design in five blocks. In the Augmented design four checks were replicated in each block and also randomized throughout the experimental area irrespective of blocks Figure 3.1 (Season 1) and figure 3.2 (Season2). Each genotype was sown in two rows of 4m length plot and 60 cm apart by adopting a spacing of 45 x 15cm. Standard agronomic practices and plant protection measures were followed to maintain the crop stand.

3.3. Characterization of germplasm for morphological traits

The observations on the germplasm grown for morphological characterization were recorded as per the NBPGR minimal descriptors and Bioversity International descriptors. A total of 34 traits (14 quantitative and 20 qualitative) were used for recording of observations.

3.3.1. Morphological traits recorded

The details of quantitative traits which were recorded on five randomly selected plants in each genotype and utilized for statistical analyzes are presented below in Table 3.2 and 20 qualitative traits in the Table 3.3.

Table 3.2. List of 14 Quantitative traits

S.no	Plant Traits	Code	Stage of recording observation
1	Plant height (cm)	PHT	Measured from ground level to tip of main shoot (average of 5 random plants)
2	Petiole length	PTL	After full flowering
3	Days to first flowering	DFF	From date of Sowing to the stage when first plant flowered in the row
4	Days to 50% flowering	50%F	From date of Sowing to the stage when 50% plants flowered in the row
5	Days to 80% flowering	80%F	From date of Sowing to the stage when 80% plants flowered in the row
6	Days to 80% pod maturity	80%M	From date of Sowing to the stage when 80% pod maturity
7	Pod length(cm)	PDL	At complete maturity of pods
8	Pod width(cm)	PDW	At complete maturity of pods
9	No. of Primary	NPB	As total number of branches on main stem

	branches		(average of 5 random plants)
10	No. of pods per plant	NPP	At completion of pod formation stage (average of 5 random plants)
11	No. of seeds per pod	NSP	At completion of pod formation stage (average of 5 random pods)
12	Seed length(cm)	SDL	Using Vernier caliper
13	Seed width (cm)	SDW	Using Vernier caliper
14	100 seed weight(g)	HSWT	Measured as weight of 100 random seeds

Table 3.3. List of 20 Qualitative traits

S.no	Plant Traits	Code	Descriptor state (Score)	Stage of recording observation
15	Seedling pigmentation	SDLG_PIG	1. No pigmentation 2. Slightly pigmented 3. Pigmented 4. Highly pigmented	During 1st week of emergence
16	Plant growth habit	GR_HBT	1. Prostrate 2. Spreading 3. Semi-erect 4. Erect	At completion of vegetative stage
17	Flower color	F_CLR	1. White 2. White blue 3. Blue 4. Grey 5. Light yellow 6. Yellow 7. Pink 8. Orange 9. Red 10. Violet-blue 11. Violet 99. Other	At the fully flowering stage
18	Plant vigor	PLT_VGR	3. Poor 5. Medium 7. Good	Recorded at 8-10 leaf stage

19	Plant pigmentation	P_PIG	0. Absent 1. Present	Recorded at 50% flowering
20	Leaf size	LF_SZ	1. Small 2. Medium 3. Large	
21	Pod pubescence	POD_PUB	0. Absent 3. Low 5. Medium 7. High	Recorded at physiological maturity
22	Pod wings	POD_WING	0. Absent 3. Narrow 5. Medium 7. Wide	Recorded at physiological maturity
23	Immature pod color	IM_PD_CLR	1. Yellow-cream 2. Light green 3. Green 4. Dark green 5. Green-purple 6. Light purple 7. Purple 99. Other	Recorded on a fully expanded immature pod from end of last loculus up to the pod tip
24	Pod shape	PD_SHP	1. Oblong-elliptical 2. Medium oblong-elliptical 3. Curved 4. Beaded 5. Broad-linear 6. Broad-elliptical 99. Other	Recorded at physiological maturity
25	Pod dehiscence	PD_DEHS	0. No shattering 3. Low shattering 5. Medium shattering 7. High shattering	Scored one week after maturity
26	Constriction of pods between seeds	CONS B/W PODS	0. Absent 3. Slight 5. Medium 7. Pronounced	Recorded at physiological maturity

27	Seed shape	SD_SHP	1. Oblate or flattened 2. Triangular 3. Rhomboid 4. Square 5. Obtriangular 6. Spherical 99. Other	Recorded at physiological maturity
28	Seed size	S_SZE	3. Small 5. Medium 7. Larg	Within three months of harvesting
29	Seed coat color	SD CT CLR	1. Greyed-white 2 Grey 3 Yellow-white 4 Brown 5 Yellow-green 6. Pink 7 Red-purple 8 Black 9 Grey mottled 10 Green mottled 99 Other (specify in descriptor	Within three months of harvesting
30	Seed coat surface	S_CT_SUR	1 Smooth 2 Tubercular	Within three months of harvesting
31	Seed coat pattern	S_CT_PTN_CLR	0 Absent 1 Marbled 2 Dotted 3 Streaked 4 Mixture	Within three months of harvesting
32	Seed coat pattern color	S_CT_PTN_CLR	1 Cream 2 Green 3 Brown 4 Red-purple 5 Black 99 Other	Within three months of harvesting
33	Seed ornamentation	SD_ORN	0 Absent 1 Present	Within three months of harvesting
34	Cotyledon color	COT_CLR	1 Yellow	Within three months of

			2 Orange 99 Other	harvesting

Figure 3.1. Season 1- Experimental field

Figure 3.2. Season 2- Aerial view of experimental field

3.3.2. Data analysis

Agro-morphological data of both the years (2019-20 and 2020-21) were pooled to compute range, mean, variance and standard deviation after checking for the homogeneity of variances by GENSTAT software. The adjusted mean values were used for the further analysis.

3.3.3. Statistical methods

3.3.3.1. Analysis of variance

In augmented completely randomized design standard varieties (checks) were replicated 'r' times randomized uniformly throughout the experiment area irrespective of the blocks and then test genotypes are replicated once, were placed randomly throughout the experimental area. Let,

g = No of genotypes

c = No of checks

r = No of replication for checks

n = Total no of entries i.e., g+ (c x r)

Source of variation	**Degrees of freedom**	**Mean sum of squares**	**Expectation of mean squares**
Among entries	(g + c) – 1	M_1	$\sigma^2_e + r\sigma^2_c + \sigma^2_g$
Among checks	c – 1	M_2	$\sigma^2_e + r\sigma^2_c$
Among test genotypes	g – 1	M_3	$\sigma^2_e + \sigma^2_g$
Test genotypes vs. checks	1	M_4	σ^2
Error	c (r – 1)	M_5 or EMS	
Total	n – 1		

3.3.3.2. Estimation of genetic variability and association analysis

The genetic parameters *viz.,* genetic variability, heritability, genetic advance and association analysis were estimated for the entire germplasm accessions of 168 by including the 14

quantitative traits. The various genetic parameters like variability, GCV, PCV, heritability and genetic advance as per cent mean were calculated by adopting the formulae given by Johnson *et al.* (1955).

a) Phenotypic and genotypic variance

In augmented design, according to (Federer, 1961) the experimental error exists only from the check varieties used and not from the test entries/lines. Hence the error mean square was considered as the environmental variance (σ^2_e). The variation existing in the germplasm lines was considered as the phenotypic variance (σ^2_p). Genotypic variance was calculated by using the following formula.

Genotypic variance (σ^2_g) = Phenotypic variance (σ^2_p)- Error variance (σ^2_e)

$= M_3 - M_5$ or EMS

b) Phenotypic and genotypic coefficient of variation (PCV and GCV)

Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were calculated by using the formulae as suggested by Burton (195)

$$\text{PCV} = \frac{\sqrt{\text{Phenotypic variance}}}{\text{Grand mean}} \times 100$$

$$\text{GCV} = \frac{\sqrt{\text{Genotypic variance}}}{\text{Grand mean}} \times 100$$

The PCV and GCV were classified as proposed by Sivasubramanian and MadhavaMenon (1973)

PCV and GCV	Category
Less than 10	Low
10 – 20	Moderate
More than 20	High

c) Heritability

In general sense, heritability specifies the proportion of the total variability that is due to genetic causes, or the ratio of genotypic variance to the total variance. It is a good index of the transmission of the characters from parents to their offspring (Falconer, 1960).

Heritability (h^2) in the board sense was calculated according to (Lush, 1940).

h^2 (B.S.) = (σ^2_g / σ^2_p) x 100

Where,

σ^2_g = Genotypic variance

σ^2_p= Phenotypic variance

Range of heritability was categorized as suggested by (Johnson *et al.* 1955)

Low: Less than 30 %

Moderate: 30-60 %

High: More than 60 %

d) Genetic advance (GA)

It is the measure of genetic gain under selection. Genetic advance is defined as the difference between the mean genotypic value of the selected lines and the mean genotypic value of the parental population. Genetic advance was estimated by the method formulated by Johnson *et al.* (1955) and expressed as percentage of mean.

Genetic advance = $k \times h^2 \times \sqrt{\sigma_p^2}$

Where

h^2 – Heritability in broad sense

$\sqrt{\sigma_p^2}$ – Phenotypic standard deviation

k – Selection differential [at 5% selection intensity *i.e.*, 2.06 (Falconer, 1960)]

e) Genetic advance as percent of mean

Genetic advance was expressed as percentage of mean by using the formula suggested by

Johnson *et al*. (1955).

$$GAM = \frac{\text{Genetic advance}}{\text{Grand Mean}} \times 100$$

The following classification was utilized in the study as suggested by Johnson *et al*. (1955).

Low: Less than 10 %

Moderate: 10-20 %

High: More than 20 %

3.3.3.3. Correlation studies

The simple correlation between yield and other component traits as well as among the component traits were estimated in the germplasm entries as suggested by Johnson *et al*. (1955).

$$r_{p(x,y)} = \frac{Cov._{p\,(xy)}}{\sqrt{\sigma^2_{p\,x} \times \sigma^2_{p\,y}}}$$

Where,

$r_{p(x,y)}$ = phenotypic correlation coefficient between the traits x and y.

$Cov._{p(xy)}$ = phenotypic covariance between the trait's 'x' and 'y'.

σ^2_{Px} = phenotypic variance of the trait – 'x'.

σ^2_{Py} = phenotypic variance of the trait – 'y'

Significance of correlation was tested using t statistic at n-2 degrees of freedom as given

$$t = \sqrt{\sigma^2 p_x \times \sigma^2 p_y}$$

Where,

'r' is estimate obtained from 'n' pairs

3.3.3.4. Frequency distribution

The raw data is grouped into classes and the frequency in each class is recorded. The bell-shaped curve is the characteristics of normal distribution. In the normal distribution, the mean, mode and

median will coincide and it will be in the center. More population are around the mean. Least Population are around two extremes.

3.3.3.5. Principal component analysis

Principal component analysis was analyzed for 14 quantitative characters in 168 genotypes using R software. PCA is defined as "a method of data reduction to clarify the relationships between two or more characters and to divide the total variance of the original characters into a limited number of uncorrelated new variables". This will allow visualization of the differences among the individuals and identify possible groups. The reduction is achieved by linear transformation of the original variables into a new set of uncorrelated variables known as principal components (PCs) (Mohammadi and Prasanna, 2003). The first step in PCA is to calculate eigen values, which define the amount of total variation that is displayed on the PC axes. The first PC summarizes most of the variability present in the original data relative to all remaining PCs. The second PC explains most of the variability not summarized by the first PC and uncorrelated with the first, and so on.

3.3.3.5.1. Eigen values and eigen vectors

The eigen value and eigen vector pairs created from data matrix were utilized to identify the principal components. Eigen values defined the amount of total variation that was displayed on principal components. The eigen vectors defined the correlation of each variable with the principal components. The proportion of variation accounted for each principal component (PC) was expressed as the eigen value divided by the sum of the eigen values.

Percentage of variance explained for PCI

$$= \frac{\text{Eigen value of PCI}}{\text{Sum of eigen value}}$$

The cumulative percentage of contribution to variation of different traits to eigen vectors were also computed.

This is informative about the proportion of total variation that variation can be accounted for the i^{th} principal component. The correlation between the i^{th} original variable Xi and the j^{th} principal component Y_i is given by

$$\rho\,(X_i, Y_j) = \frac{Aij.\sqrt{\lambda j}}{\sqrt{Si}}$$

Where, Si is the standard deviation of Xi.

Thus, a principal component is linear function of the test variables and are given as follows

Principal component = $ax_1 + bx_2 + \ldots + hx_n$; where, a, b… h are coefficients and x_1, $x_2 \ldots x_n$ etc.., are the variables in such a way that the principal component has a unit variance.

3.3.3.6. Cluster analysis

"Cluster analysis" refers to "a group of multivariate techniques whose primary purpose is to group individuals or objects based on the characteristics they possess, so that individuals with similar descriptions are mathematically gathered into the same cluster" (Hair Jr *et al.* 1995). Hierarchical clustering methods was employed for 168 grasspea germplasm for 34 traits (both quantitative and qualitative) through Ward's linkage method with Euclidean distance in R software using the adjusted means obtained from the GENSTAT. Euclidean or straight-line measure of distance is the most commonly used statistic for estimating genetic distance (GD) between individuals (genotypes or populations) by morphological data. Euclidean distance between two individuals I and j, having observations on morphological characters denoted by x_{1k}, x_{2k}, …, x_{ik}, … x_{kk} can be calculated by the following formula:

$$dij = \sqrt{\sum_1^k (Xik - Xjk)^2}$$, where k is the number of genotypes

3.4. Molecular characterization of grasspea

3.4.1. Plant Genomic DNA extraction

Fresh leaves from all the 100 accessions of adzuki bean were collected from the experimental field and stored in the deep freeze (-20°C). DNA extraction from the collected leaves were carried out by following the modified Cetyltrimethylammonium bromide (CTAB) method in the Figure 3.3.

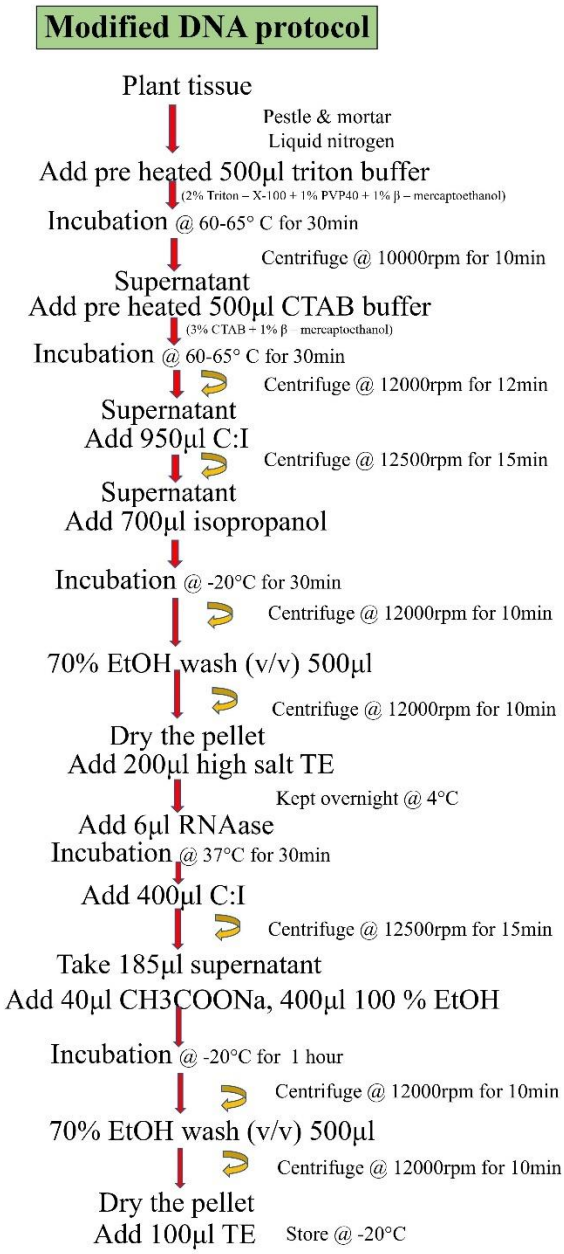

Figure 3.3. Modified DNA protocol of grasspea

3.4.2. DNA quantification

The concentration of the DNA was estimated by gel electrophoresis in 0.8% agarose gel were loaded with 10µl of sample (3µl DNA+2µl loading dye + 5µl nuclease free water) 100kb ladder was used and was run under 90 V at constant mA for 2 hours. The DNA quantification

was done using Nanodrop, in which the concentration of DNA has a maximum absorbance at about 260 nm and based on the OD value, the samples were diluted with TE buffer (100µl). The absorbance value of the sample in spectrophotometer was recorded at 260/280 nm using TE as blank which is used to dissolve the DNA pellet at the last step (Mandal et al. 2014). The DNA concentration of the sample was calculated as follows:

Concentration of DNA (µg/ml) = OD value@ 260nm × 50 × dilution factor.

3.4.3. PCR standardization

The DNA samples were subjected to PCR amplification with SSR primers after dilution with nuclease free water. Initially 70 SSR markers were used for screening and 20 markers found to be polymorphic and it is given in the Table 3.4. The primers used here have already shown to be polymorphic by Yang et al. 2014. The total reaction volume was 20µl (2µl 10XPCR buffer + 0.4µl 2.5 mM dNTPs + 0.8µl primer (100 ng) + 0.6µl Taq Polymerase + 5µl genomic DNA + 8.8µl double distilled sterile water) having approximately 20-30 ng DNA. In case of using master mix (from Gbiosciences), the total reaction volume of 15µl (7.5µl master mix + 4.5µl nuclease free water + 1µl primer + 2µl genomic DNA). The PCR reaction condition was programmed as follows: initial hold at 95°C for 5 min, then 36 cycles for denaturation at 95°C for 45s, annealing of primer at 54.3°C for 45s for SSR extension at 72°C for 10 min. The resulting amplified PCR products were subjected to gel electrophoresis in 3 % metaphor gel and run for 2½ hour at fixed voltage of 100 V. The amplification patterns of the samples were captured on gel documentation system.

Table 3.4. List of polymorphic SSR primers

S.no.	Marker	Sequence F- forward, R- reverse	Annealing

			temperature (◦C)
1	LTR1	F-AAGGAGCAGCAGCATTTGTT R-TAATAATGGGGAGCCGATCA	54.6
2	LTR2	F- CACAACCAGTTGCATCAGTG R- TGGCTCACATGATGGTTTGT	54.6
3	LTR4	F- CAACCAGAGCAACCACAAGA R- GGTTGCAAGAGGTTGCAGAT	56.4
4	LTR5	F-CAGGTCCGGCTTATCTCTCA R- TTGGTTTCAACCCACTCCTC	52.2
5	LTR8F	F-ACGCACACACGGAAGAAAG R- GTGTGCGCATGTGTGTATGA	51
6	LTR11	F- CACCCTCTTCACTGCCTAGC R- TTGGGGGTTGTAGAAGGAAC	51
7	LTR12	F-GCACACAAGGGCACACTG R- TGCGTCGTGTGTATGTGTTG	51
8	LTR28	F- GGGCAGTGGACCAGTTAGAG R- CCGAGGGAATAAACGACAAA	51
9	LTR30	F- CAATCCGAAAATCACCACCT R- GCACTCACATGCACACAAAC	51
10	LTR31	F- CCTTTCGGAGCAATCAAGAC R- TGCCTAAGCATTGGCTTTCT	51
11	LTR33	F- CAACCAGAGCAACCACAAGA R- GGTTGCAAGAGGTTGCAGAT	51
12	LTR35	F- TCAAGCCCAAAGTGAGATGA R- TTTTGTGTTGCTTGCTGACC	51
13	LTR37	F- ATCTTACCGGGGATCCATTC R- CTTCCCCATTCTCTGGTGTT	51
14	LTR58	F- AAACTGGCCCTGCATTTTC	55.2

		R- GGTCATGGCAATTTGAGACA	
15	LTR62	F- CGTTGGTTGTTAGTCGGTCA R- GAACGAAACAACGACGACAA	55
16	LTR70	F- TTTGTGCGGTTGATGTTGTT R- CTACGTCAGCCCGTCATACC	54.6
17	LTR32	F- ACAGCAAGAAGCAGCAACAG R- AGTTGGTTGTTGTGTCGTTGT	55
18	LTR68	F- TTTTTGGTATTGTTGTTGTCGT R- CTGCAGCAATAACAGCATCAG	52
19	LTR41	F- CCCTTACCGAGTGCAGAAAA R- CACCACGACTTGCTCACCTA	54
20	LTR40	F- GGGCACACATTCTCACACAC R- TGTCGTCGTGTCGTAGTCGT	52

3.4.4. Population Structure Analysis and cluster analysis

Population stratification of 168 genotypes of grasspea panel was done using the Bayesian model-based programme, software STRUCTURE 2.3.4 (Pritchard *et al.* 2000). The number of clusters (K) investigated, in this study, ranged from two to ten and three replications were run for analysis of each K value. The model following correlated allele frequency and admixture with 100,000 steps of burn-in period and followed by 100,000 Monte Carlo Markov Chain (MCMC) replications were used for each replication. The membership of each studied *L. sativus* germplasm was estimated for range of genetic clusters from value of K = 2-10. Ln (PD) was obtained for each K and plateau of DK values are derived after plotting Ln (PD) (Evanno *et al.* 2005). Output of analysis was collected using STRUCTURE harvester-an online available programme (http:// taylor0.biology.ucla.edu) for obtaining the final population structure of the studied germplasm. K means hierarchical clustering was performed using Ward's linkage method with Euclidean distance and the data set were also subjected to

Principal Coordinate Analysis (PCoA) and analysis for molecular variation (AMOVA) using software suing R software.

3.5. Biochemical Characterization

A total of 168 accessions along with four checks (total 172), both seed and leaf powder were scanned using the FOSS NIRS 6500, and the reflectance spectra from 400 to 2400 nm was recorded. Ward's method was used to do hierarchical clustering on the normalized spectral data of 172 samples using a squared Euclidean distance of 5. Major clusters and sub-clusters were identified and separated in the same manner. To account for all possible variability in the data set, 40 accessions each of seed and leaf with a wide range of characteristics were chosen. These accessions were subjected to wet chemistry experiments. The flour that was produced after these materials were homogenized, ground, and sieved through a 1 mm sieve in the FOSS cyclotec was utilized for NIRS scanning and analyzing protein, starch, sugar, TDF, phenols, amino acids, mineral estimation and β-ODAP estimation.

3.5.1. Determination of Protein content using DUMAS method:

The grain samples were homogenized using Foss Cyclone mill equipped with 1mm sieve. homogenized flour was used for estimation of total nitrogen content by DUMAS method.

Principle:

The Dumas approach is based on the complete combusting of the entire sample inside an oxygen-enriched environment at higher temperatures which convert nitrogen present in the sample into gaseous NO_x by complete combustion then reduced to N_2 and measured using the thermal conductivity detector.

Methodology

1.The flour samples of about 0.1 g up to 4 decimal places were weighed using a scientific balance (Sartorius ATX224R)

2.The weighed flour was fed to rapid MAX N (Elementar) which analyze nitrogen content in the flour using DUMAS method

3.Percentage of nitrogen in the sample was determined using the formula

% of N=(28 X volume of Nitrogen gas at STP X 100)/(22400 X weight of the sample)

Calculation:

Protein g per 100g = Reading value of N% x 6.25 (Conversion Factor)

Acceptance of the results:

Duplicate results should not differ by more than 5 % of the mean.

3.5.2. Total Phenols Content estimation by Spectrophotometric method:

Principle:

Phenols respond to phosphomolybdate in Folin-Ciocalteu reagent under alkaline medium which acts as oxidizing agent. As a result of the reaction blue colored complex (Molybdenum Blue) is developed, which is estimated colorimetrically estimated at 650 nm (Bray and Thorpe,1954).

Reagents:

Folin-Ciocalteu reagent (FCR),

80% Ethanol,

20% Na2CO3

Galic Acid Standard solution (1.0mg/ml)

Galic Acid Working solution (0.1mg/ml)

Working standard solution

Sample preparation:

1. Weigh (0.1g) of flour in calibrated weighing machine and put inside a 15 ml centrifuge tube.
2. Then add 5ml of the 80% ethanol and put it in rotator for continuous rotation for overnight.
3. Then place in hot water bath kept at 80°C for about 30 minutes.
4. Then keep it for cooling for 5 minutes and centrifuge the homogenate at 10,500 rpm for 10 minutes.
5. Collect the Supernatant in separate centrifuge tube.
6. Again extract the residue with 5ml volume of 80% ethanol and keep it for rotation for overnight.
7. Then repeat steps 3 &4.
8. After final extraction, volume was made up to 10 ml.
9. Keep the residue for starch analysis in -20°C refrigerator.

Procedure:

1. Pipette out 0.5ml of aliquot in clean and dried test tubes in duplicate and keep it for drying in hot water bath at 99°C.
2. After complete evaporation, add 3ml of the distilled water.

3. Add 0.5ml FCR reagent in each test tube and after 3 minutes, add 2.0ml 20% Na_2Co_3 in each test tube.

4. Vortex the test tubes and keep for incubation at room temperature for 1hr.

5. Then take the readings in spectrophotometer at 650nm

6. Plot the standard graph with concentration of standard on X-axis and absorbance on Y-axis.

7. With the help of calculated slope value from graph, calculate the total amount of phenols present in the samples.

Calculation:

Amount of phenols in sample (%) = (Phenol value from graph in mg x total volume of extract in ml) x 100/ (Aliquots used x weight of sample in mg)

Acceptance of the results:

Duplicate results should not differ by more than 5 % of the mean.

3.5.3. Estimation of total sugar content by spectrophotometric method:

Principle:

Principle for the estimation of the sugar content is based on anthrone reagent and this reaction is the basis of convenient and quick determination of aldopentoses, hexoses, and hexuronic acid either in free form or in polysaccharide form. After dehydration with the concentrated sulphuric acid sugars are converted into furfural. Anthrone (10-keto-9, 10-dihydro-anthracene) and furfural together form a blue-green colored complex that is estimated at 630 nm.

Reagents required:

Anthrone reagent

Glucose standard stock solution (1mg/ml)

Glucose working solution (0.1mg/ml)

80% ethanol

Concentrated Sulphuric acid

Sample preparation:

1. Weigh (0.1g) of flour in calibrated weighing machine and put inside a 15 ml centrifuge tube.

2. Then add 5ml 80% ethanol and put it in rotator for continuous rotation for overnight.

3. Then place in hot water bath kept at 80°C for about 30 minutes.

4. Then keep it for cooling for 5 minutes and centrifuge the homogenate at 10,500G for 10 minutes.

5. Collect the Supernatant in separate centrifuge tube.

6. Again extract the residue with 5ml 80% ethanol and keep it for rotation for overnight.

7. Then repeat steps 3 &4.

8. After final extraction, make up the volume to 10 ml.

9. Keep the residue for starch analysis in -20°C refrigerator.

Procedure:

1. Take 0.1ml of the aliquot in the test tube and keep it in boiling water bath for drying.

2. Cool the dried tubes and add 1.0 ml of distilled water.

3. Then prepare the standards by taking 0, 0.2, 0.4, 0.8, 1.0 ml of standard working solution.

4. Then add 4.0ml of ice cold Anthrone reagent in each test tube along with standards and mix it properly.

5. Put the tubes in boiling water bath for 8 minutes and then keep it for cooling.

6. Then take the absorbance reading of green to dark green color at 630 nm

7. Draw a standard graph by plotting standard's concentration on X-axis and absorbance on Y-axis.

8. Calculate the number of sugars using slope value from the graph.

Calculation:

Amount of sugars in sample (%) = (Sugar value from graph in mg x Total volume of extract in ml) x 100/ (Aliquot samples used x weight of sample in mg).

Acceptance of the results:

Duplicate results should not differ by more than 5 % of the mean.

3.5.4. Determination of total starch content by Enzymatic method:

Principle:

α- amylase is an enzyme are that hydrolyses the starch into soluble unbranched and branched maltodextrins

Starch + H_2O ⟶ maltodextrins (in presence of α-amylase, pH 7.0, 100°C)

Resistant starch + H_2O ⟶ maltodextrins (KOH than neutralization + α amylase)

Maltodextrins ⟶ D-glucose (in presence of amyloglucosidase)

D-glucose + O_2+ H_2O ⟶ D-gluconate + H_2O_2

2 H_2O_2 + para hydroxybenzoic acid + 4-aminoantipyrine ⟶ quinoneimine dye +4H_2O (peroxidase)

Apparatus

Positive displacement pipette

Centrifuge

Analytical balance

Spectrophotometer

Vortex mixer

Water bath

Test tubes

Reagents:

Thermostable α- amylase

Amyloglucosidase

GOPOD reagent buffer

GOPOD reagent enzymes

D-glucose standard solution in 0.2% benzoic acid

Sodium acetate buffer (100mM, pH 5.0)

Sodium acetate buffer (1.2M, pH 3.8)

Potassium hydroxide solution (2M)

Sodium acetate buffer (200 mM, pH 4.5)

Procedure:

1. Take the residue of the sample from total soluble sugar extraction step.
2. Add few drops of ethanol (80%v/v) to the residue. Stir the tubes on a vortex mixer.
3. Then, add 3.0ml of thermostable α-amylase in the tubes and incubate the tubes in hot water bath for 6 minutes. At frequent intervals of 2min, 4min, 6min stir the tubes.
4. Then add 0.1 ml of the amyloglucosidase enzyme in the tubes.
5. Vortex the mixture and incubate for 30 minutes at 50°C in hot water bath.
6. After 30 minutes transfer the entire content of the test tube into 50ml volumetric flask.
7. Adjust to the volume of volumetric flask with distilled water using a wash bottle and mix thoroughly.
8. Then transfer the mixed aliquot into a 2.0 ml centrifuge tube and centrifuged at 3000 rpm for 10 minutes. Use the clear, undiluted filter for the assay.
9. Transfer duplicate 0.1ml aliquots from the centrifuge tube to the glass test tubes.

10. Then add 3.0 ml GOPOD reagent in each test tube including the D-Glucose standard and blanks and incubate the tubes for 20 minutes at 50°C.

11. Prepare the D-glucose control by adding 0.1ml of D-glucose standard and 3.0ml of the GOPOD reagent and blank was prepared by adding 0.1ml of water and 3.0ml of the GOPOD reagent.

12. Read the absorbance at 510 nm for each sample and D- Glucose standard.

Calculations

Starch % = (Sample Absorbance x FV x 0.9) / (Slope of standard x W)

Were,

FV= Final volume made after enzymatic hydrolysis

Slope of standard = Absorbance of standard / Standard amount in µg

W = Weight of sample in mg.

Acceptance of the results:

Duplicate results should not differ by more than 5 % of the mean.

3.5.5. Determination of total dietary fibre by enzymatic method

a) Principle:

On duplicate samples of dried and defatted (if the fat content > 10%) material, the total dietary fibre (TDF) is calculated. Selected samples are incubated at 100ºC with heat stable α-amylase to gelatinise, hydrolyse and depolymerization the starch. The samples are then incubated with protease at 60ºC (to depolymerise protein) and amyloglucosidase (to hydrolyse starch to glucose). Followed by treatment with 4 volume ethanol to precipitate soluble fibre and remove depolymerized protein and glucose. The residue is filtered, washed with 75% ethanol, dried and weighed. One part is analyzed for protein and the other part is incubated at 525ºC to determine ash. The TDF is then determined by the weight of the filtered and dried residues less the weight of protein and ash.

b) Apparatus

i. Beakers 400 ml and 600 ml.
ii. Fritted crucible.
iii. Filtering flask
iv. Vacuum source
v. Water bath
vi. Weighing balance

vii. pH meter
viii. Cylinder
ix. Magnetic stirrers
x. Muffle furnace

c) Reagents:

i. Ethanol 95% V/V
ii. Acetone, reagent grade
iii. α-amylase
iv. Protease
v. Amyloglucosidase
vi. Celite
vii. TRIS buffer 0.05 M each, Ph 8.2 at 24ºC
viii. Hydrochloric acid solution,0.561 N (Add 93.5 ml of 6N HCl to approx. 700 ml of distilled water in 1L volumetric flask. Dilute it to 1L)

d) Procedure:

i. In duplicate, 1.0 g samples were weighed with a 1 mg precision and put into 400 mL tall beakers.
ii. To depolymerize and hydrolyze starch molecules, phosphate buffer (50 mL; pH 6) and 50 µL thermotolerant α-amylase were introduced to the beakers.
iii. The beakers were then wrapped in aluminum foil and submerged for 15 minutes with moderate shaking in a water bath heated to 98–100ºC.
iv. The pH of the solution was adjusted to 7.5±0.1 by 0.275 N NaOH solution after reaching room temperature.
v. After that, 100 µL of protease solution was added to depolymerise, solubilize, and hydrolyze proteins.
vi. The beakers were incubated for 30 minutes at 60ºC while being continuously shaken.
vii. Following the addition of 200 µL of amyloglucosidase to hydrolyze the 1,4-linked terminal α-D-glucose residues from poly- and malto-oligosaccharides and liberate free glucose, the pH was further adjusted to 4.5±0.2 by adding 0.325 N HCl solution.

viii. The beakers underwent an additional 30 minutes of 60°C incubation. To get rid of the depolymerized proteins and glucose, 280 mL of pre-heated 95% ethanol was added to the beakers and left for 12 hours, allowing the soluble fibres to entirely precipitate.

ix. Through a crucible with a celite bed, the solution was vacuum filtered. The duplicate fractions were gathered and baked at 60ºC for 24 hours.

x. By using a Foss Tecator 2300 Kjeltec nitrogen auto-analyzer (FOSS Analytical, Denmark) on one duplicate, proteins were determined; the second duplicate was incubated at 525 °C to measure the ash content.

xi. The weight of the filtered and dried fractions was subtracted from the weight of ash and proteins to determine the TDF. The data were given in units of g per 100g DWB.

e) Calculations

$$\text{Dietary Fibre (\%)} = \frac{\frac{R1+R2}{2} - p - A - B}{\frac{m1+m2}{2}} \text{X100}$$

Where,

R1= Residue weight 1 from m1, m1= Sample weight 1

R2= Residue weight 2 from m2, m2= Sample weight 2

A= Ash weight from R1

P= Protein weight from R2

B= Blank = $\frac{BR1+BR2}{2} - BP - BA$

BR= Blank residue, BP= Blank protein from BR1, BA= Blank ash from BR2

3.5.6. Mineral concentration estimation

The mineral contents like iron, zinc, copper, calcium and magnesium content are estimated using Atomic Absorption Spectrophotometer (AAS) as per the method given by the Association of Official Agricultural Chemists (AOAC) method 999.11 for both the seeds and leaves.

Principle:

Atomic Absorption AAS is founded on the idea that atoms and ions can take in light or energy at different unique wavelengths. When a particular wavelength of light is applied to the sample, it

absorbs the energy and its level of absorption is measured. AAS measurement is performed by counting the number of absorbing species generated at a specific analytical wavelength.

AAS quantification principles are based on the Beer-Lambert equation, which states that an increase in absorbance (measured as a reduction in transmittance) has a linear relationship with the concentration of gas-phase atoms. The atomic lines produced by the radiation source must be thin enough in width to overcome the instrument's limited bandpass.

Apparatus used

- Microwave Digester
- Atomic Absorption Spectrophotometer

Reagents used

- Double distilled water
- Nitric Acid
- Hydrogen Peroxide

Procedure

- The grain from the accessions was dehulled manually.
- Dehulled grains were homogenised using Foss Cyclone mill equipped with 1 mm sieve to obtain the flour.
- The obtained flour was digested using a microwave digester (Model ETHOS UP Maxim digester - 44) and filtered.
- To digest the wheat flour were weighed 25 mg in a laboratory weighing balance. The weighed flour was added with 5 ml of nitric acid (HNO_3) and 1 ml of hydrogen peroxide (H_2O_2)
- The above solution was placed in the microwave digester at a temperature of 170^oC for 45 minutes and allowed to cool for 25 minutes.
- The obtained solution was vented for 2 to 3 hours so that the fumes of concentrated acids escape.
- The obtained solution was then diluted to 25 ml using double distilled water.
- The diluted solution was filtered by Ashless Whatman filter paper 42 and the filtered solution was fed to the atomic absorption spectrophotometer

- The atomic absorption spectrophotometer (Agilent 240FS AA) consists of a SIPS sample introduction pump, flame chamber, lamp compartment and discharge unit
- SIPS sample introduction pump was used for the introduction and dilution of the sample for the atomic absorption flame.
- The flame chamber produces oxyacetylene flame which was used for the atomisation of the sample introduced via SIPS sample introduction pump.
- The lamp chamber consists of cathode lamps which were used to generate the wavelength of the required element.
- The concentration of the element of interest was obtained using Agilent SpectrAA software.
- The obtained readings of the elements were used to calculate the concentration of the element in mg/kg using the formula

$$Concentration\ \left(\frac{mg}{Kg}\right) = \frac{Reading\ of\ the\ element\ X\ Dilution\ factor(25\ ml)}{weight\ of\ the\ element}$$

- The accessions which had shown high grain iron and grain zinc content were selected and the grains of those accessions were cross-validated to ensure that no contaminations have done while milling the grains.

3.5.7. Determination of amino acids using UPLC (Ultra High-Profile Liquid Chromatography)

A modern liquid chromatographic system known as UPLC is a modification of HPLC. The basic principle of UPLC is the same as HPLC except the particle size of sorbent of the column which is less than 2µm which requires pressure to work with. This offers better resolution, speed and sensitivity as compared to HPLC and effectiveness of the column, which doesn't reduce on increasing the rate of flow (Chesnut and Salisbury, 2007).

a) Procedure

Step-1 (Mobile Phase Preparation)

Mobile Phase A: 10 mM Na2HPO4: 10 mM Na2B4O7, pH 8.2; 5 mM NaN3

1.4 g anhydrous Na2HPO4 and 3.8 g Na2B4O7.10H2O were added in 1L water along with 32 mg

NaN3 adjusted to about pH 8.4 with 1.2 mL conc HCl. The solution is then filtered through 0.2 µm regenerated cellulose membrane.

Mobile Phase B: Acetonitrile: Methanol: Water (45:45:10, v: v: v)

All mobile-phase solvents are HPLC grade. It is convenient to make 2L of A for every 1L of B since mobile phase A is consumed at a faster rate than B.

Injection diluent: 100 mL of mobile phase A with 0.4 mL conc H3PO4 in a 100 mL bottle is stored at 4ºC.

0.1 N HCl: 4.2 ml of conc HCl (36 %) was added to a 500 mL volumetric flask having partially filled water. The solution is mixed and filled with water up to the mark. Store at 4ºC.

Derivatization: Borate buffers, OPA and FMOC.

Step-2 (Preparing the amino acid standards)

Amino Acid Standards (10 pmol/µL to 1 nmol/µL): For calibration curves solutions of 23 amino acids are available from agilent. Each 1 mL ampule of standards is divided into 100 µL portions in conical vial inserts.

Extended amino acid (EAA) stock solution: To make the extended amino acid stock solution, weigh; 59.45 mg aspargine, 59 mg hydroxyproline, 65.77 mg glutamine, 91.95 mg tryptophan and add to a 25 ml volumetric flask following by addition of 0.1 HCl and shaken or sonicated until dissolved. Then water is filled up to the mark for a total concentration of 18 nmol/µL of each amino acid.

Internal standards (ISTD) stock solution: For primary amino acids, norvaline was weighed 58.58 mg and added into a 50 mL volumetric flask. And for secondary amino acid sarcosine was weighed 44.54 mg and added into the same 50 mL flask. 0.1 N HCl was added into that flask and shaken or sonicated until dissolved. Then water is filled up to the mark for a total concentration of 10 nmol/µL of each amino acid.

Step-3 (Preparation of the samples)

i) 900 mg sample was weighed in a screw cap test tube.

ii) Then 4 ml of 6N HCl and 0.5 ml of 0.1N HCl, was added to the test tube

iii) 50 µL of phenol was added and kept for hydrolysis at 110^{o} C overnight

iv) The hydrolysate was completely transferred into 10 mL volumetric flask and 0.5 mL of NaOH was added.

v) The volume was made up to 10 ml with 0.1N HCl and filtered with the help of nylon syringe filters (0.25 µm).

Sl No	Characteristics	Specifications
1	Column	Agilent ZORBAX Eclipse Plus C18 stationary phase column (2.1x150mm, 3.5µm)
2	Column temperature	40^{o} C
3	Sample temperature	5-7^{o}C
4	Flow	0.5 ml/min
5	Run time	27 mins
6	Detection	DAD (262nm, Em 325nm (For FMOC derivatized AA)

3.5.8. Determination of β-ODAP using UPLC (Ultra High-Profile Liquid Chromatography)

a) Procedure

Step-1 (Mobile Phase Preparation)

Mobile Phase A: Acetonitrile: Methanol (50:50 v/v): Formic acid (0.1%)

The solution is then filtered through 0.2 µm regenerated cellulose membrane.

Mobile Phase B: Methanol: Formic acid (100:0.1v/v)

All mobile-phase solvents are HPLC grade. The solution is then filtered through 0.2 µm regenerated cellulose membrane.

Step-2 (Preparing the β-ODAP standards)

β-ODAP Standards (10 pmol/µL to 1 nmol/µL): For calibration, single point calibration was caried out as 100 µL /ml for β-ODAP standard (> 98% HPLC grade) obtained from Sigma Aldrich.

Step-3 (Preparation of the samples)

- 400 mg sample was weighed in a screw cap test tube.
- Then 10 ml of Solvent A was added to the test
- The mixture was extracted using ultra-turrax for 2 min at 10000 rpm which is kept in an ice bath.
- Extracted samples were centrifuged at 4°C at 4000 rpm for 10 min
- The supernatant was passed through a 0.2-µm PTFE membrane filter.

- Filtered samples were diluted with a mobile phase and injected at 2µL volumes to UPLC

Sl No	Characteristics	Specifications
1	Column	Agilent ZORBAX Eclipse SB C18 Rapid resolution, stationary phase column (4.6x100mm, 1.8µm)
2	Column temperature	40^{o} C
3	Sample temperature	5-7^{o}C
4	Flow	0.3 ml/min
5	Run time	12 mins
6	Detection	DAD (254nm)

CHAPTER IV

RESULTS

Grasspea is considered as drought-tolerant hardy crop, and is grown in low-rainfall regions under rainfed conditions, during winter when lentil and chickpea are not expected to give good yields. The crop has unique tolerance ability against stress environmental conditions not only drought but also for water logging. In addition to use as dal and chapatti, it is usually grown as fodder crop. Lathyrus leaves about 36-48 kg/ha nitrogen economy for the succeeding cereal.

The present study was carried out to characterize 168 germplasm for its agro-morphological traits, biochemical trait and genetic diversity analysis by molecular characterization using SSR markers. The experiment was conducted at Pusa Research Farm of ICAR - National Bureau of Plant Genetic Resources at Indian Agricultural Research Institute, New Delhi during *Rabi* 2019- 2020 and 2020-2021. In view with this, an attempt was made and discussions of results are given below.

4.1. Agro-morphological characterization of germplasm

Characterization of genotypes is a pre-requisite for differentiating it from the available germplasm. It helps us to classify the existing genotypes, to measure the genetic diversity and to maintain genetic purity. It also provides information about the magnitude of genetic variability available in the germplasm for further exploitation. These quantitative characters are affected by environment also. The morphological variability of grasspea is given in the Figure 4.1.1. The frequency distribution of quantitative traits is given in the figure 4.1.2.

4.1.1. Characterization and frequency distribution of Quantitative traits

4.1.1.1. Plant Height

The height of the plant from the ground level to the tip of the main axis at the time of harvest was measured and recorded in centimetre. The value was ranged between 48.28 to 119.45cm. The accession BANG285 (119.45) had the highest plant height followed by IFLA1870 (90.98cm). Accessions with desirable range can be selected for further breeding programmes.

4.1.1.2. Petiole length

The petiole length in the randomly selected plants were taken. The value was ranged from 1.18 (IC0634666) to 3.13cm (IC208434). The accessions with desirable range can be selected for further breeding programmes.

4.1.1.3. Days to first flowering

Number of days taken from the date of sowing to the first flower opening is called as Days to first flowering. The value was ranged between 63.34 (IC525179, BANG270) to 86.65 days (IFLA151). The accessions having earliness can be selected for further breeding programmes.

4.1.1.4. Days to 50% flowering

Number of days from sowing to stage when 50% of plants have begun to flower in a row were taken. The value was ranged between 70.81 to 93.66 days. The accessions having earliness can be selected for further breeding programmes.

4.1.1.5. Days to 80% flowering

Number of days from sowing to stage when 80% of plants have begun to flower in a row were taken. The value was ranged between 87.64 (BANG31, BANG32) to 110.24 days (IC0634667). The accessions having earliness can be selected for further breeding programmes.

4.1.1.6. Days to 80% maturity

From sowing to when 80% of plants have mature pods in a row were taken. The value was ranged between 112.35 (BANG31) to 147.47 days (IC0634667). The accessions having earliness can be selected for further breeding programmes.

4.1.1.7. Pod length

The length of the pod was taken. The value was ranged between 3.69 (BANG37) to 4.14cm (IFLA2861). The accessions with desirable range can be selected for further breeding programmes.

4.1.1.8. Pod width

The width of the pod was taken. The value was ranged between 0.82 (IC0634664) to 1.22cm (IFLA2765). The accessions with desirable range can be selected for further breeding programmes.

4.1.1.9. Number of primary branches

Counted at first pod maturity (only pod-bearing branches) were taken. The value was ranged between 5.20 (IFLA143) to 7.68 (IFLA1864, IFLA126). The accessions with desirable range can be selected for further breeding programmes.

4.1.1.10. Number of pods per plant

The number of pods in the randomly selected plants is taken. The value was ranged between 43.98 (IC0634666) to 87.08 (IFLA276) pods. The accessions with desirable range can be selected for further breeding programmes.

4.1.1.11. Number of seeds per Pod

The number of seeds per pod in the randomly selected plant was taken. The value was ranged from 2.85 (IC395836) to 5.01(IC525185) seeds. The accessions with desirable range can be selected for further breeding programmes.

4.1.1.12. Seed length

The length of the seed was taken. The value was ranged between 3.73 (IC347378) to 8.24mm (IC525185). The accessions with desirable range can be selected for further breeding programmes.

4.1.1.13. Seed width

The width of the seed was taken. The value was ranged between 3.48 (IC0634660) to 7.45mm (IFLA118). The accessions with desirable range can be selected for further breeding programmes.

4.1.1.14. 100 Seed weight

The weight of the 100 randomly selected, well filled grains was recorded and expressed in gram. The value was ranged from 4.48 (BANG224) to 14.02 g (IFLA1439). The accessions with desirable range can be selected for further breeding programmes.

Agro-morphological characterization identified promising accessions for important traits like hundred seed weight – IFLA1439 (14.02g), IFLA432 (13.38g), IC634674 (13.384g) and IFLA143 (12.32g); early flowering accessions- BANG31 (64.41days), IC525179 (63.34days), BANG208 (69.28days), BANG285, IC0634660, IC0634661 (69.31days); higher plant height- BANG285 (119.45cm); number of pods per plant- IFLA276 (87.08 pods), IC489623 (86.27pods); days to 80% maturity- BANG31 (112.35days).

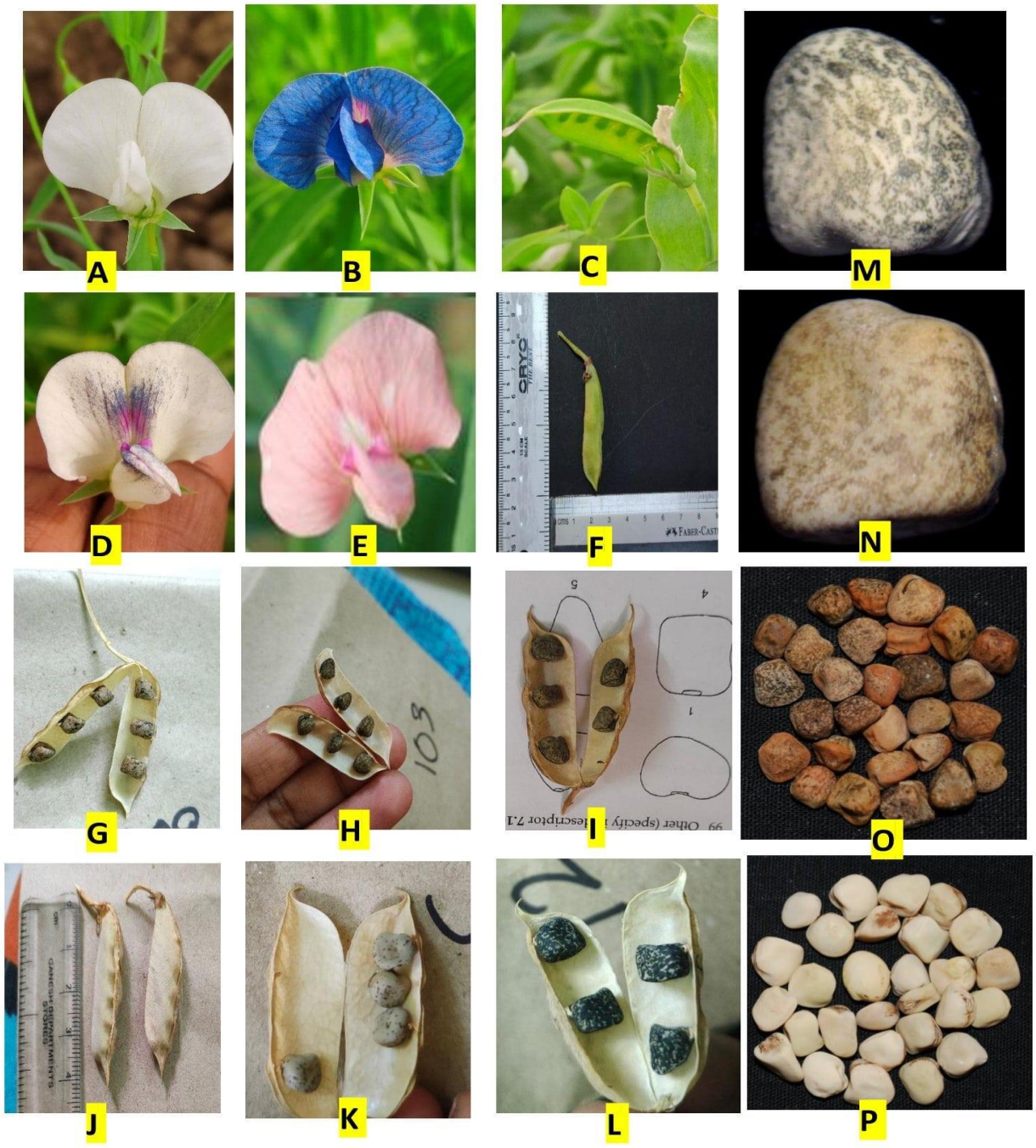

Figure 4.1.1. Morphological variations in 168 Grasspea accessions (A- white flower; B-blue violet; D- white with stripes; E- pink flower (Mahateora); C- immature pod color; J- mature pod color; F- medium oblong-elliptical shape; G, H, I, K, L, M, N, O, P- shows different color, shape, number of seeds per pod and size of grasspea seeds (M-Prateek; N-Mahateora)

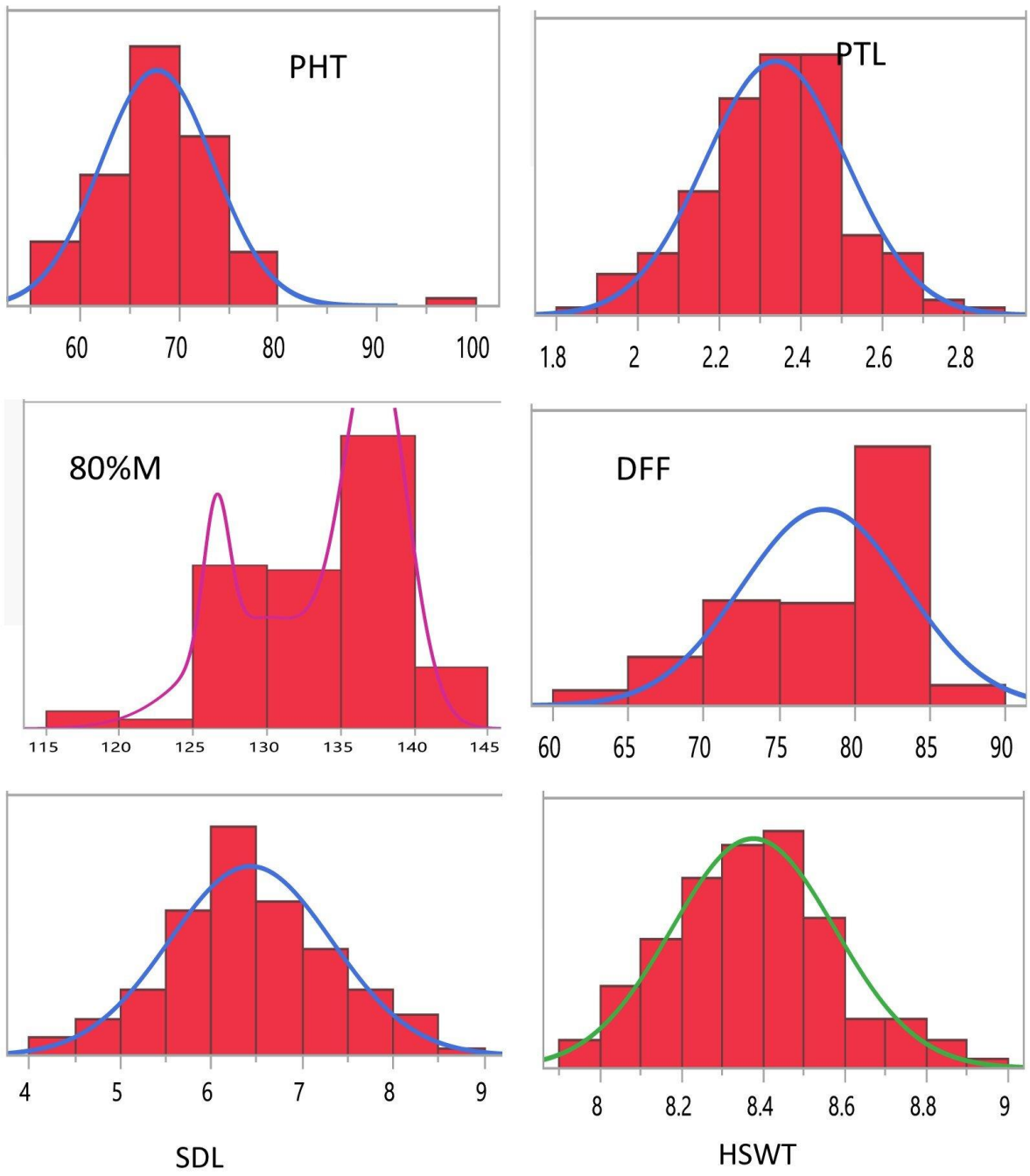

Figure 4.1.2. Frequency distribution of selected quantitative traits

4.1.2. Characterization and frequency distribution of Qualitative traits

The characterization was carried out for qualitative traits like seedling pigmentation, plant growth habit, flower color, plant vigour, plant pigmentation, leaf size, pod pubescence, pod wings, immature pod color, pod shape, pod dehiscence, constriction of pods between seeds, seed shape, seed size, seed coat color, seed coat surface, seed coat pattern, seed coat pattern color, seed ornamentation and cotyledon color. The accessions with desired characters can be chosen and used for future breeding activities though it hereditary. The frequency distribution and percentage of contribution was given in the table 4.1.1. The entire data of 20 qualitative data were given in the Table 3 of annexure. Frequency distribution of qualitative traits are given in the 4.1.3.

4.1.2.1. Seedling pigmentation

The pigmentation of the seedling after seven days of emergence were taken. Most of the accessions were slightly pigmented with purple color.

4.1.2.2. Plant growth habit

All the grasspea accessions showed the semi-erect plant growth habit

4.1.2.3. Flower color

In the present study, four different colors of flowers were are observed namely violet blue, violet, white, pink, white with blue stripes. Violet blue is the most commonly observed flower color in the 168 accessions and only pink color flower is observed in check Mahateora.

4.1.2.4. Plant Vigour

Plant vigour was recorded at 8-10 leaf stage. Most of the accessions shows medium plant vigour in their growth stage.

4.1.2.5. Plant pigmentation

The pigmentation of the plant was taken. Most of the accessions were slightly pigmented with purple color.

4.1.2.6. Leaf size

In the present study, the leaf size was recorded which varies from small to medium and also larger leaf size also observed and it is one of the traits considered in importance with fodder and forage, green leafy vegetable.

4.1.2.7. Pod pubescence

All the grasspea accessions showed the pubescence in their pods slightly

4.1.2.8. Pod wings

All the grasspea accessions showed the medium to large (score) wingedness in their pods

4.1.2.9. Immature pod color

All the grasspea accessions showed the green to light green color in their pods

4.1.2.10. Pod shape

The pod shape varies from oblong-elliptical, medium oblong-elliptical, beaded and the most observed shape is the medium oblong-elliptical

4.1.2.11. Pod dehiscence

About 97% of the population showed low pubescence in their pods

4.1.2.12. Constriction of pods between seeds

All the grasspea accessions showed the slight to medium constriction of pods between the seeds

4.1.2.13. Seed shape

The different seed shapes observed in the accessions are rhomboid, square, obtriangular and spherical. The shape of seed predominantly varies within the accession and among the accessions.

4.1.2.14. Seed size

The observed seed size of the grasspea accessions varies from medium to large and the predominantly medium is the size of the seed

4.1.2.15. Seed coat color

The seed coat color varies from greyed-white, grey, brown, grey mottled and the predominant is the latter one.

4.1.2.16. Seed coat surface

The seed coat surface varies from smooth to tubercular. Almost 99% showed tubercular seed coat surface

4.1.2.17. Seed coat pattern

The observed seed coat pattern varies from dotted to marbled and mostly dotted one is the predominant one.

4.1.2.18. Seed coat pattern color

The observed seed coat pattern color varies from cream, brown, red-purple and black and brown is the most observed seed coat pattern color.

4.1.2.19. Seed ornamentation

The observed seed ornamentation almost present in all the accessions which accounts for 86%

4.1.2.20. Cotyledon color

In general, *L. sativus* species and all the accessions which was taken under study shows yellow cotyledon color.

Table 4.1.1. Frequency distribution and percent contribution of 20 qualitative traits.

S.no	Plant Traits	Code	Descriptor state (Score)	Frequency	Percentage
1	Seedling pigmentation	SDLG_PIG	1. No pigmentation	38	22.09
			2. Slightly pigmented	78	45.35
			3. Pigmented	50	29.07
			4. Highly pigmented	6	3.49
2	Plant growth habit	GR_HBT	1. Prostrate	0	0.00
			2. Spreading	0	0.00
			3. Semi-erect	168	100.00
			4. Erect	0	0.00
3	Flower color	F_CLR	1. White	1	0.58

			2. White blue	11	6.40
			3. Blue	1	0.58
			4. Grey	0	0.00
			5. Light yellow	0	0.00
			6. Yellow	0	0.00
			7. Pink	1	0.58
			8. Orange	0	0.00
			9. Red	0	0.00
			10. Violet-blue	158	91.86
			11. Violet	0	0.00
			99. Other	0	0.00
4	Plant vigor	PLT_VGR	3. Poor	28	16.28
			5. Medium	123	71.51
			7. Good	21	12.21
5	Plant pigmentation	P_PIG	0. Absent	92	53.49
			1. Present	80	46.51
6	Leaf size	LF_SZ	1. Small	25	14.53
			2. Medium	88	51.16
			3. Large	59	34.30
7	Pod pubescence	POD_PUB	0. Absent	5	2.91
			3. Low	167	97.09
			5. Medium	0	0.00
			7. High	0	0.00
8	Pod wings	POD_WING	0. Absent	0	0.00
			3. Narrow	0	0.00
			5. Medium	171	99.42
			7. Wide	1	0.58
9	Immature pod color	IM_PD_CLR	1. Yellow-cream	0	0.00
			2. Light green	34	19.77
			3. Green	138	80.23
			4. Dark green	0	0.00

			5. Green-purple	0	0.00
			6. Light purple	0	0.00
			7. Purple	0	0.00
			99. Other	0	0.00
10	Pod shape	PD_SHP	1. Oblong-elliptical	14	8.14
			2. Medium oblong-elliptical	153	88.95
			3. Curved	0	0.00
			4. Beaded	4	2.33
			5. Broad-linear	1	0.58
			6. Broad-elliptical	0	0.00
			99. Other	0	0.00
11	Pod dehiscence	PD_DEHS	0. No shattering	60	34.88
			3. Low shattering	112	65.12
			5. Medium shattering	0	0.00
			7. High shattering	0	0.00
12	Constriction of pods between seeds	CONS B/W PODS	0. Absent	0	0.00
			3. Slight	142	82.56
			5. Medium	29	16.86
			7. Pronounced	1	0.58
13	Seed shape	SD_SHP	1. Oblate or flattened	0	0.00
			2. Triangular	0	0.00
			3. Rhomboid	37	21.51
			4. Square	70	40.70
			5. Obtriangular	63	36.63
			6. Spherical	2	1.16

			99. Other	0	0.00
14	Seed size	S_SZE	3. Small	0	0.00
			5. Medium	102	59.30
			7. Large	70	40.70
15	Seed coat color	SD CT CLR	1. Greyed-white	20	11.63
			2. Grey	22	12.79
			3 Yellow-white	0	0.00
			4 Brown	58	33.72
			5 Yellow-green	0	0.00
			6. Pink	0	0.00
			7 Red-purple	0	0.00
			8 Black	0	0.00
			9 Grey mottled	72	41.86
			10 Green mottled	0	0.00
			99 Others	0	0.00
16	Seed coat surface	S_CT_SUR	1 Smooth	2	1.16
			2 Tubercular	170	98.84
17	Seed coat pattern	S_CT_PTN_CLR	0 Absent	26	15.12
			1 Marbled	40	23.26
			2 Dotted	106	61.63
			3 Streaked	0	0.00
			4 Mixture	0	0.00
18	Seed coat pattern color	S_CT_PTN_CLR	1 Cream	22	12.79
			2 Green	1	0.58
			3 Brown	130	75.58
			4 Red-purple	11	6.40
			5 Black	8	4.65
			99 Other	0	0.00
19	Seed ornamentation	SD_ORN	0 Absent	25	14.53
			1 Present	147	85.47
20	Cotyledon color	COT_CLR	1 Yellow	172	100.00

2 Orange	0	0.00
99 Other	0	0.00

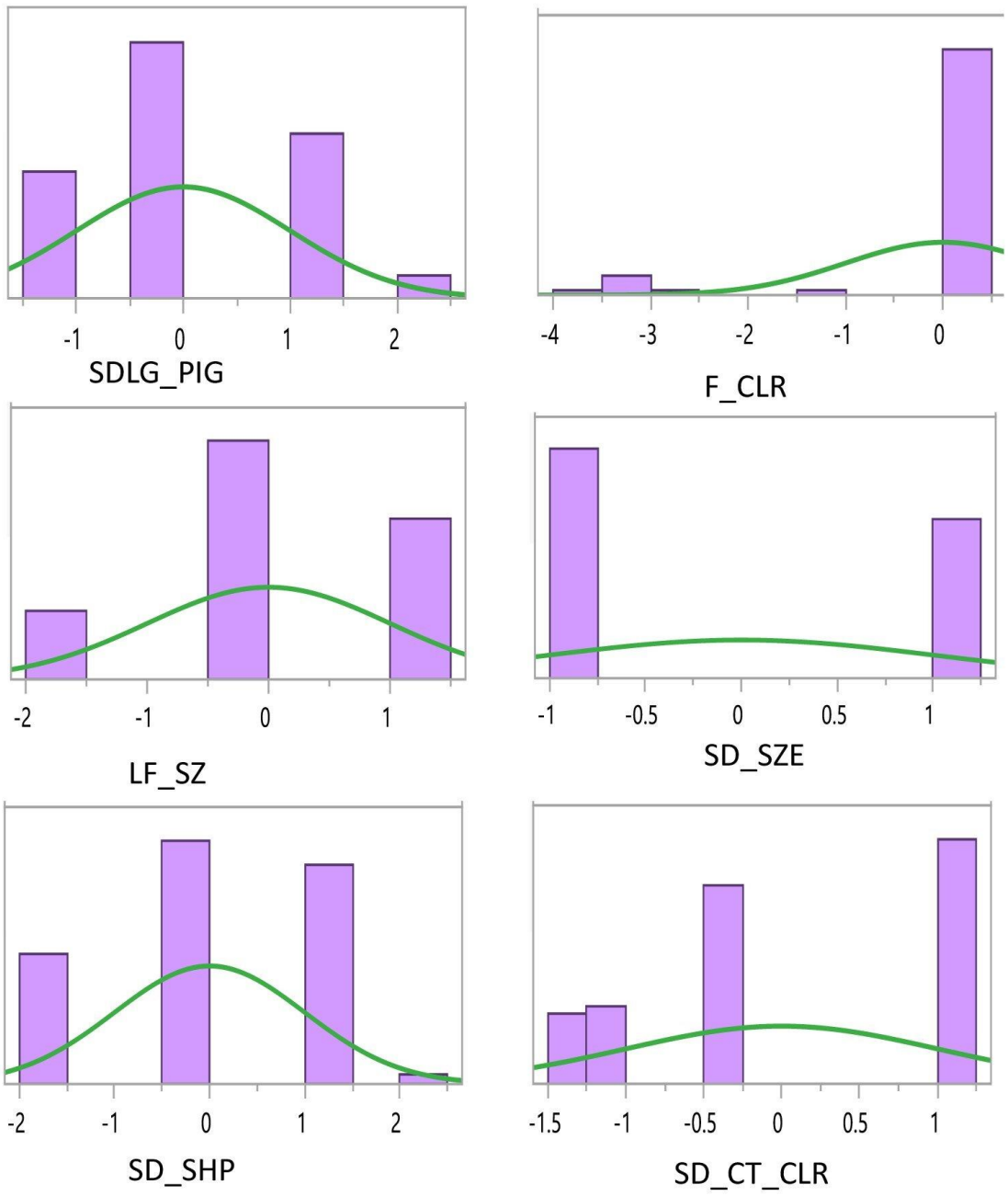

Figure 4.1.3. Frequency distribution of selected qualitative traits

Table 4.1.2. Adjusted means from GENSTAT for 14 quantitative traits

Accessions	PHT	PTL	DFF	50%F	80%F	80%M	PDL	PDW	NPB	NPP	NSP	SDL	SDW	HSWT
IFLA 143	83.68	2.06	83.25	87.05	104.34	134.23	3.83	1.07	5.2	65.72	4.11	6.76	5.76	12.32
IFLA276	68.88	2.66	84.52	89.36	106.6	137.23	3.91	1.02	5.77	87.08	3.96	5.79	5.19	9.68
IFLA1795	65.98	2.81	78.56	83.57	100.93	129.73	3.84	1.05	5.77	65.72	4.11	5.64	5.23	9.3
IFLA1870	90.98	2.56	84.95	90.13	107.36	138.23	3.9	0.99	6.16	64.08	3.61	6.35	5.3	9.6
IFLA2026	82.98	2.76	85.38	90.52	107.74	138.73	3.78	1.03	6.16	84.61	3.81	6.53	6.05	11.52
IFLA151	62.88	3.11	86.65	92.06	109.25	140.73	3.88	1.01	6.35	80.51	3.61	7.14	5.85	12.8
IFLA2282	70.68	3.06	84.1	88.2	105.47	135.73	3.82	1	6.35	71.47	3.96	5.92	5.34	9.19
IFLA2475	70.88	2.86	75.15	80.1	97.53	125.23	4.01	1.05	6.35	74.76	3.76	5.38	5.21	7.83
IFLA274	72.98	2.46	77.71	82.03	99.42	127.73	3.83	1.03	6.35	70.65	4.31	5.06	4.82	8.34
IFLA2765	74.88	2.41	84.1	89.75	106.98	137.73	3.85	1.22	6.35	73.11	4.56	5.87	5.64	7.9
IFLA2998	69.38	2.06	81.97	86.27	103.58	133.23	3.93	1.01	6.35	78.04	4.96	6.61	5.53	8.12
IFLA2736	60.38	2.41	75.58	80.1	97.53	125.23	3.92	1.04	6.35	76.07	4.31	5.44	4.5	9.5
IFLA2968	75.78	1.96	83.25	87.05	104.34	134.23	4	1	6.35	77.22	4.11	5.84	5.11	8.24
IFLA2924	79.28	2.06	75.58	80.87	98.29	126.23	3.92	1.01	6.35	66.54	4.41	6.16	6.25	9.8
IFLA172	76.18	2.76	75.15	80.87	98.29	126.23	3.83	1.02	6.35	50.73	4.01	6.54	5.84	11.38
IFLA1720	71.48	2.31	85.38	92.06	109.25	140.73	3.8	1.01	6.16	73.11	3.96	6.27	5.62	10.34
IFLA128	74.13	1.86	85.8	92.83	110.01	141.73	4.03	1.01	6.16	80.92	4.11	4.38	5.45	10.71
IFLA2636	69.38	1.71	85.38	92.06	109.25	141.23	3.8	0.99	6.16	69.42	3.76	6.59	5.42	9.12
BANG211	86.58	2.41	78.13	83.19	100.56	129.73	3.89	1.01	6.16	58.74	4.46	5.4	4.73	6.62
BANG184	60.58	1.86	72.6	77.79	95.26	123.23	3.83	0.99	6.16	71.88	4.36	5.92	5.25	6.18

BANG291	76.98	1.51	82.39	87.05	104.34	135.23	3.83	1.04	6.16	70.24	3.76	5.3	5.01	7.72
BANG297	70.53	1.46	82.39	86.27	103.58	134.23	3.81	1.02	6.16	66.95	4.41	3.98	3.92	7.06
BANG308	54.73	1.61	78.56	83.96	101.31	131.23	3.82	1.01	6.16	75.17	4.11	5.65	5.09	6.29
BANG218	48.43	1.76	74.3	81.26	98.67	127.73	3.83	0.98	6.16	77.63	4.41	5.48	4.9	5.78
BANG198	66.98	2.31	82.39	87.05	104.34	135.23	3.8	1.01	6.16	71.88	3.96	5.72	5.19	9.32
BANG224	69.78	1.61	70.47	77.01	94.51	122.23	3.99	0.98	6.16	74.35	3.76	4.88	4.42	4.48
BANG292	58.58	1.36	75.15	81.64	99.42	128.23	3.8	0.96	6.16	77.63	4.11	4.86	4.7	5.52
BANG299	68.58	1.71	75.15	81.64	99.42	128.23	3.85	1.04	6.16	66.95	4.41	5.5	4.95	7.5
BANG227	58.58	1.56	77.28	83.19	100.93	130.23	3.84	1.02	6.16	77.63	4.61	5.69	5.63	6.5
BK154	83.08	1.71	76	80.87	99.04	127.23	3.82	0.87	6.16	68.6	3.61	6.02	5.43	7.78
BK148	70.18	1.66	79.41	84.73	102.82	132.23	3.83	1.02	6.16	70.24	3.96	6.32	5.64	8.69
IFLA1522	48.28	1.96	73.87	78.94	97.15	124.73	3.85	0.99	6.16	69.42	3.96	6.41	5.79	9
IFLA2750	70.88	2.41	71.32	77.01	95.26	122.23	3.84	0.87	6.16	74.51	4.26	7.37	5.88	8.64
IFLA1926	68.2	2.28	83.79	92.12	108.73	145.47	4.03	0.97	6.86	62.87	4.36	6.38	5	9.64
IFLA1857	61.52	1.99	76.52	86.11	102.82	132.85	3.84	0.98	7.49	66.9	3.84	8.12	5.83	10.62
IFLA432	59.67	2.74	74.81	84.18	100.93	130.35	3.86	1.01	7.49	52.11	4.34	6.93	5.36	13.38
IFLA2341	69.12	2.74	82.05	90.35	106.98	138.85	3.78	1.04	7.49	69.36	3.84	6.87	4.76	10.38
IFLA2025	59.42	1.74	82.05	90.74	107.35	139.35	3.96	0.99	7.49	69.36	3.64	7.65	5.78	12.32
IFLA1864	56.42	2.74	80.78	89.2	105.84	137.35	3.79	1.01	**7.68**	72.64	3.84	7.64	6.01	11.5
IFLA2687	54.42	2.59	82.05	91.51	108.11	140.85	3.92	1	**7.68**	66.9	3.49	7.9	5.74	11.14
IFLA126	73.02	1.94	82.05	91.13	107.73	140.35	3.89	1.02	**7.68**	64.43	3.64	6.89	5.56	7.54
IFLA1813	50.02	2.79	73.96	82.64	99.41	129.35	3.86	1.02	7.68	51.29	3.84	7.47	6.03	10.5

IFLA1715	66.72	2.04	82.05	89.2	105.84	137.85	3.88	0.99	7.49	66.9	3.84	7.93	6.27	12.06
IFLA1193	72.12	2.59	79.92	86.88	103.57	134.85	3.82	1.04	7.49	70.18	3.99	7.54	5.68	12
IFLA144	55.12	2.69	80.78	88.43	105.08	136.85	3.79	1.07	6.72	73.88	3.64	5.87	5.37	5.62
IFLA127	55.42	2.79	82.05	89.97	106.6	138.85	3.75	1.02	6.72	70.59	3.84	6.63	5.51	8.38
IFLA2129	67.42	2.54	82.05	90.35	106.98	139.35	3.81	0.96	6.91	73.06	3.84	6.63	5.59	8.98
IFLA1849	73.22	2.14	72.26	81.48	98.28	127.85	3.88	0.9	6.91	76.34	3.49	6.87	5.15	9.16
BANG208	61.72	2.54	**69.28**	77.62	94.5	122.85	3.84	1.13	6.91	65.66	3.49	6.09	4.89	6.4
BANG206	71.92	2.29	**69.7**	78.39	95.25	123.85	3.86	1	6.91	76.34	3.64	5.91	4.68	6.78
BANG257	62.12	2.74	78.65	86.5	103.19	134.35	3.83	1.02	6.91	67.31	4.84	5.49	4.68	5.9
BANG268	49.22	2.54	71.41	80.71	97.52	126.85	3.78	0.96	6.15	68.95	4.64	6	5.06	6.04
BANG263	56.42	2.34	79.92	88.43	105.08	136.85	3.87	1.05	6.91	64.51	4.49	6.46	4.88	5.22
BANG286	54.52	2.84	80.78	88.81	105.46	137.35	3.8	0.97	6.91	68.95	3.84	6.54	5.1	6.22
BANG284	62.02	2.84	82.05	90.74	107.35	139.85	3.79	0.96	6.91	68.13	4.19	6.82	5.13	7.23
BK551	76.32	2.34	78.65	86.11	102.82	133.85	3.77	1	6.91	53.34	4.19	5.94	5.13	8.19
BK401	79.52	1.69	70.55	79.55	96.39	125.35	3.81	0.88	6.91	70.59	4.14	6.73	5.32	8.64
BK3111	67.12	2.64	76.52	84.57	101.3	131.85	3.77	1	6.91	70.59	4.14	6.83	5.51	9.06
BK101	76.22	2.19	79.07	87.27	103.95	135.35	3.78	0.97	6.91	73.88	3.84	6.6	5.43	9.38
BK391	74.42	2.79	78.65	86.88	103.57	134.85	3.8	1.01	6.91	68.13	4.34	6.68	5.15	8.12
BK541	70.92	2.29	70.13	79.17	96.01	124.85	3.86	1.03	6.91	65.66	3.64	6.45	5.5	9.38
BK301	80.47	2.69	77.79	85.34	102.06	132.85	3.86	1	6.72	52.52	3.79	6.49	5.06	9.05
IFLA479	71.52	2.29	81.2	89.2	105.84	137.85	3.86	0.98	6.72	68.13	4.34	6.42	5.02	8.79
IFLA220	57.37	2.69	77.79	86.11	102.82	133.85	3.91	0.94	6.72	71.41	3.29	6.45	4.87	7.67

IFLA190	62.22	2.54	79.07	88.43	105.08	136.85	3.79	1.06	6.72	64.84	3.99	6.31	5.3	7.78
IFLA2861	61.82	2.29	71.83	80.32	97.14	126.35	4.09	1.02	6.72	86.2	3.34	6.79	5.27	6.42
IFLA2460	68.22	2.29	78.22	86.88	103.57	134.85	3.86	0.97	6.72	64.84	3.94	6.04	4.86	7.42
BIO520	57.22	2.64	82.05	91.13	107.73	141.35	3.88	0.95	6.72	63.2	3.64	6.54	4.67	8.44
IFLA123	85.32	2.59	81.2	91.51	108.11	141.85	3.87	0.94	6.72	83.73	3.49	7.59	5.88	12.94
IFLA118	81.61	2.43	71.37	80.44	97.24	130.6	3.83	1.05	6.12	79.7	4.9	8.13	7.45	11.34
IFLA1826	72.51	2.68	81.6	91.24	107.82	144.6	3.9	0.97	6.12	70.66	3.9	7.8	6.25	11.52
IFLA2441	66.01	2.83	78.19	86.23	102.91	138.1	3.86	0.91	6.12	73.95	4.7	7.44	6.29	10.32
IFLA3001	71.81	2.38	70.09	78.13	94.97	127.6	3.84	1.02	6.12	69.84	3.9	7.56	6.36	10.62
SeI190	68.81	2.43	71.8	78.51	95.35	129.1	3.92	0.97	6.12	77.23	3.9	7.4	6.38	10.84
IFLA2990	55.81	3.03	72.22	79.28	96.1	130.1	3.81	0.98	6.12	68.83	4.05	7.31	6.31	10.78
IFLA1439	68.81	2.73	74.35	81.21	97.99	132.6	3.79	0.95	6.5	76.41	4.05	8.15	6.89	14.02
IFLA1913	68.81	2.53	79.89	87.77	104.42	141.1	3.93	0.95	6.5	79.7	4.05	8.06	7.03	12.54
BANG309	52.61	2.88	74.35	81.6	98.37	133.1	3.86	1	6.5	68.2	4.2	6.11	5.26	9.94
BANG310	54.61	2.43	**69.24**	76.58	93.46	126.6	3.98	0.95	6.5	57.52	4.05	6.68	5.87	9.14
BANG186	70.81	3.03	72.65	79.28	96.1	130.1	4.01	0.96	6.5	70.66	3.9	6.71	5.68	9.22
BANG249	58.81	2.78	77.76	83.53	100.26	135.6	3.93	1.03	6.69	70.66	4.25	7	5.97	9.11
BANG182	73.01	2.38	78.19	85.07	101.77	137.6	3.88	0.97	6.69	73.95	4.2	6.52	5.69	9.7
BANG267	67.81	2.33	80.32	88.16	104.8	139.1	3.89	0.97	6.5	83.8	4.15	6.75	5.75	9.69
IC525182	51.41	2.48	78.61	85.07	101.77	137.6	3.8	0.99	6.69	65.73	4.05	7.63	5.61	9.39
IC148364	69.66	2.58	79.47	86.23	102.91	139.1	3.88	0.95	6.69	52.59	3.75	6.63	5.57	9.91
IC148352	53.31	2.98	80.32	88.16	104.8	141.6	4	1.02	6.69	68.2	4.2	7.27	6.15	9.3

IC574469	69.01	2.63	81.6	89.32	105.93	143.1	3.88	1.02	6.69	71.48	4.4	6.27	5.72	8.24
IC148356	67.51	2.68	80.32	88.54	105.18	142.1	3.8	0.97	6.69	64.91	4.2	6.15	5.51	8.06
IC489623	73.91	2.53	80.32	88.54	105.18	139.6	4	0.96	6.69	86.27	3.7	6.51	5.55	10.88
IC574470	62.71	3.03	80.32	88.54	105.18	139.6	3.87	0.96	6.69	64.91	3.9	5.81	5.26	11.34
IC208429	73.31	2.58	78.61	86.23	102.91	136.6	3.85	0.96	6.5	63.27	4.35	6.91	5.89	8.75
IC208430	58.31	2.38	**68.39**	75.43	92.32	125.1	3.86	0.93	6.69	68.2	3.55	7.52	5.95	9.24
IC208433	71.31	2.18	80.32	87.77	104.42	138.6	3.81	0.97	6.5	79.7	4.05	7.15	6.25	9.45
IC208434	74.31	3.13	80.32	88.16	104.8	139.1	3.83	0.94	6.5	70.66	4.4	7.14	6.24	8.52
IC208436	79.66	2.68	77.34	85.07	101.77	135.1	3.85	1	6.5	73.95	3.55	7.3	6.34	10.44
IC208437	65.56	2.68	79.89	88.16	104.8	139.1	3.82	0.99	6.5	69.84	3.75	6.86	5.8	9.44
IC212672	55.56	2.78	80.32	88.93	105.55	140.1	3.82	0.97	6.5	72.3	4.2	6.93	5.38	8.52
IC212649	59.91	2.48	80.32	89.32	105.93	138.1	3.9	0.98	6.5	77.23	3.4	6.19	5.08	7.95
IC415656	60.11	2.48	78.19	86.23	102.91	134.1	3.77	0.96	6.5	69.02	3.55	6.89	5.63	7.76
IC415658	74.01	2.49	79.89	88.54	105.18	137.1	3.86	0.99	6.5	76.41	4.25	6.63	5.59	7.54
IC415678	66.31	2.73	73.5	81.21	97.99	127.6	3.78	0.98	6.5	65.73	4.05	6.95	6.17	7.85
IC395836	68.01	2.14	71.8	78.9	95.72	124.6	3.81	0.93	6.69	66.39	2.85	6.87	5.92	9.28
IC396712	71.91	2.03	72.65	80.06	96.86	126.1	3.81	0.98	6.69	74.6	4.05	7.17	5.83	9.68
IC470982	75.81	2.78	72.65	80.06	96.86	126.1	3.91	1.01	6.69	77.07	4.85	7.95	6.44	9
IC427679	83.78	2.54	72.51	77.37	94.07	120.85	3.79	1	7.3	73.91	4.5	5.39	5.82	8.84
IC427710	81.63	2.19	79.75	84.7	101.25	130.35	3.74	1	7.3	76.38	4.35	4.66	5.51	8.75
IC421914	83.18	2.04	79.75	85.09	101.63	130.85	3.75	1.03	7.3	79.66	4.2	4.64	5.39	8
IC349809	85.38	2.44	80.18	84.7	101.25	130.35	3.83	1.03	7.3	56.66	3.35	3.96	4.87	6.36

IC347378	92.78	2.54	80.18	83.93	100.5	129.35	3.7	1.02	7.3	79.66	3.85	3.73	4.69	7.58
IC18879	82.78	2.53	73.36	78.14	94.83	121.85	3.75	1.01	7.3	70.63	4.35	4.73	5.07	7.64
IC567350	88.38	2.59	72.51	76.98	93.69	120.35	3.8	0.92	7.11	67.1	3.85	4.16	4.83	7.04
BANG27	66.28	2.64	83.58	87.79	104.28	134.35	3.8	0.95	7.11	75.31	3	5.8	6.28	11
BANG31	57.18	2.78	**64.41**	70.81	87.64	112.35	3.78	0.94	7.11	77.77	3.55	6.03	6.13	10.49
BANG15	67.18	2.53	72.93	79.3	95.96	123.35	3.7	1	7.11	72.02	3.85	6.95	6.42	9.16
BANG20	61.18	2.63	82.31	87.79	104.28	134.85	3.74	1.02	7.11	74.49	3	7.69	6.72	9.17
BANG21	66.48	2.38	82.31	87.79	104.28	134.85	3.79	1.04	7.11	77.77	3.85	5.65	5.9	8.23
BANG26	56.38	2.54	81.45	87.02	103.52	133.85	3.71	1.05	7.11	67.1	3.7	5.42	6.11	8.29
BANG37	71.29	2.03	82.31	87.02	103.52	133.85	3.69	1.02	7.11	77.77	4.15	5.04	5.61	8.17
BANG9	60.4	1.94	82.31	87.02	103.52	133.85	3.72	1.02	7.11	70.63	4	6.63	5.97	8.79
BANG18	62.39	2.74	70.38	75.44	92.18	118.85	3.81	0.97	7.11	72.27	3.85	7.64	5.74	7.93
BANG28	75.98	2.14	82.31	86.24	102.77	134.35	3.79	0.93	7.11	71.45	4.2	5.69	5.64	8.73
BANG29	83.18	2.69	82.31	86.24	102.77	134.35	3.75	0.94	7.11	76.38	4	6.24	5.8	7.82
BANG30	74.65	2.73	84.44	90.1	106.55	139.35	3.72	0.94	6.73	72.27	4.35	6.04	6.89	10.44
BANG3	89.28	2.59	82.31	90.1	106.55	139.35	3.78	0.99	6.73	71.45	3.85	7.17	6.22	11.38
BANG4	70.48	2.59	84.86	90.1	106.55	139.35	3.78	0.98	6.73	56.66	3.5	6.92	5.82	9.4
BANG5	60.08	2.04	84.44	87.02	103.52	135.35	3.78	1	6.73	73.91	3.35	6.4	6.51	8.57
BANG13	72.58	2.88	84.44	87.02	103.52	135.35	3.81	0.99	6.73	73.91	3.5	6.63	6.45	8.02
BANG22	70.28	2.34	72.93	78.53	95.2	**124.35**	3.79	0.97	6.73	77.2	4.35	6.79	7.23	7.76
BANG32	77.28	2.93	**64.41**	70.81	87.64	114.35	3.72	0.93	6.73	71.45	4.2	7.12	6.29	8.25
BANG33	77.83	2.93	84.01	87.79	104.28	136.35	3.72	0.99	6.73	68.98	4.35	5.69	5.78	7.79

BANG38	75.83	2.54	**66.12**	71.58	88.4	115.35	3.8	0.97	6.73	55.84	4.35	6.37	6.22	9.29
BANG41	87.83	2.48	70.38	76.21	92.94	122.85	3.79	0.96	6.73	54.78	4.35	5.25	5.81	9.76
BANG43	66.83	2.14	70.38	76.21	92.94	122.85	3.71	0.98	6.73	72.02	3.65	3.89	4.86	9.24
BANG44	68.83	2.53	70.38	77.76	94.45	124.85	3.79	1.04	7.11	72.02	4.35	6.02	6.14	7.87
BANG45	67.83	2.28	84.86	88.56	105.03	138.85	3.89	1.01	7.11	75.31	4	6.08	5.78	8.84
IC424511	60.83	2.54	82.31	86.24	102.77	135.85	3.78	1.13	7.11	68.94	4.35	5.27	6.38	10.08
BANG209	60.83	2.14	70.38	76.21	92.94	122.85	3.7	1.05	7.11	71.82	3.85	4.92	4.92	10
IC574468	63.33	2.58	70.38	75.44	92.56	121.85	3.8	0.97	7.11	73.67	4.15	4.91	5.48	6.83
IC525179	57.95	2.63	**63.34**	72.06	89.45	119.47	3.9	0.96	7.24	66.57	4.01	6.3	5.12	9.44
BANG270	62.45	2.78	**63.34**	71.28	88.69	118.47	3.82	0.98	7.24	69.04	3.66	6.21	6.08	7.1
BANG285	119.45	2.08	**69.31**	77.46	93.98	126.47	3.81	0.96	7.24	67.8	3.66	7.1	4.86	10.57
BANG198	51.45	1.78	**69.31**	78.23	95.12	127.47	3.76	0.95	7.24	63.7	3.86	6.46	5.24	9.11
BANG219	49.95	2.58	81.23	91.35	107.97	144.48	3.79	0.97	7.24	70.68	3.51	7.76	5.08	6.45
BANG204	55.45	2.28	81.23	92.12	108.73	145.47	3.88	0.95	7.24	60.82	3.16	8.19	3.84	6.18
BANG149	56.45	1.93	83.36	91.35	107.97	144.47	3.91	0.9	7.24	66.98	4.16	6.81	5.15	6.98
IC525185	51.45	1.43	81.23	89.03	105.71	141.48	3.83	0.97	6.86	65.38	5.01	8.24	5.65	7
IC525181	65.65	2.33	81.23	91.35	107.97	144.47	3.84	0.98	6.86	70.35	3.86	7.34	4.84	7.02
IC0634654	66.4	2.33	**69.31**	78.23	95.5	127.47	3.85	0.83	6.86	61.64	3.81	8.2	5.95	9.25
IC0634655	71.15	2.08	81.23	89.03	105.71	141.47	3.79	1	6.86	61.23	4.21	6.79	5.71	7.89
IC0634656	65.45	2.13	71.86	80.54	97.39	130.48	3.83	1	6.86	59.59	3.86	8.08	5.24	6.77
IC0634657	65.85	1.58	81.23	89.8	106.46	142.48	3.81	0.92	6.86	68.01	4.16	7.4	5.72	6.58
IC0634658	61.7	1.83	81.23	90.58	107.6	143.48	3.88	0.97	6.86	63.08	4.36	6.2	5.6	7.55

IC0634659	63.4	1.38	81.23	89.03	106.08	141.47	3.93	1.01	6.86	68.21	4.16	7.72	4.76	5.68
IC0634660	72.95	1.38	81.23	89.03	105.71	141.48	3.85	0.98	6.86	63.49	4.86	7.75	3.48	6.12
IC0634661	58.8	2.13	81.23	89.03	105.71	141.48	3.85	1	6.86	65.75	4.36	6.85	4.96	6.54
IC0634662	50.8	1.93	**69.31**	79	95.5	128.48	3.83	0.95	6.86	63.7	4.16	7.75	6.19	10.59
IC0634663	55.3	1.98	81.23	89.42	106.08	141.97	3.88	0.97	7.05	62.75	2.86	5.72	4.84	9.5
IC0634664	57.8	1.58	81.23	89.03	105.71	141.48	3.81	0.82	6.86	65.75	3.86	6.09	6.17	8.95
IC0634665	63.8	1.63	81.23	89.03	105.71	141.47	3.89	0.99	6.86	60	3.86	6.01	5.11	8
IC0634666	62.3	1.18	81.23	89.8	106.46	142.48	3.93	0.94	6.86	43.98	4.21	5.99	5.49	8.83
IC0634667	59.3	2.53	83.79	93.66	110.24	147.47	3.81	0.97	6.86	64.93	4.01	7.03	5.38	8.01
IC0634668	66.65	2.13	81.23	88.26	104.95	140.47	3.87	1.02	6.86	64.52	3.51	6.22	4.46	9.46
IC0634669	60.3	2.13	81.23	89.03	105.71	141.47	3.83	0.97	6.86	69.86	4.51	6.25	4.72	9.22
IC0634670	66.15	1.78	79.53	86.72	103.44	138.48	3.86	0.98	6.86	65.34	3.71	5.25	4.29	8.7
IC0634671	69.8	1.68	83.79	92.12	108.73	145.48	3.82	1	6.86	60.62	4.51	5.98	4.91	7.87
IC0634672	75	1.23	81.23	89.8	106.46	142.48	3.88	0.99	6.86	60.41	4.36	6.21	4.6	6.21
IC0634673	76.75	1.83	83.79	92.89	109.49	146.48	3.91	1	6.86	67.39	4.51	6.56	4.36	7.9
IC0634674	73.43	2.21	75.15	82.03	100.18	128.73	3.8	0.99	6.16	70.24	4.11	6.35	5.15	13.84
Min	48.28	1.18	63.34	70.81	87.64	112.35	3.69	0.82	5.2	43.98	2.85	3.73	3.48	4.48
Max	119.45	3.13	86.65	93.66	110.24	147.47	4.09	1.22	7.68	87.08	5.01	8.24	7.45	14.02

4.1.2. Variability studies

4.2.1. Analysis of variance

The two-year data were pooled using general linear mixed model and adjusted means of 14 quantitative traits using GENSTAT software were used for the further analysis. The mean value data for 14 quantitative traits of two seasons rabi 2019-20 and 2020-21 was given in the annexure table 1 and 2. The adjusted mean values are given in the Table 4.1.2. The mean sum of squares due to different sources of variation for 14 characters namely plant height (PHT), petiole length (PTL), days to first flowering (DFF), days to 50% flowering (50%F), days to 80% flowering (80%F), days to 80% maturity (80%M), pod length (PDL), pod width (PDW), number of primary branches (NPB), number of pods per plant (NPP), number of seeds per plant (NSP), seed length (SDL), seed width (SDW) and hundred seed weight (HSWT) are presented in the table 4.1.3. To study the extent of variation the value of phenotypic and genotypic coefficient of variation for different characters were estimated and furnished in table 4.1.4. The PCV and GCV showed the degree of variability with respect to various characters studied.

Table 4.1.3. Mean sum of squares due to different sources of variation for 14 quantitative traits

	Sources of variation					
Traits	**Germplasm (df=171)**	**Germplasm: Check (df=3)**	**Treatment: Test (df=167)**	**Treatment: Test vs. Check (df=1)**	**Block (df=4)**	**Residuals (df=12)**
PHT	113.06*	7.08	114.26	229.3	136.99	38.02
PTL	0.17*	0.17	0.18*	0.04	0.46**	0.05
DFF	27.06**	43.84**	26.88**	5.73	2.81	5.27
50%F	26.25*	36.29*	26*	37.24	6.17	9.29
80%F	24.92*	34.41	24.67*	37.78	7.51	10.17
80%M	47.92**	24.56	48.4**	37.79	13.73	12.15
PDL	0.01	0.14*	0.30	0.08	0.01	0.03
PDW	0.02	0.11	0.24	0.09	0.02	0.04
NPB	0.06	0.01	0.06	0.04	0.54**	0.05
NPP	44.17	21.03	44.49	60.08	24.3	36.31
NSP	0.15	0.26*	0.15	0.19	0.04	0.07
SDL	0.63*	0.1NS	0.65*	0.27	0.82*	0.23
SDW	0.3*	0.99**	0.29*	0.01	0.54*	0.11
HSWT	4.87**	0.93	4.97**	0.35	10.49**	1.05

*, ** Significance at 5 and 1 per cent level, respectively

NOTE:

PHT - Plant height (cm)	80%F- Days to 80% flowering	SDL- Seed length (mm)
PTL - Petiole length(cm)	80%M- Days to 80% maturity	SDW- Seed width (mm)
DFF- Days to first flowering	NPB- Number of primary branches	HSWT- Hundred seed weight (g)
50%F- Days to 50% flowering	NPP- Number of pods per plant	

4.2.2. Phenotypic and genotypic co-efficient of variation

The phenotypic and genotypic coefficients of variation exhibited wide range for 14 characters. For all the traits under study, the phenotypic coefficient of variance (PCV) was found to be higher than genotypic coefficient of variance (GCV). In general, for all the traits studied, the phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV).

Table 4.1.4. Genetic variability for 14 quantitative traits

Traits	Range		Mean	GCV%	PCV%	Heritability%	GA	GAM%
	Min	Max						
PHT	48.28	119.45	67.94	12.85	15.73	66.72	14.71	21.66
PTL	1.18	3.13	2.34	15.04	17.88	70.69	0.61	26.08
DFF	63.34	86.65	77.98	5.96	6.65	80.39	8.60	11.03
50%F	70.81	93.66	84.99	4.81	6.00	64.28	6.76	7.96
80%F	87.64	110.24	101.85	3.74	4.88	58.79	6.02	5.92
80%M	112.35	147.47	133.65	4.50	5.21	74.89	10.75	8.04
PDL	3.69	4.14	3.84	5.84	9.89	34.82	0.29	7.11
PDW	0.82	1.22	0.99	6.98	8.22	72.19	0.12	12.24
NPB	5.2	7.68	6.73	1.89	3.69	26.32	0.13	2.00
NPP	43.98	87.08	69.5	4.11	9.60	18.37	2.53	3.64
NSP	2.85	5.01	4	6.95	9.72	51.18	0.41	10.26
SDL	3.73	8.24	6.44	9.97	12.48	63.76	1.06	16.42
SDW	3.48	7.45	5.52	7.64	9.72	61.90	0.68	12.41
HSWT	4.48	14.02	8.8	22.50	25.33	78.93	3.63	41.25

Note:

PHT - Plant height (cm)	80%F- Days to 80% flowering	SDL- Seed length (mm)
PTL - Petiole length(cm)	80%M- Days to 80% maturity	SDW- Seed width (mm)
DFF- Days to first flowering	NPB- Number of primary branches	HSWT- Hundred seed weight (g)
50%F- Days to 50% flowering	NPP- Number of pods per plant	

The range, mean, genetic variability and heritability for the traits are given in the Table 4.1.4. The highest plant height recorded was 119.45cm, days to 80% maturity ranges from 112.35 to 147.47 days, the highest number of pods per plant was 96.78 pods and the highest hundred seed weight recorded was 14.02g. The ranges of the observed PCV are from 26.06 to 4.65%; high PCV was recorded for hundred seed weight (25.33); moderate PCV observed for petiole length (17.88), plant height (15.73), seed length (12.48), number of seeds per pod (13.81), number of pods per plant (12.73), seed width (12.04); and for remaining all the traits like days to first flowering, days to 50% flowering, days to 80% flowering, days to 80% maturity, pod length, pod width, number of primary branches, number of seeds per pod, number of pods per plants and seed width it was observed low PCV. The high GCV was observed for hundred seed weight (22.50) and medium range of GCV was obtained for the trait's petiole length (15.04) and plant height (12.85) and for the remaining traits like days to first flowering, days to 50% flowering, days to 80% flowering, days to 80% maturity, pod length, pod width, number of primary branches, number of pods per plant, number of seeds per pod, seed length and seed width, low GCV were observed.

4.2.4. Heritability and Genetic advance

Heritability in broad sense and genetic advance as per cent of mean for 14 characters were estimated and the results are presented in table 4.1.4.

4.2.4.1. Heritability (%)

High heritability was observed for the traits HSWT (78.93%), SDL (63.76%), SDW (61.90%), PHT (66.72%), PTL (70.69%), DFF (80.39%), 50%F (84.28%), 80%M (74.89%) and PDW (72.19%).

4.2.4.2. Genetic advance as per cent of mean (%)

High GAM observed for the traits PHT (21.66%), PTL (26.08%), HSWT (41.25%); medium GAM was observed for the traits like DFF (11.03%), NSP (10.26%), SDL (16.42%) and SDW (12.41%) and low GAM was observed for the traits like 50%F, 80%F, 80%M, PDL, PDW, NPB and NPP.

4.3. Association studies

The study of association of yield with other component characters helps the breeder to select the most important characters and to construct a suitable plant type leading to higher yield. phenotypic

correlation was worked out for ten characters and the results are presented in table 4.1.5.

4.3.1. Correlation between hundred seed weight and other biometrical traits

The present study revealed that the HSWT showed significant positive correlation with the traits like PTL, SDL and SDW. The trait PHT showed significant negative correlation with SDL; PTL showed significant positive correlation with SDW; DFF, 50%F and 80%M showed positive significant correlation with each other; days to 80%M showed significant positive correlation with PDL and SDL; days to 80%F shows significant negative correlation with SDW; PDL shows significant positive correlation with SDL and significant negative correlation with NPB and SDW. PDW showed significant negative correlation with SDL; NPB showed significant negative correlation with NPP and NSP; NPP showed significant positive correlation with SDW. The traits like SDL and SDW showed significant positive correlation with each other. The correlation plot was given in the figure 4.1.4

Table 4.1.5. Correlation table for the 14 quantitative data

	PHT	PTL	DFF	50%F	80%F	80%M	PDL	PDW	NPB	NPP	NSP	SDL	SDW
PTL	0.05												
DFF	0.04	-0.09											
50%F	-0.05	-0.09	0.94**										
80%F	-0.05	-0.11	0.95**	1.00**									
80%M	-0.12	-0.11	0.86**	0.96**	0.95**								
PDL	-0.09	-0.10	0.05	0.13	0.14	0.20**							
PDW	0.00	0.09	0.20*	0.12	0.12	0.02	-0.03						
NPB	-0.05	0.05	-0.04	0.04	0.00	0.03	-0.21**	-0.05					
NPP	0.11	0.11	0.07	-0.01	0.00	-0.07	0.01	0.07	-0.21**				
NSP	0.06	-0.13	-0.01	-0.03	-0.02	0.01	-0.01	0.13	-0.21**	-0.03			
SDL	-0.20**	0.15	-0.04	0.14	0.13	0.24**	0.17*	-0.24**	0.09	-0.06	-0.06		
SDW	0.03	0.34**	-0.11	-0.15	-0.16*	-0.13	-0.16*	-0.09	-0.04	0.21**	0.00	0.41**	
HSWT	0.11	0.31**	0.09	0.08	0.08	0.09	0.05	0.00	-0.03	0.09	-0.12	0.28**	0.47**

Significance levels: p < .01 '**', p < .05 '*'

Note

PHT - Plant height (cm)

PTL - Petiole length(cm)

DFF- Days to first flowering

50%F- Days to 50% flowering

80%F- Days to 80% flowering

80%M- Days to 80% maturity

NPB- Number of primary branches

NPP- Number of pods per plant

SDL- Seed length (mm)

SDW- Seed width (mm)

HSWT- Hundred seed weight (g)

Table 4.1.6. Path Analysis for the 14 quantitative traits

	PHT	PTL	DFF	50%F	80%F	80%M	PDL	PDW	NPB	NPP	NSP	SDL	SDW	Correlations HSWT
PHT	0.154	0.011	-0.002	0.170	-0.167	-0.037	-0.006	0.000	-0.005	-0.004	-0.007	-0.008	0.012	0.110
PTL	0.008	0.210	0.003	0.311	-0.366	-0.036	-0.007	0.005	0.005	-0.004	0.015	0.006	0.161	0.312
DFF	0.007	-0.018	-0.038	-3.153	3.057	0.275	0.003	0.010	-0.004	-0.002	0.001	-0.001	-0.052	0.086
50%F	-0.008	-0.020	-0.036	-3.342	3.222	0.306	0.009	0.006	0.004	0.000	0.003	0.006	-0.071	0.080
80%F	-0.008	-0.024	-0.036	-3.334	3.229	0.304	0.010	0.006	0.000	0.000	0.002	0.005	-0.077	0.078
80%M	-0.018	-0.023	-0.032	-3.195	3.067	0.320	0.014	0.001	0.003	0.002	-0.001	0.010	-0.060	0.088
PDL	-0.013	-0.021	-0.002	-0.421	0.457	0.064	0.072	-0.001	-0.021	0.000	0.001	0.007	-0.074	0.049
PDW	0.000	0.020	-0.007	-0.387	0.392	0.007	-0.002	0.051	-0.005	-0.002	-0.015	-0.010	-0.042	-0.002
NPB	-0.008	0.011	0.002	-0.145	0.006	0.010	-0.015	-0.003	0.100	0.007	0.025	0.004	-0.018	-0.026
NPP	0.017	0.023	-0.003	0.030	-0.008	-0.024	0.001	0.003	-0.021	-0.033	0.003	-0.003	0.099	0.085
NSP	0.009	-0.028	0.000	0.093	-0.061	0.003	-0.001	0.007	-0.021	0.001	-0.116	-0.002	0.000	-0.118
SDL	-0.032	0.032	0.001	-0.468	0.416	0.078	0.012	-0.012	0.009	0.002	0.007	0.041	0.193	0.279
SDW	0.004	0.072	0.004	0.505	-0.534	-0.041	-0.011	-0.004	-0.004	-0.007	0.000	0.017	0.468	0.469

Residuals: 0.56

The last column is the correlations with the dependent variable.

Note:

PHT - Plant height (cm)

PTL - Petiole length(cm)

DFF- Days to first flowering

50%F- Days to 50% flowering

80%F- Days to 80% flowering

80%M- Days to 80% maturity

NPB- Number of primary branches

NPP- Number of pods per plant

SDL- Seed length (mm)

SDW- Seed width (mm)

HSWT- Hundred seed weight (g)

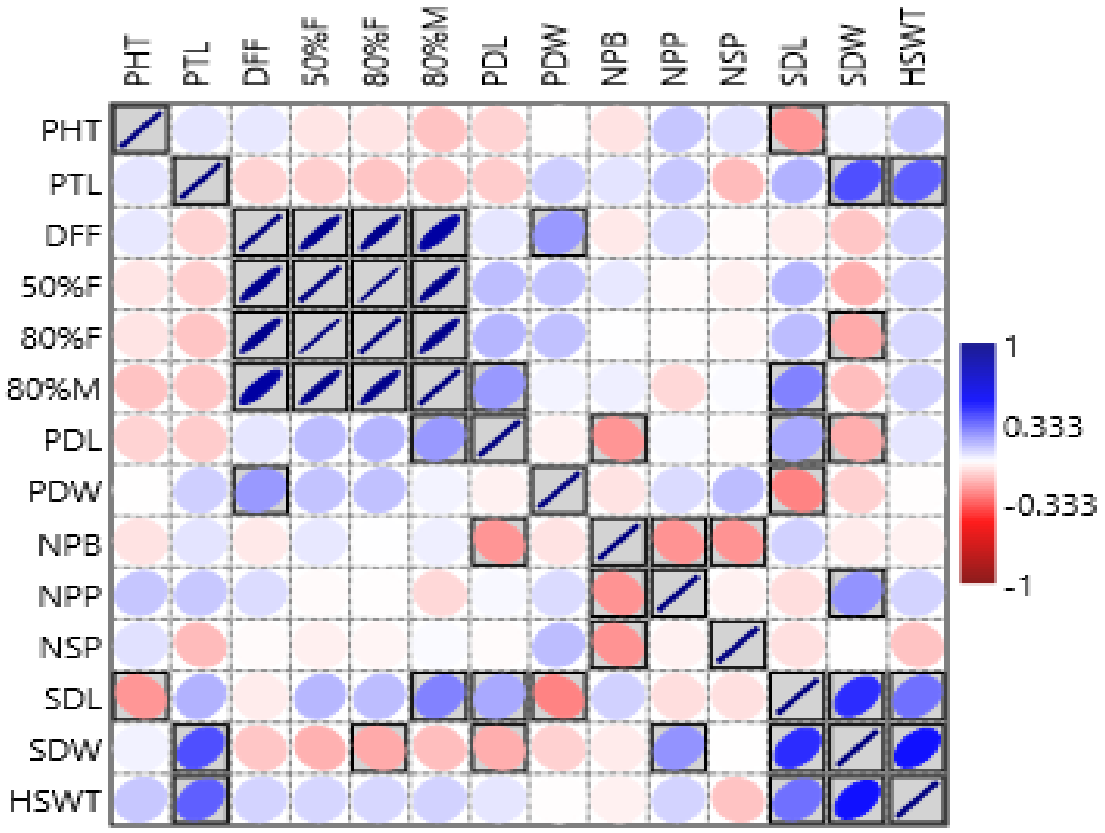

Figure 4.1.4. Correlation plot of 14 quantitative traits

Path coefficient was worked out based on the HSWT as a dependent variable and all the other 13 characters as independent variables and the residual effect was (0.56) presented in the table 4.1.6. The traits like 50%F, 80%F showed very high direct effects on the HSWT. SDW and 80%M showed high direct effects; PTL showed moderate direct effects and PHT, NPB and NSP showed low direct effects to the HSWT whereas remaining traits like DFF, PDL, PDW, NPP and SDL showed low direct effects; similar results were obtained by Tyagi et al. 2011; Singh et al. 2012 in lentil. Through 50%F, traits like DFF, 80%F and 80%M showed very high indirect effects and PTL, PDL, PDW, SDL and SDW showed high indirect effects; low indirect effect by NPB on HSWT. Through 80%F traits like DFF, 50%F, 80%M showed very high indirect effect on HSWT; traits like PTL, PDL, PDW, SDL and SDW showed high direct effects; PHT showed low indirect effect through 80%F on HSWT. Through 80%M, traits like 50%F, 80%F showed high indirect

effect; DFF showed moderated indirect effect on HSWT. through SDW, traits like PTL and SDL showed low indirect effect on HSWT. The wholesome direct and indirect effects of different traits are shown in the Figure 4.1.5.

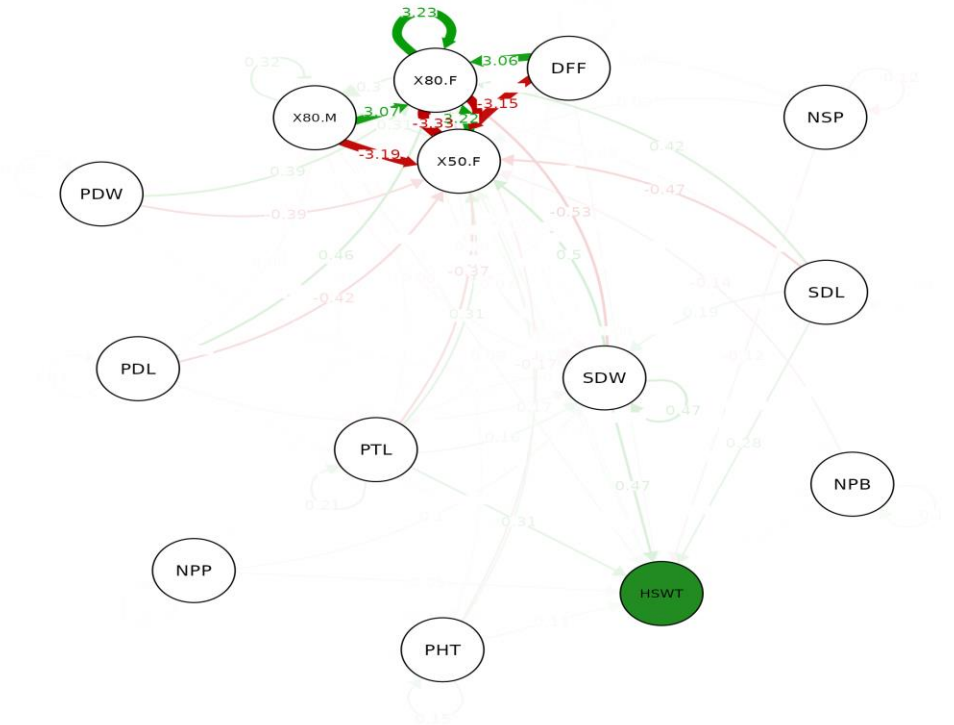

Figure 4.1.5. Simple path diagram of 13 quantitative traits with hundred seed weight

4.4. Genetic diversity studies

4.4.1. Principal component analysis (PCA)

Principal component analysis or canonical (vector) analysis is a sort of multivariate analysis where canonical vectors or roots representing different axes of such axes, respectively, are derived (Rao, 1952). It is called principal component analysis as it reflects the importance of the largest contributor to the total variation at each axis of differentiation. Differentiation among population results from gradual divergence accumulated over time under the influence of both natural and artificial evolutionary forces. But differentiation occurs in stages or in other words, in different axes of diversification which account for total divergence. Theoretically, as many as axes of differentiation can be envisaged as there are characters contributing to total variation. At each stage, different traits tend to contribute to divergence in varying degrees. Thus, for four

traits, there can be four axes, but it is not absolutely necessary. May be that most of the variation is accounted for by the first two axes of differentiation. The tool has a practical application in the selection of elite genotypes for breeding programmes.

The mean data of 14 quantitative characters of 168 germplasm were subjected to principal component analysis to identify the characters which contributes most towards the observed variation. Total variance, extraction of eigen factors, per cent of variation for four principal components of 168 germplasm were presented in the table 4.1.7 and the first five principal components (PC's) of scree plot on all the studied traits of 168 germplasm were depicted in table 4.1.8. figure 4.1.6. Only PCs with Eigen values which were more than one considered in determining the agro-morphological variability in the accessions.

Table 4.1.7. Principal component and extracted eigen value

Components	PC 1	PC 2	PC 3	PC 4	PC 5
Eigen Value	3.94	2.07	1.53	1.32	1.03
Proportion of variance (%)	28.14	14.81	10.91	9.42	7.35
Cumulative variance (%)	28.14	42.94	53.86	63.28	70.63

Table 4.1.8. Component matrix showing latent vectors associated with the first five principal components

	Components				
	1	**2**	**3**	**4**	**5**
50%F	0.99	0.04	0.01	0.07	-0.01
80%F	0.99	0.02	0.03	0.04	-0.01
80%M	0.97	0.07	-0.11	-0.04	0.03
DFF	0.94	-0.01	0.20	0.13	-0.03
NPB	0.00	0.06	-0.50	0.65	0.04

NPP	-0.03	0.21	0.57	-0.14	-0.34
NSP	-0.01	-0.23	0.30	-0.35	0.63
PDL	0.20	-0.02	-0.12	-0.68	-0.25
PDW	0.14	-0.17	0.50	0.21	0.46
PHT	-0.08	0.00	0.50	0.21	-0.41
PTL	-0.14	0.59	0.20	0.28	0.12
SDL	0.14	0.65	-0.45	-0.30	0.16
SDW	-0.19	0.80	0.15	-0.04	0.18
HSWT	0.08	0.74	0.17	0.00	-0.04

Note

PHT - Plant height (cm)	80%F- Days to 80% flowering	SDL- Seed length (mm)
PTL - Petiole length(cm)	80%M- Days to 80% maturity	SDW- Seed width (mm)
DFF- Days to first flowering	NPB- Number of primary branches	HSWT- Hundred seed weight (g)
50%F- Days to 50% flowering	NPP- Number of pods per plant	

The PCA revealed that the first five Principal components had accounted eigen value of more one. The variability on the first principal component (28.14 %) was accounted by high positive loading for 50%F (0.99), 80%F (0.99), 80%M (0.97) and DFF (0.94)

The variability on the second PC (14.81 %) was accounted by positive loading for SDW (0.80), HSWT (0.74), SDL (0.65) and PTL (0.59). The variability on the third PC (10.91 %) was accounted by NPP (0.57), PDW (0.50), PHT (0.50) and NSP (030) in positive loading. The variability on the fourth PC (9.42 %) was accounted by NPB (0.65). The variability on the fifth PC (7.35%) was accounted by NSP (0.63) and PDW (0.46) and these where the traits in different axes contribute more to the diversity. The value of five different PC's and its rotational view of PC components are given figure 4.1.7.

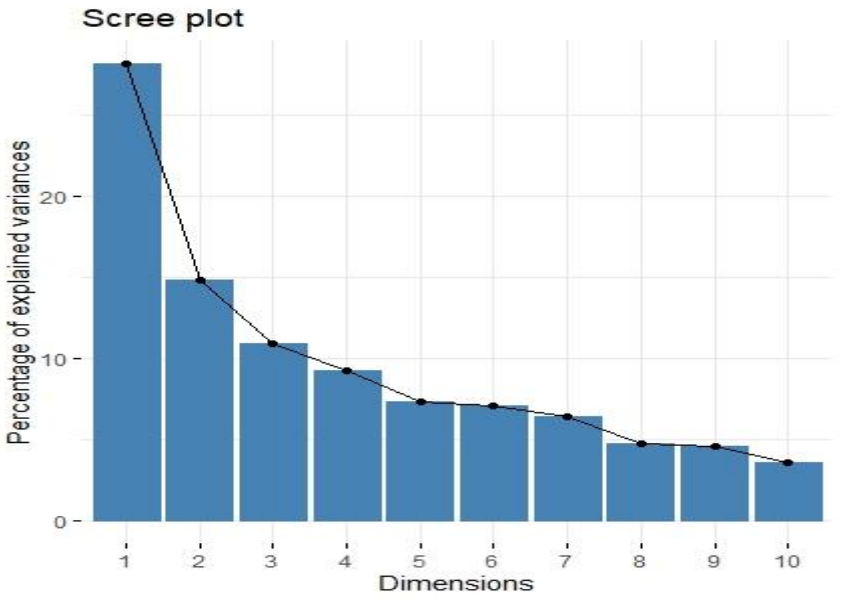

Figure 4.1.6. Scree plot of PCA for 14 quantitative traits

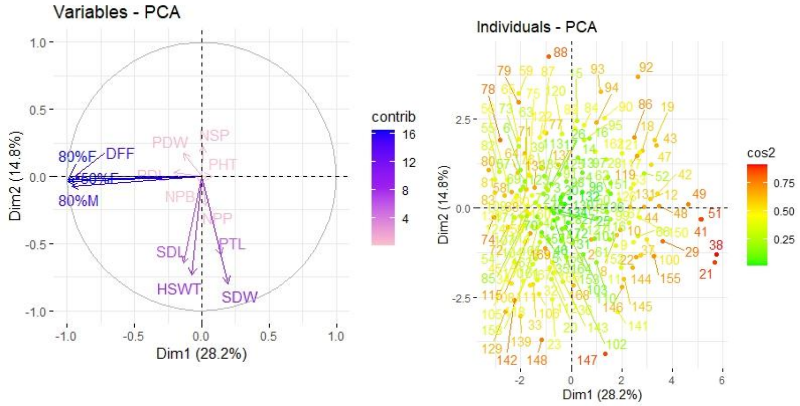

Figure 4.1.7. PCA plot showing different axes

Cluster analysis

The cluster analysis based on both quantitative traits (adjusted means) and qualitative traits (transformed values used) were divided all the 168 accessions of grasspea into two major clusters. At the distance of 35, all accessions were grouped into four clusters viz., cluster I to cluster IV (Figure 4.1.8). The mean values of all the ten quantitative traits for each cluster were given in the Table 4.1.9.

Table 4.1.9. Number of genotypes and average values of four clusters for 14 different quantitative traits

Clusters	Count	PHT	PTL	DFF	50%F	80%F	80%M	PDL	PDW	NPB	NPP	NSP	SDL	SDW	HSWT
I	21	70.08	2.60	75.26	82.86	99.67	132.47	3.87	0.97	6.44	74.10	3.95	7.26	6.12	10.62
II	57	69.67	2.28	74.62	80.87	97.99	127.12	3.83	1.00	6.64	69.11	4.07	5.86	5.37	8.39
III	61	65.77	2.30	81.06	88.89	105.61	139.30	3.84	0.99	6.82	68.22	4.00	6.69	5.35	8.66
IV	29	67.04	2.35	80.01	86.25	102.90	135.25	3.81	0.98	6.92	69.89	3.91	6.46	5.73	8.62

Note

PHT - Plant height (cm)
PTL - Petiole length(cm)
DFF- Days to first flowering
50%F- Days to 50% flowering
80%F- Days to 80% flowering
80%M- Days to 80% maturity
NPB- Number of primary branches
NPP- Number of pods per plant
SDL- Seed length (mm)
SDW- Seed width (mm)
HSWT- Hundred seed weight (g)

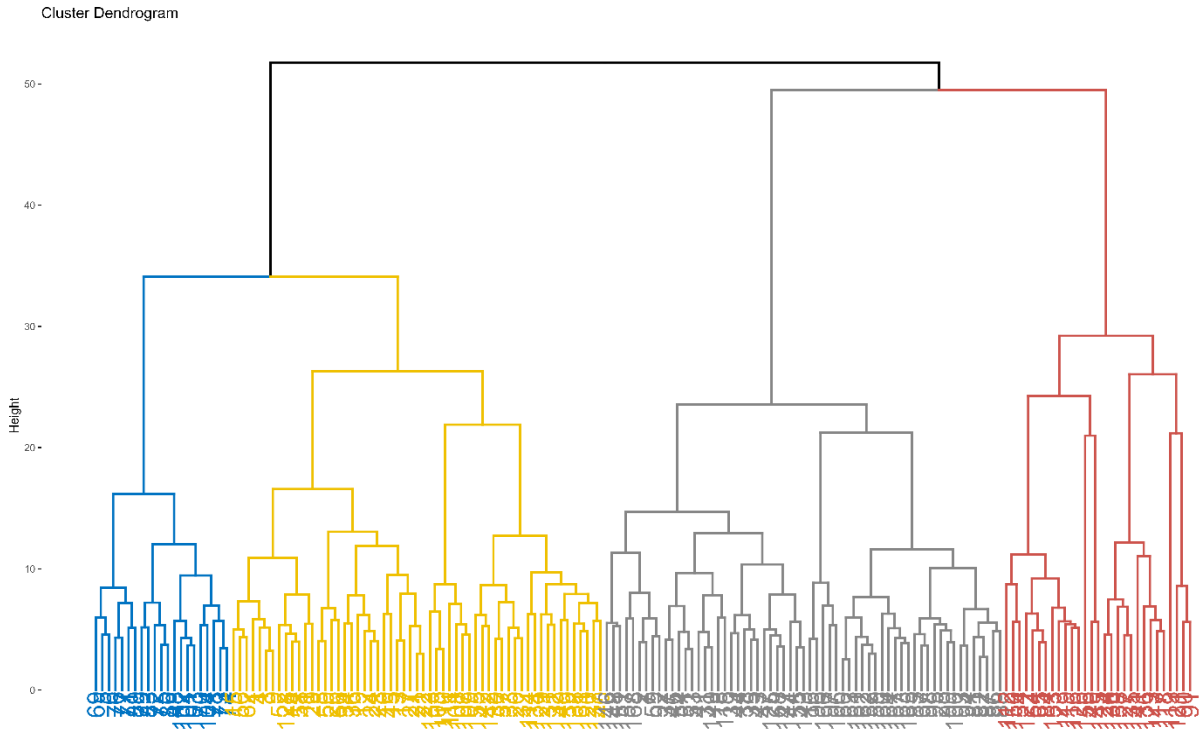

Figure 4.1.8. Cluster dendrogram of 168 accessions using 34 traits

Among all the clusters, cluster I was the smallest cluster comprises 21 genotypes, and distinguished by accessions (average values of all accessions in the cluster) having higher plant height (70.08cm), petiole length (2.60cm), pod length (3.87cm), number of pods per plant (74.10), seed length (7.26mm) and hundred seed weight (10.62g) with other traits in moderate values. Cluster II was the second largest one with distinguished by accessions (average values of all accessions in the cluster) having early flowering genotypes (74.62 days), early maturing genotypes with 80%M (127.12 days), higher pod width (1.00cm) and higher number of seeds per pod (4.07 seeds). 61 accessions makeup Cluster III, which was the first largest cluster distinguished by the accessions (average values of all accessions in the cluster) having late flowering (81.06 days), late maturing (139.30 days) genotypes with second higher values of pod length (3.84cm), pod width (0.99cm), number of primary branches (6.82), number of seed per pod (4.00), seed length (6.69mm) and hundred seed weight (8.66g). Cluster IV contains 29 genotypes which was characterized by accessions (average values of all accessions in the cluster) having higher number of primary branches (6.92), second higher seed width (5.73mm) and lower values of pod length (3.81cm), pod width (0.98mm), number of seeds per plant (3.91) and lowest hundred seed weight (8.62g) among all the clusters obtained.

4.2. Molecular characterization and genetic diversity by SSR markers

The result on the 20 polymorphic primers were presented and described here to explain the genetic diversity in 168 grasspea genotypes. Molecular profile of 168 grasspea accessions revealed by SSR primer LTR70 is depicted in Figure 4.2.1 and the accessions details were mentioned in the Table 3.1. The Polymorphic Information Content (PIC) values for each 20 selected polymorphic primers were given in the Table 4.2.1.

Table 4.2.1. List of SSR primers used for genotyping of grasspea and its PIC

S.no.	Marker	Sequence F- forward, R- reverse	PIC
1	LTR1	F-AAGGAGCAGCAGCATTTGTT R-TAATAATGGGGAGCCGATCA	0.55
2	LTR2	F- CACAACCAGTTGCATCAGTG R- TGGCTCACATGATGGTTTGT	0.45
3	LTR4	F- CAACCAGAGCAACCACAAGA R- GGTTGCAAGAGGTTGCAGAT	0.46

4	LTR5	F-CAGGTCCGGCTTATCTCTCA R- TTGGTTTCAACCCACTCCTC	0.50
5	LTR8F	F-ACGCACACACGGAAGAAAG R- GTGTGCGCATGTGTGTATGA	0.43
6	LTR11	F- CACCCTCTTCACTGCCTAGC R- TTGGGGGTTGTAGAAGGAAC	0.14
7	LTR12	F-GCACACAAGGGCACACTG R- TGCGTCGTGTGTATGTGTTG	0.67
8	LTR28	F- GGGCAGTGGACCAGTTAGAG R- CCGAGGGAATAAACGACAAA	0.30
9	LTR30	F- CAATCCGAAAATCACCACCT R- GCACTCACATGCACACAAAC	0.30
10	LTR31	F- CCTTTCGGAGCAATCAAGAC R- TGCCTAAGCATTGGCTTTCT	0.50
11	LTR33	F- CAACCAGAGCAACCACAAGA R- GGTTGCAAGAGGTTGCAGAT	0.50
12	LTR35	F- TCAAGCCCAAAGTGAGATGA R- TTTTGTGTTGCTTGCTGACC	0.36
13	LTR37	F- ATCTTACCGGGGATCCATTC R- CTTCCCCATTCTCTGGTGTT	0.18
14	LTR58	F- AAACTGGCCCTGCATTTTC R- GGTCATGGCAATTTGAGACA	0.32
15	LTR62	F- CGTTGGTTGTTAGTCGGTCA R- GAACGAAACAACGACGACAA	0.02
16	LTR70	F- TTTGTGCGGTTGATGTTGTT R- CTACGTCAGCCCGTCATACC	0.27
17	LTR32	F- ACAGCAAGAAGCAGCAACAG R- AGTTGGTTGTTGTGTCGTTGT	0.19
18	LTR68	F- TTTTTGGTATTGTTGTTGTCGT R- CTGCAGCAATAACAGCATCAG	0.18
19	LTR41	F- CCCTTACCGAGTGCAGAAAA R- CACCACGACTTGCTCACCTA	0.28
20	LTR40	F- GGGCACACATTCTCACACAC R- TGTCGTCGTGTCGTAGTCGT	0.21

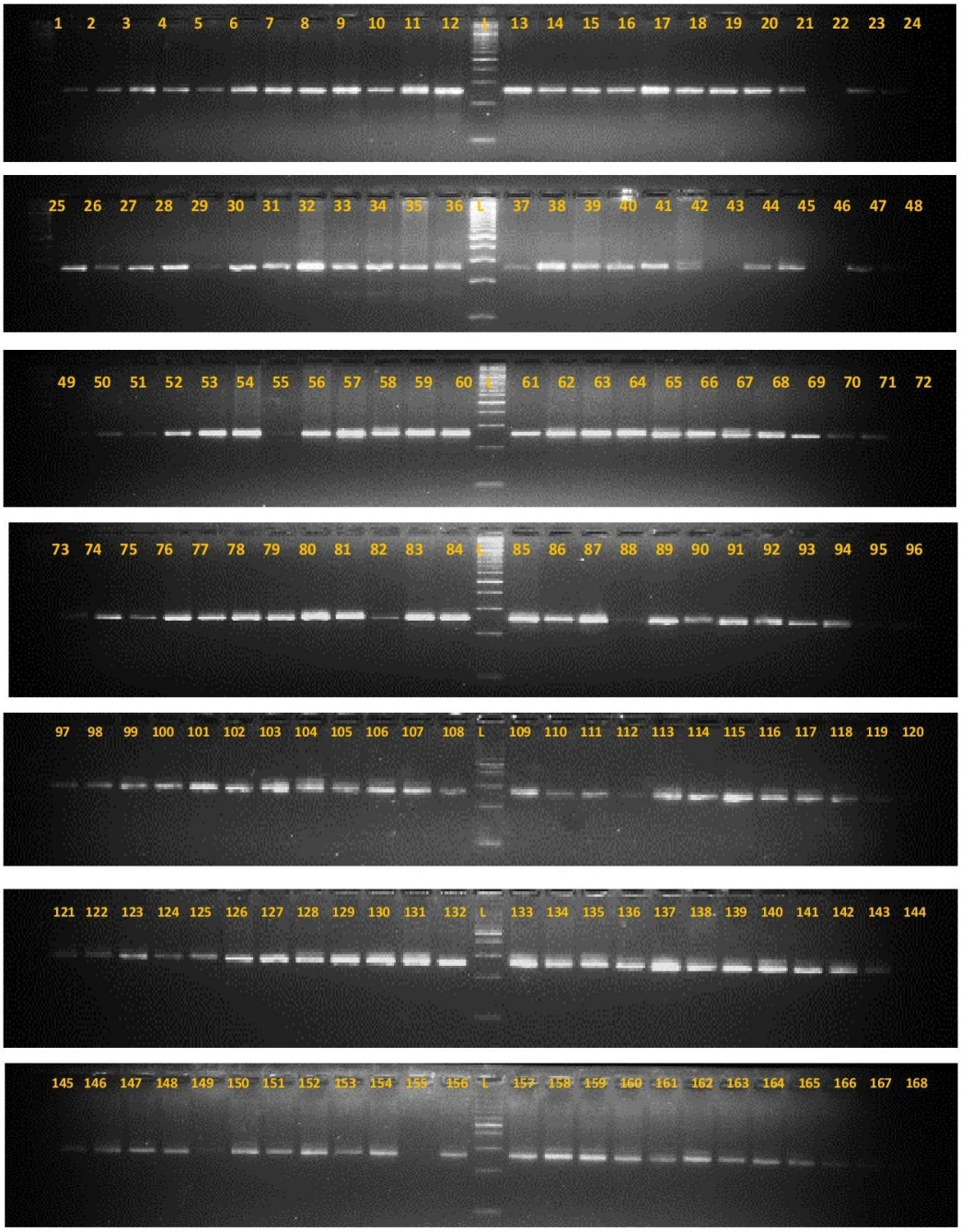

Figure 4.2.1. Profile of 168 accessions revealed by primer LTR70. L- Ladder (100bp);1-168- grasspea accessions

Polymorphic information content (PIC) value was ranged from 0.02 to 0.67 with an average of 0.34. The maximum PIC was observed for primer LTR12 (0.67) and the minimum for LTR62 (0.02). The PIC value of primers indicated greater diversity within and between studied grasspea

accessions and also revealed that selected primers are very informative and can be utilized in the further studies.

4.2.3. Population structure

Population structure studies helps to understand the genetic kinship and forms the basis for successful genetic relationship studies. To analyze the 168 accessions of grasspea, a model- based population structure analysis was employed. To reach convergence, 100,000 iterations of K values from 2 to 10 were tried. The (delta) LnPD values were plotted against the corresponding K values in order to determine the ideal K (Figure 4.2.2). Since K=4 was the observed peak value, all further interpretations were made after K=4. 100 genotypes of grasspea were divided into three genetically diverse sub-populations (K = 4) based on the model- based clustering, which is consistent with the results as shown in the dendrogram (Figure 4.2.4).

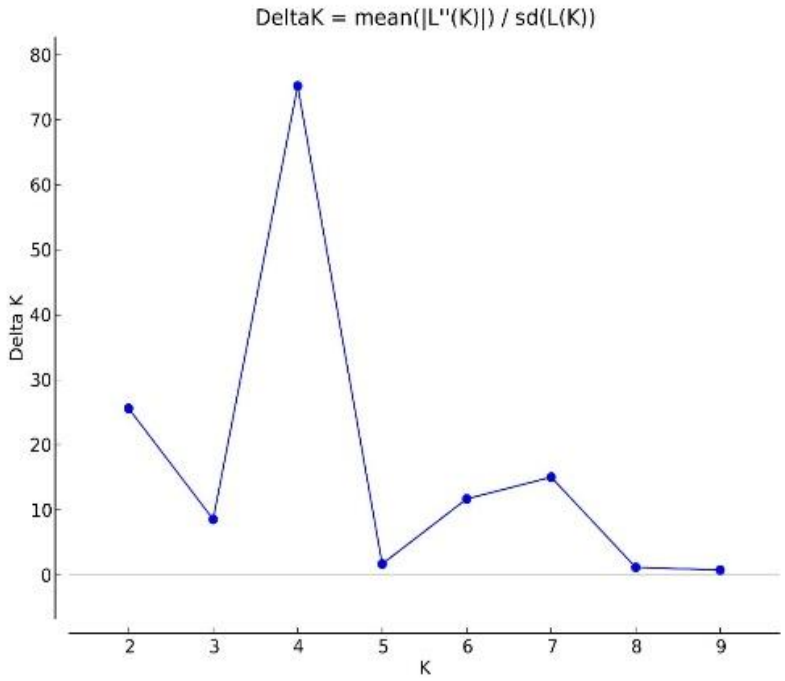

Figure 4.2.2. Population estimation by using LnP (D) derived delta k for k from 2 to 10

The 100 accessions got distributed into four populations. Population 1 consists of 41 admix and 11 pure individuals; population 2 consists 37 admix and 23 pure; and population 3 consists 16 admix and 17 pure individuals and population four consists of 14 admix and 9 pure individuals (Table 4.2.2 and Figure.4.2.3).

Table 4.2.2. Distribution pattern of accessions across genetic populations identified by population genetic structure analysis

Population	Pure individuals	Admixture	Total
P1	11	41	52
P2	23	37	60
P3	17	16	33
P4	9	14	23

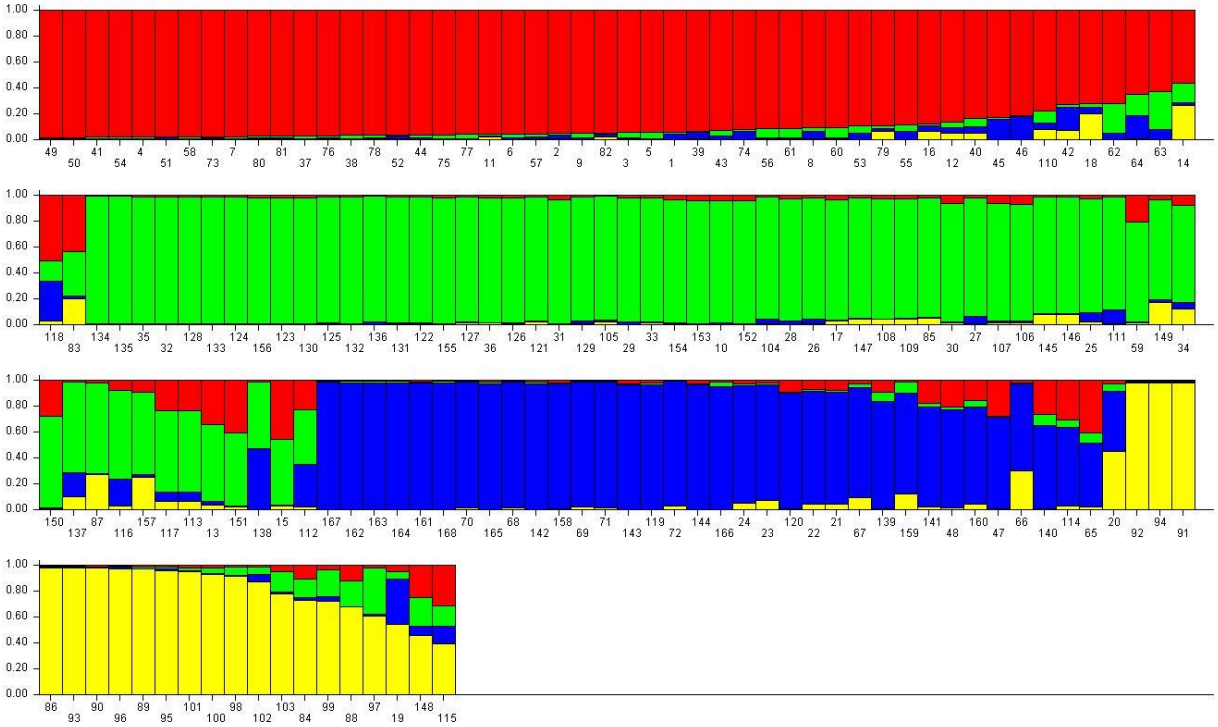

Figure 4.2.3. Model-based clustering of 168 accessions of grasspea detected based on 20 SSR markers. An accession is represented by each individual bar. The various color bars (S1–S4, respectively) reflect various genetic groups, while the combination of colors in a bar indicates admixture.

By F-statistics mean Fst as fixation indices (Fst) measures the amount of differentiation among sub-populations derived from the subdivision of an original population (Wright 1978). The values for Fst range from 0 for non-differentiation to 1 for complete differentiation between an original population and its subpopulation, respectively. The range from 0 to 0.05 may be considered as indicating little genetic differentiation, the range from 0.05 to 0.15, 0.15 to 0.25and values of Fst above 0.25 indicate moderate, great, and very great genetic differentiation, respectively. In this study, mean Fst value was 0.122 indicting moderate genetic diversity present in the accessions (Table 4.2.3).

Table 4.2.3. Fst, Fis and Fit values for 168 accessions of grasspea evaluated by 20 polymorphic primers

F-Statistics	Value	P(rand >= data)
Fst	0.122	0.001
Fis	0.654	0.001
Fit	0.781	0.001

4.2.4. Analysis of molecular variance (AMOVA) of grasspea

AMOVA of 168 genotypes was executed to analyze the genetic diversity distribution within and between the populations of grasspea. The analysis revealed maximum diversity within individuals was 48%, diversity among individuals was 40% and diversity among populations was 12%. Variation within individuals was highest (1.419), among individual was (1.189) and lowest among populations (0.364) (Table 4.2.4).

Table 4.2.4. Analysis of molecular variance (AMOVA)

Source	Df	SS	MS	Est. Var.	%
Among Populations	3	54.75	27.37	0.364	12
Among Individuals	164	364.3	3.796	1.189	40
Within Individuals	168	140.5	1.419	1.419	48
Total	335	559.7		2.972	100

4.2.2. Hierarchical cluster analysis

In the present experiment, the DNA polymorphism revealed by 20 primers was used for studying the clustering pattern of grasspea accessions. All the 168 accessions were grouped into four (Figure 4.2.4).

Cluster I is the second largest and is composed of two subclusters, having 55 genotypes. Cluster II is the largest cluster and again subclustered into two clusters, comprising total of 87 genotypes. Cluster III again subclustered as two cluster and each subcluster has eight and 10 genotypes accounting for total of 18 genotypes in this cluster. Cluster IV is the smallest cluster having eight genotypes into it.

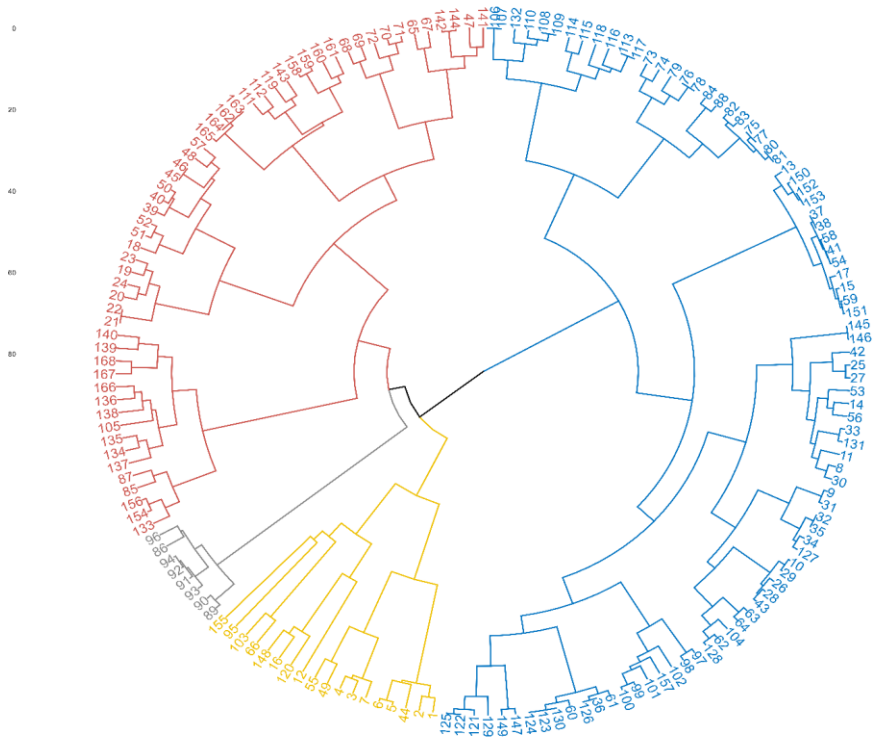

Figure 4.2.4. Dendrogram depicting genetic diversity and relationship between 168 accessions of grasspea as revealed by polymorphism of 20 SSR

4.2.5. Principal Coordinate Analysis (PCoA)

To describe the genetic variance using multidimensional association, a principal coordinate analysis (PCoA) was carried out. The first three PCoA axes explained 13.85%, 9.20%, 8.83%of variation, respectively. The PCoA also grouped 168 accessions of grasspea into four clusters, which compliments the results of cluster analysis as depicted in Figure 4.2.2. The results of PCoA disclosed high genetic variability exists in grasspea genotypes (Table 4.2.5 and Figure 4.2.5)

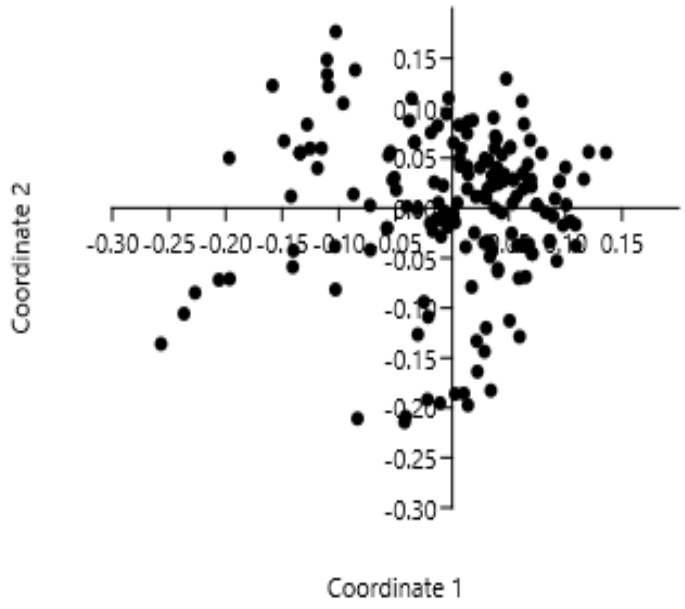

Figure 4.2.5. Principal Coordinate Analysis (PCoA) of 168 accessions of grasspea

Table 4.2.5. Percentage of variation in the first 3 axes by 20 SSR markers

Axis	% Variance	Cumulative Variance
1	14.15	13.85
2	9.42	9.20
3	9.05	8.83

4.3. Biochemical characterization of Grasspea

A total of 168 accessions along with four checks (total 172), both seed and leaf powder were scanned using the FOSS NIRS 6500, and the reflectance spectra from 400 to 2400 nm was recorded. Ward's method was used to do k means clustering (k=20) on the normalized spectral data of 172 samples using a squared Euclidean distance of 5. Major clusters and sub-clusters were identified and separated in the same manner. To account for all possible variability in the data set, 40 accessions each of seed and leaf with a wide range of characteristics were chosen. These accessions were subjected to wet chemistry experiments. The flour that was produced after these materials were homogenised, ground, and sieved through a 1 mm sieve in the FOSS cyclotec was utilised for NIRS scanning and analysing protein, starch, sugar, TDF, phenols, amino acids, mineral estimation and β-ODAP estimation. Figure 4.3.1 and 4.3.2 represents the dendrogram based on the spectra using unscrambler software of seed and leaf respectively.

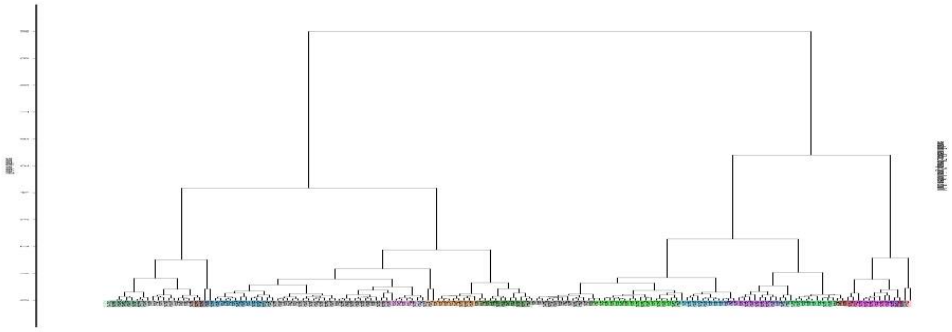

Figure 4.3.1. Dendrogram from spectra of 168 grasspea seeds

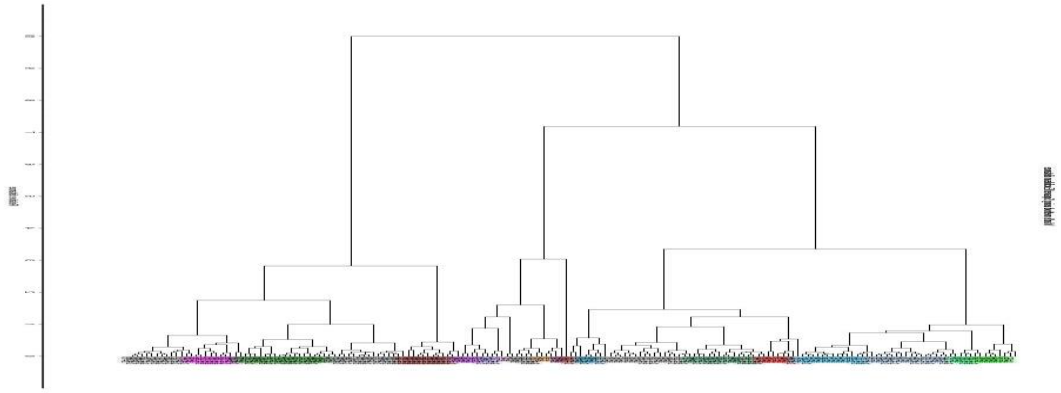

Figure 4.3.1. Dendrogram from spectra of 168 grasspea leaf

The list of accessions chosen from each cluster (two from each cluster making a total of 40 accessions from 20 cluster with four checks Ratan (C1), Prateek (C2), Mahateora (C3) and Narayangon (C4) of seed and leaf are given in the table 4.3.1

Table 4.3.1. List of selected accessions used for the wet lab analysis

S.no	Accessions	
	Seed	**Leaf**
1	IFLA3001	IFLA1193
2	IC421914	IC470982
3	IC0635672	IC208430
4	BANG-291	IC0634674
5	IFLA2765	IC567350
6	BANG-208	IFLA2475
7	BANG-292	IC0634659
8	IFLA2924	BANG285
9	Bio520	IC0634662
10	BANG-218	BANG184
11	BANG-227	IFLA1439
12	IFLA151	IFLA128
13	IFLA1870	IC0634754
14	BANG-31	IC148352
15	SeI190	BANG206
16	IC0634654	IFLA 143
17	BANG-267	IC574469

18	IFLA276	BANG31
19	IC0634754	BANG224
20	IC208434	IFLA2026
21	BANG-310	BANG33
22	BANG-206	IFLA2750
23	BANG-227	BANG22
24	IFLA2736	IC0634654
25	IFLA2129	BANG26
26	IC148364	IC0634666
27	BK-30-1	IFLA2765
28	IFLA1864	IC208437
29	BK-31-1-1	IC574468
30	BANG-270	IC349809
31	IFLA2750	BANG37
32	BANG-31	BANG208
33	IFLA2998	BANG227
34	IFLA 143	BANG198
35	BANG-285	IFLA118
36	IC0634662	IFLA276
37	IFLA1864	IFLA1715
38	IFLA1193	BANG27
39	BANG-224	IC18879
40	IC208430	IFLA479

4.3.1. Descriptive statistics (Seed): The data on the biochemical compounds viz., mean, range (minimum and maximum), standard error (SE), coefficient of variation and standard deviation were calculated and presented in the Table 4.3.2 (Seed) and the mean values of biochemical parameters in Table 4.3.3.

Table 4.3.2. Descriptive statistics of proximate compounds of 40 accessions grasspea (seed)

	Protein	Starch	Sugar	Phenol	TDF	Moisture	Ash	Fe	Cu	Zn	Ca	Mg
Min	25.23	26.2	3.87	0.39	6.75	6.34	1.76	64.92	6.45	44.01	230.5	1162.83
Max	32.02	39	8.83	1.19	19.91	11.5	3.81	164.4	12.97	101.86	557.7	1677.59
Mean	27.59	32.9	6.48	0.7	15.1	8.54	2.58	101.1	9.26	67.82	377.4	1405.87
SE	0.22	0.56	0.21	0.03	0.55	0.23	0.08	3.55	0.25	1.76	11.76	19.23
SD	1.39	3.54	1.31	0.19	3.49	1.44	0.49	22.46	1.61	11.11	74.41	121.64

Note : Protein, starch, sugar, phenol are expressed in g/100g on dry weight basis; While Fe, Cu, Zn and Ca are expressed in ppm (parts per million) and TDF, Moisture and Ash in %

Table 4.3.3. Mean values of proximate compounds of 40 accessions grasspea (seed)

S.no	Protein	Starch	Sugar	Phenol	TDF	Moisture	Ash	Cu	Fe	Zn	Ca	Mg
1	25.84	28.1	5.41	0.55	6.75	6.49	2.82	105.1	10.06	64.33	463.31	1634.07
2	28.76	38.86	3.87	0.93	6.75	9.61	2.63	140.79	8.18	65.5	402.81	1541.74
3	29.73	35.35	4.56	0.88	8.98	7.48	2.05	109.79	8.89	66.29	408.17	1437.1
4	28.42	33.9	4.78	0.7	9.08	6.34	3.13	106.2	12.97	53.81	324.24	1411.87
5	26.61	38.85	6.04	0.89	10.51	6.69	3.15	154.19	11.36	71.99	376.32	1455.94
6	27.08	33.28	4.07	1.02	10.66	7.1	3.81	112.73	11.61	69.83	557.7	1545.65
7	26.83	35.65	5.1	0.39	11.8	7.48	3.15	103.38	8.97	44.01	386.57	1445

8	27.03	38.79	6.84	0.63	11.83	7.36	1.94	97.81	6.45	67.13	377.02	1335.46
9	28.28	33.54	8.46	0.73	12.98	9.42	2.7	109.38	11.61	101.86	237.55	1491.75
10	27.82	36.55	8.12	0.91	13.34	10.25	3.17	64.92	8.7	72	413.23	1677.59
11	28.01	30.85	8.62	0.62	13.56	11.52	2.34	104.14	12.23	68.65	457.51	1439.34
12	29.66	33.34	5.34	0.81	13.57	9.07	2.69	101.52	9.86	72.66	391.63	1469.5
13	27.45	32.75	8.22	1.19	13.95	10.28	2.54	83.41	11.04	87.33	390.46	1396.28
14	32.02	36.6	8.1	1.09	13.98	8.44	2.52	106.32	9.14	64.77	409.24	1182.96
15	25.23	36.74	6.34	0.82	14	9.3	2.55	103.58	9.05	88.47	249.87	1452.03
16	28.85	39	7.02	0.56	14.13	9.39	2.34	115.69	8.68	80.83	386.15	1470.13
17	26.36	32.92	7.82	0.58	14.81	8.17	3.18	93.69	9.59	71.74	387.69	1374.21
18	25.65	32.66	5.62	0.64	14.9	8.77	2.01	74.24	8.43	57.71	407.74	1381.82
19	29.37	35.9	6.58	0.64	15.07	7.16	2.75	79.55	7.36	62.23	298.65	1223.12
20	26.66	33.39	7.37	0.69	15.09	8.4	2.34	73.36	9.01	53.41	265.94	1434.8
21	26.43	36.87	6.29	1.04	15.83	7.39	2.14	79.46	10.65	63.6	492.46	1488.03
22	26.95	30.75	5.36	0.58	16.6	11.12	2.73	164.35	9.95	68.37	285.69	1458.91
23	25.45	30.69	5.5	0.6	16.7	8.47	1.76	88.03	11.31	70.4	384.06	1554.1
24	28.4	26.42	6.85	0.52	16.72	6.87	2.7	113.35	11.61	77.16	389.49	1484.01
25	27.35	28.69	6.4	0.64	16.73	6.67	1.89	116.12	9.55	55.41	386.76	1315.66
26	28.05	28.66	8.64	0.74	17.05	7.87	2.05	140.34	8.98	72.09	445.54	1302.94
27	26.53	31.05	8.05	0.62	17.22	7.83	2.32	99.81	8.39	60.09	249.82	1209.44
28	26.6	32.32	6.33	0.69	17.63	7.06	2.29	78.54	9.22	65.27	432.62	1544.67
29	27.12	31.8	7.04	0.53	17.67	8.23	2.28	95.63	9.74	81.33	420.45	1404.53
30	28.05	34.06	6.98	0.86	17.99	10.56	3.05	86.38	8.49	72.47	413.07	1413.42

31	27.24	37.06	6.92	0.55	18	11.27	2.42	103.14	7.03	46.19	295.86	1229.16
32	29.6	32.25	5.12	0.53	18.13	9.2	2.28	83.06	7.64	54.91	286.54	1248.7
33	26.19	32.12	4.86	0.55	18.25	10.12	3.05	110.14	6.75	66.17	316.17	1402.88
34	28.52	27.33	5.28	0.55	18.51	11.29	3.35	82.75	8.72	74.88	466.36	1348.33
35	29.06	26.16	5.81	0.58	18.62	8.88	3.43	83.2	6.55	57.03	230.5	1162.83
36	27.8	28.98	6.33	0.79	18.74	8.41	2.58	136.53	7.99	71.11	426.33	1527.86
37	28.54	30.05	6.53	0.57	18.83	7.94	2.06	82.28	10.61	75.26	430.83	1408.23
38	30.25	32.97	6.4	0.74	19.52	7.52	2.35	69.59	7.5	64.59	404.15	1225.7
39	26.52	33.1	7.23	0.4	19.68	7.6	2.94	98.4	7.99	69.87	330.41	1271.58
40	29.89	28.04	8.83	0.61	19.91	8.45	1.89	93.14	8.39	62.16	416.63	1433.52
C1	27.62	32.02	5.33	0.76	**13.21**	7.41	2.26	**103.38**	8.14	**87.86**	182.31	**1445.12**
C2	26.31	30.8	5.04	0.45	11.25	7.94	3.11	89.36	**8.96**	56.32	201.56	1243.41
C3	25.45	29.06	5.98	0.63	15.51	7.22	3.01	111.1	10.32	74.13	**378.36**	1341.02
C4	26.01	28.21	6.02	0.57	12.62	6.25	2.85	97.65	9.11	67.24	232.78	1069.55

Note : Protein, starch, sugar, phenol are expressed in g/100g on dry weight basis; While Fe, Cu, Zn and Ca are expressed in ppm (parts per million) and TDF, Moisture and Ash in %

4.3.1.1. Total protein content: The range of variations in protein content exhibited by accessions was ranged between 25.23 % (Sel190) to 32.02 % (BANG31), with a mean value of 27.59 %, and best performing check Ratan 27.62 % (Table 4.3.3).

4.3.1.2. Total starch content: Grasspea genotypes exhibited significant variations among the accessions in starch content ranging from 26.16 (BANG285) to 39.00 g/100g (IC0634654), mean value of 32.91 g/100g, with value of best performing checks Ratan 32.02/100g (Table 4.3.3).

4.3.1.3. Total soluble sugar (TSS): The TSS content of grasspea genotypes ranged from 3.87 g/100 g (IC421914) to 8.83 g/100 g (IC208430) with a mean value of 6.48 g 100g−1, which is higher than the value of best performing check Narayangon 6.02 g/100g.

4.3.1.4. Phenol content: The total phenolic content of grasspea genotypes varied between 0.39 g/100g (BANG292) to 1.19 g/100g (IFLA1870) with an average value of 0.70 g/100g (Table 4.3.3). The best performing check Prateek has lower phenol content 0.45 g/100g than the genotypes.

4.3.1.5. Total Dietary fibre (TDF): The TDF content of grasspea genotypes ranged from 6.75 % (IFLA3001, IC421914) to 19.91% % (IC208430) with a mean value of 15.10 % (Table 4.3.3). The best performing check having higher TDF was ratan having 13.21%.

4.3.1.6. Moisture content: The moisture content of grasspea genotypes ranged from 6.34 % (BANG291) to 11.52 % (BANG227) with a mean value of 8.54 % (Table 4.3.3). The best performing check Narayangon has lower moisture content 6.25% than the genotypes.

4.3.1.7. Ash content: The ash content of grasspea genotypes ranged from 1.76 % (BANG227) to 3.81% (BANG208), mean value of 2.58 % (Table 4.3.3), with best performing Narayangon (3.11%).

4.3.1.8. Fe content: The Fe content of grasspea genotypes ranged from 64.92 (BANG218) to 164.35ppm (BANG206) with a mean value of 101.10ppm (Table 4.3.3). The best performing check is Ratan 103.38ppm

4.3.1.9. Cu content: The Cu content of grasspea genotypes ranged from 6.45 (IFLA2924) to 12.97ppm (BANG291) with a mean value of 9.26 ppm (Table 4.3.3). The best performing check is mahateora 10.32ppm

4.3.1.10. Zn content: The Zn content of grasspea genotypes ranged from 44.01 (BANG292) to 101.86ppm (Bio520) with a mean value of 67.82 (Table 4.3.3). The best performing check is ratan 87.86 ppm

4.3.1.11. Ca content: The Ca content of grasspea genotypes ranged from 230.50 (BANG285) to 557.70ppm (BANG208) with a mean value of 377.39 (Table 4.3.3). The best performing check is mahateora 378.36 ppm

4.3.1.12. Mg content: The Mg content of grasspea genotypes ranged from 1162.83 (BANG285) to 1677.59ppm (BANG218) with a mean value of 1405.87 (Table 4.3.3). The best performing check is ratan 1445.12 ppm

4.3.1.13. Aminoacid profiling of grasspea seeds

Amino acid profiling has been done for selected grasspea accessions using UPLC. 23 total amino acids have been identified in the studies which includes three non-protein aminoacids. The mean, range (minimum and maximum), standard error (SE), coefficient of variation and standard deviation were calculated and presented in the table 4.3.4. The standard calibration curve of aminoacid and the sample chromatogram was given in the figure 4.3.2 and figure 4.3.3. In the aminoacid profiling of seeds, sarcosine accounts for highest (11.64 g/100g protein) followed by cysteine (8.91 g/100g protein), glutamic acid (6.79 g/100g protein) and valine accounts for the lowest (0.23 g/100g protein)

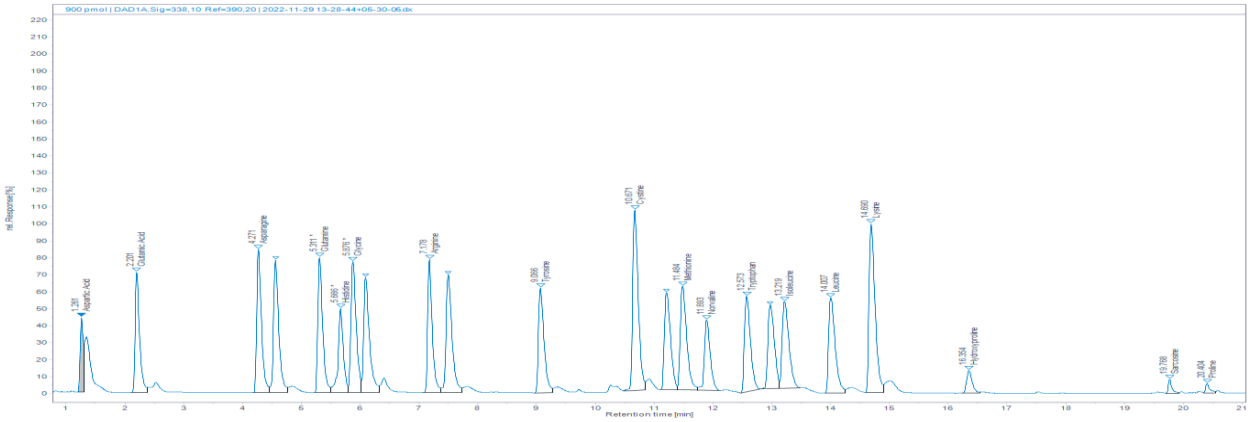

Figure 4.3.2. Standard calibration curve of aminoacid

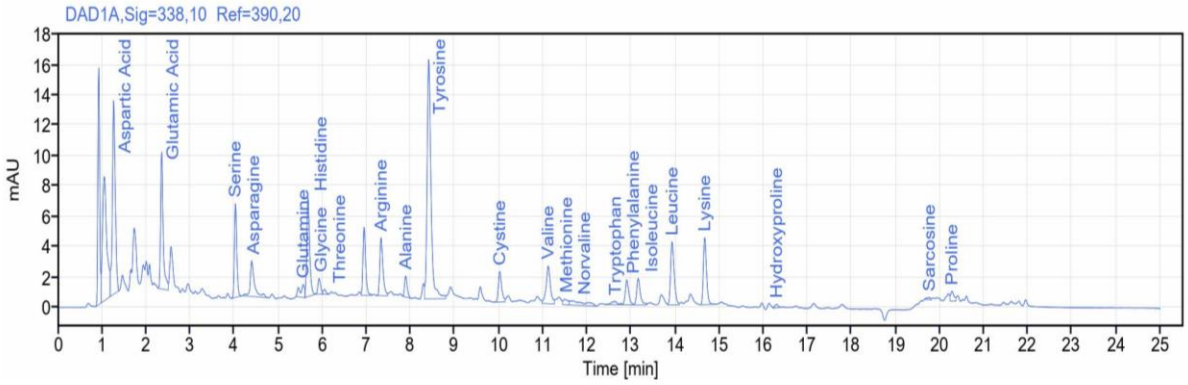

Figure 4.3.3. Chromatogram of aminoacid profiling of seed (IFLA1864)

Table 4.3.4. Descriptive statistics of 23 aminoacids in 40 accessions of grasspea (seed)

	Min*	Max*	Mean	SE	Variance	SD
Alanine	0.06	0.41	0.154	0.013	0.006	0.08

Arginine	0.14	0.96	0.395	0.026	0.027	0.164
Aspartic Acid	0.5	2.76	1.779	0.073	0.213	0.461
Cystine	0.72	8.91	5.307	0.271	2.942	1.715
Glycine	0.11	0.21	0.053	0.006	0.002	0.039
Histidine	0.06	0.96	0.17	0.025	0.025	0.16
Isoleucine	0.62	0.94	0.248	0.027	0.03	0.172
Leucine	0.26	1.74	0.764	0.038	0.056	0.238
Lysine	0.45	1.36	0.473	0.044	0.076	0.276
Methionine	0.20	0.42	0.091	0.01	0.004	0.062
Phenylalanine	0.09	0.19	0.12	0.008	0.003	0.051
Proline	0.27	1.01	0.524	0.032	0.041	0.202
Serine	0.03	0.13	0.082	0.003	0.005	0.02
Threonine	0.19	1.21	0.878	0.037	0.056	0.236
Tryptophan	0.12	0.31	0.11	0.009	0.003	0.056
Tyrosine	0.1	2.08	0.492	0.05	0.098	0.313
Valine	0.11	0.23	0.078	0.008	0.003	0.05
Glutamic acid	4.95	6.79	5.94	0.065	0.17	0.413
Asparigine	0.19	1.23	0.799	0.027	0.028	0.169
Sarcosine	1.1	11.64	5.804	0.32	4.108	2.027
Hydroxyproline	1.23	5.31	1.223	0.156	0.977	0.988
Glutamine	0.22	0.85	0.435	0.023	0.021	0.145
Norvaline	0.08	0.7	0.398	0.028	0.031	0.176

* Unit:g/100g protein

4.3.1.14. β-ODAP Estimation in grasspea seeds:

β-ODAP profiling has been done for grasspea using UPLC for the all the accessions included in the study irrespective of the cluster. The mean, range (minimum and maximum), standard error (SE), coefficient of variation and standard deviation were calculated for the 40 accessions and presented in the table 4.3.5. The standard chromatogram and the sample chromatogram were given in the figure 4.3.4 and figure 4.3.5. The accessions which are having low β-ODAP content in their seeds include IC525182 (0.074), IC0634755 (0.078), IFLA2924 (0.088) and the accessions having high β-ODAP in the seeds include IFLA1926 (0.327) and IFLA2765 (0.340) expressed in Unit: mg/g

Table 4.3.5. Descriptive statistics of β-ODAP values of 40 accessions (seed)

	168 accessions	40 accessions
Min*	0.07	0.08
Max*	0.34	0.34
Mean	0.18	0.19
Std. error	0.01	0.01
Variance	0.005	0.005
Stand. dev	0.06	0.07

Unit: mg/g

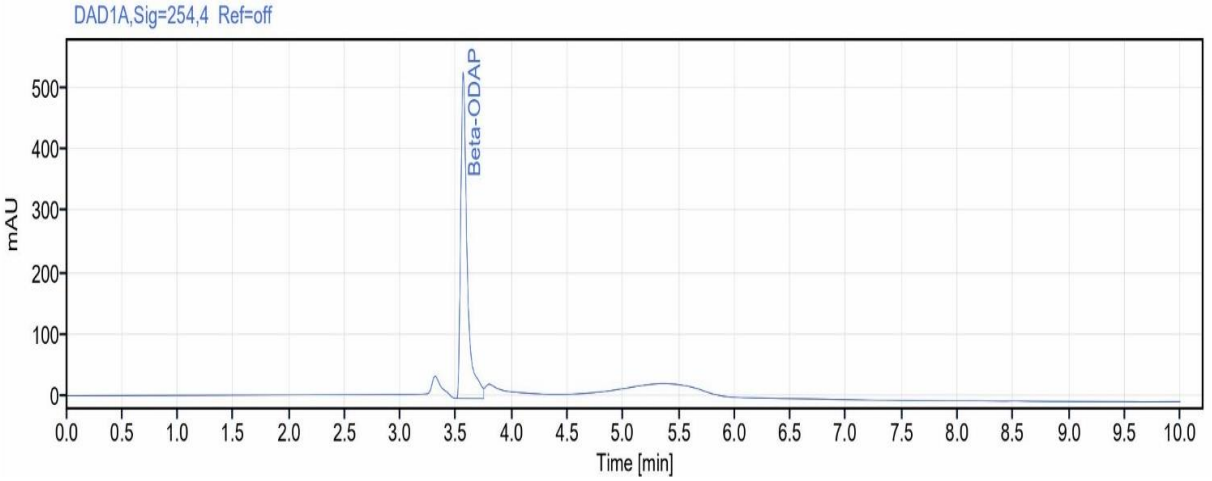

Figure 4.3.4. Standard curve of β-ODAP

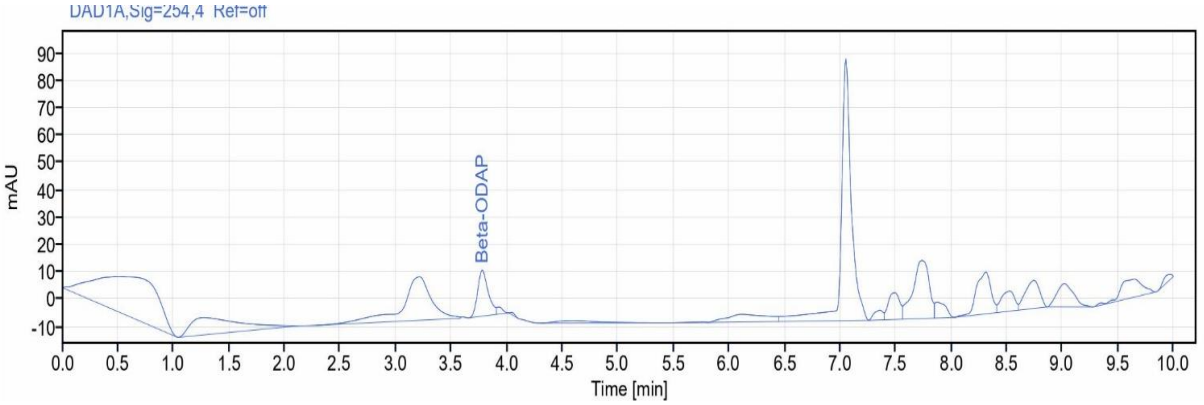

Figure 4.3.5. Chromatogram of β-ODAP profiling of seed (BANG20)

4.3.2. Descriptive statistics (Leaf): data on the biochemical compounds viz., mean, range (minimum and maximum), standard error (SE), coefficient of variation and standard deviation were calculated and presented in the Table 4.3.6 (Leaf) and the mean values of biochemical parameters in Table 4.3.7.

Table 4.3.6. Descriptive statistics of proximate compounds of 40 accessions grasspea (Leaf)

	Protein	Sugar	Phenol	TDF	Moisture	Ash	Fe	Cu	Zn	Ca	Mg
Min	20.61	2.77	0.36	15.25	12.42	5.45	338.71	6.12	31.77	607.61	1291.42
Max	33.50	7.36	1.04	31.05	17.87	13.45	795.21	23.45	90.22	2849.27	3739.89

Mean	28.79	4.18	0.51	22.79	14.91	8.37	537.77	11.68	58.18	1337.55	2585.03
SE	0.35	0.16	0.02	0.77	0.23	0.27	14.97	0.46	2.38	96.77	83.20
SD	2.22	0.98	0.13	4.88	1.45	1.73	94.68	2.94	15.06	612.00	526.19

Note : Protein, sugar, phenol are expressed in g/100g on dry weight basis; While Fe, Cu, Zn and Ca are expressed in ppm (parts per million) and TDF, Moisture and Ash in %

Table 4.3.7. Mean values of proximate compounds of 40 accessions grasspea (leaf)

S.no	Protein	Sugar	Phenol	TDF	Moisture	Ash	Fe	Cu	Zn	Ca	Mg
1	33.5	5.56	0.38	15.64	12.81	7.68	424.44	9.73	60.31	1754	1291.42
2	31.57	4.43	0.62	17.57	15.85	8.05	338.71	14.16	64.14	739.89	2992.19
3	29.37	3.42	0.76	22.73	12.63	13.37	605.75	14.27	90.22	944.08	2175.63
4	30.39	3.17	0.53	23.35	12.48	12.16	380.17	13.16	56.83	1755.81	2535.16
5	30.05	2.86	0.54	23.68	12.42	9.81	441.04	9.78	67.21	607.61	2131.74
6	28.48	2.77	0.44	15.25	13.73	9.83	440.92	9.23	72	987.95	2401.99
7	27.24	3.89	0.43	15.59	14.29	10.43	659.4	12.18	51.91	921.09	2545.96
8	31.32	4.32	0.45	16.22	14.15	10.23	514.73	7.86	36.55	1421.34	1762.59
9	28.21	5.04	0.48	16.6	15.93	6.57	562.35	11.06	48.62	1091.41	2967.6
10	29	4.73	0.64	16.69	16.63	7.65	480.37	18.09	64.6	1625.56	1940.16
11	28.38	4.28	0.66	17.43	17.64	8.02	634.98	12.05	89.68	1372.03	2243.52
12	27.25	4.13	0.55	17.64	15.29	8.34	547.95	12.71	57.78	810.67	3000.52
13	33.21	4.11	0.55	17.79	16.57	7.74	580.37	11.21	39.03	855.69	2968.26
14	28.86	3.57	0.67	18.21	15.05	8.69	559.5	10.52	36.19	693.27	2472.46
15	29.23	2.84	0.37	18.21	15.83	6.94	572.28	11.19	37.35	2819.9	2863.2

16	30.08	3.65	0.45	18.93	15.34	8.38	610.26	13.6	66.42	828.79	2780.88
17	28.33	3.3	0.46	20.31	14.64	8.71	502.13	11.91	31.77	1638.71	2647.71
18	29.09	4.79	0.54	20.65	15.32	9.62	436.61	9.75	57.16	706.65	1767.9
19	28.9	4.4	1.04	20.94	13.31	8.75	497.22	6.12	34.76	957.92	2841.34
20	24.4	3.24	0.41	21.98	14.94	8.64	572.62	12.31	53.94	1023	3150.76
21	26.89	3.03	0.57	22.36	13.95	6.64	463.91	7.62	43.97	779.72	3171.72
22	29.8	4.06	0.58	22.73	17.46	7.73	592.64	8.91	51.84	740.57	3242.03
23	27.36	3.65	0.53	23.21	14.03	13.45	577.71	12.03	90.22	1302.3	3006.91
24	28.1	3.27	0.46	23.95	13.25	8.13	516.35	9.84	59.63	2606.18	3739.89
25	31.3	4.63	0.48	24.05	13.42	8.12	512.96	9.37	40.35	1085.67	2474.29
26	28.71	4.55	0.54	24.84	14.46	8.97	523.72	11.94	63.24	2249.47	3143.52
27	29.28	3.93	0.63	24.91	14.59	6.82	467.51	23.45	52.77	2009.88	1967.92
28	28.37	3.91	0.47	25.57	13.89	7.61	575.12	13.52	57.79	2849.27	2877.41
29	28.13	5.26	0.55	26.67	14.74	8.72	614.05	11.59	61.27	1476.17	1846.45
30	26.98	5.48	0.36	26.79	16.84	8.37	672.01	13.32	80.48	1654.04	2976.86
31	28.95	4.35	0.43	27.07	17.52	6.76	595.63	11.13	58.66	826.37	2579.57
32	29.65	3.43	0.55	28.5	15.62	5.45	368.61	10.91	63.34	1073.59	1802.2
33	28.14	3.95	0.5	28.75	16.39	8.29	596.46	9.75	43.52	1074.38	2430.83
34	27.36	3.17	0.6	28.76	17.87	7.1	459.52	10.14	50.23	1526.93	2632.45
35	28.36	3.7	0.36	29.97	15.4	7.72	656.49	13.4	67.84	827.88	3270.99
36	20.61	6.35	0.36	30.77	14.69	6.48	638.91	11.13	59.88	1143.28	2892.15
37	27.65	7.36	0.4	31.05	14.54	7.43	624.58	11.52	62.03	1670.42	2772.77
38	32.1	5.27	0.38	30.26	14.23	7.78	453.98	10.39	58.85	1676.22	2151.22

39	26.89	4.38	0.37	29.63	13.87	6.23	443.53	10.46	60.61	2529.86	1992.34
40	30.21	5.15	0.39	26.36	14.91	7.57	795.21	15.96	84.23	844.41	2948.82
C1	28.14	3.35	0.32	23.21	12.39	7.29	436.61	9.76	64.25	1006.32	1767.32
C2	29.33	3.3	0.41	22.17	14.87	6.1	380.63	8.32	56.38	903.1	1452.1
C3	26.78	3.05	0.36	20.54	15.4	6.12	328.61	7.54	61.71	856.2	1320.34
C4	25.1	2.87	0.62	24.32	13.69	5.48	341.46	8.55	68.12	1065.32	1098.56

Note : Protein, sugar, phenol are expressed in g/100g on dry weight basis; While Fe, Cu, Zn and Ca are expressed in ppm (parts per million) and TDF, Moisture and Ash in %

4.3.2.1. Total protein content: The range of variations in protein content exhibited by accessions was ranged between 20.62 % (IFLA276) to 33.50 % (IFLA1193), with a mean value of 28.79 %, and best performing check was Prateek 29.33 % (Table 4.3.7).

4.3.2.2. Total soluble sugar (TSS): The TSS content of grasspea genotypes ranged from 2.77 g/100 g (IFLA2475) to 7.36 g/100 g (IFLA1715) with a mean value of 4.18 g 100g−1 (Table 4.3.7), which is higher than the value of best performing check was ratan 3.35 g/100g.

4.3.2.3. Phenol content: The total phenolic content of grasspea genotypes varied between 0.36 g/100g (IC349809) to 1.04 g/100g (BANG224) with an average value of 0.51 g/100g (Table4.3.7). The best performing check Ratan has lower phenol content 0.32 g/100g than the genotypes.

4.3.2.4. Total Dietary fibre (TDF): The TDF content of grasspea genotypes ranged from 15.25 % (IFLA2475) to 31.05 % (BANG198) with a mean value of 22.79 % (Table 4.3.7). The best performing check having higher TDF was Narayangon 24.32%.

4.3.2.5. Moisture content: The moisture content of grasspea genotypes ranged from 12.42 % (IC567350) to 17.87 % (BANG198) with a mean value of 14.91 % (Table 4.3.7), these values were significantly higher than the best performing check ratan 12.39 %.

4.3.2.6. Ash content: The ash content of grasspea genotypes ranged from 5.45 % (BANG208) to 13.45% (BANG22), mean value of 8.37 % (Table 4.3.7), with best performing ratan (7.29 %).

4.3.2.6. Fe content: The Fe content of grasspea genotypes ranged from 338.71 (IC470982) to 795.21 ppm (IFLA479) with a mean value of 537.77 ppm (Table 4.3.7). The best performing check was Ratn with 436.61ppm

4.3.2.7. Cu content: The Cu content of grasspea genotypes ranged from 6.12 (BANG224) to 23.45 (IFL2765) with a mean value of 11.88 (Table 4.3.7). The best performing check was ratan 9.76ppm.

4.3.2.8. Zn content: The Zn content of grasspea genotypes ranged from 31.77 (IC574469) to 90.22 ppm (IC208430) with a mean value of 58.26ppm (Table 4.3.7). The best performing check was Narayangon 68.12 ppm.

4.3.2.9. Ca content: The Ca content of grasspea genotypes ranged from 607.61 (IC567350) to 2849.27 (IC208437) with a mean value of 1337.55ppm (Table 4.3.7). The best performing check was Narayangon 1065.32 ppm.

4.3.2.10. Mg content: The moisture content of grasspea genotypes ranged from 1291.42 (IFLA1193) to 3739.89 (IC0634654) with a mean value of 2585.03ppm (Table 4.3.7). The best performing check was ratan having 1767.32 ppm.

4.3.2.11. Aminoacid profiling of grasspea leaves

Amino acid profiling has been done for selected 40 grasspea accessions using UPLC. 23 total amino acids have been identified in the studies which includes three non-protein aminoacids, the details have been given in table 4.3.8. The mean, range (minimum and maximum), standard error (SE), coefficient of variation and standard deviation were calculated and presented in the table 4.3. The standard chromatogram and the sample chromatogram were given in the Figure 4.3.6. In the aminoacid profiling of leaves, sarcosine accounts for highest (14.6 g/100g protein) followed by cysteine (7.85 g/100g protein), glutamic acid (6.82 g/100g protein) and the lowest observed is valine (0.3 g/100g protein)

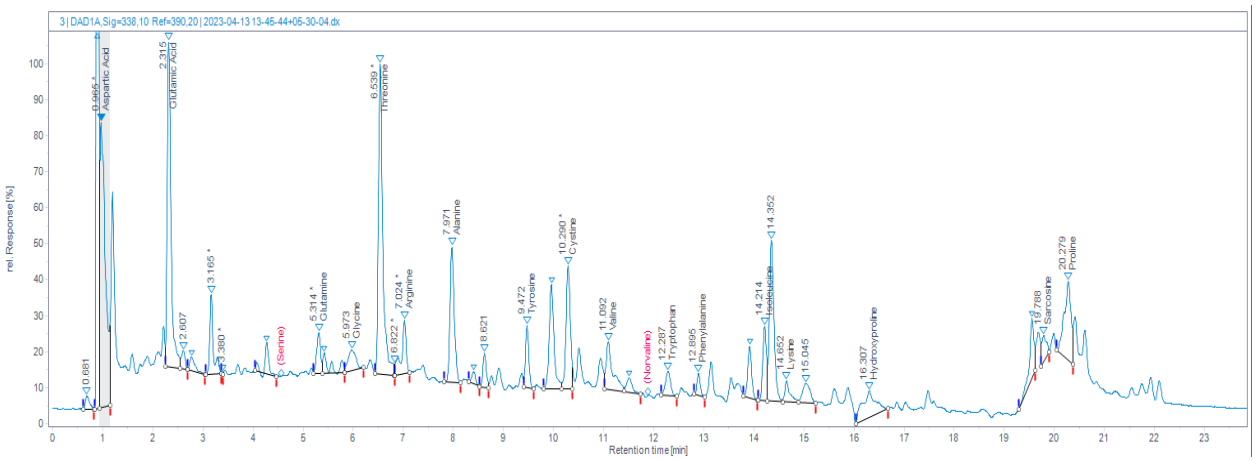

Figure 4.3.6. Chromatogram of aminoacid profiling of leaf (IC415658)

Table 4.3.8. Descriptive statistics of 23 aminoacids present in the 40 grasspea accessions (leaf)

	Min*	Max*	Mean	SE	Variance	SD
Alanine	0.06	0.41	0.154	0.013	0.006	0.08
Arginine	0.14	0.96	0.395	0.026	0.027	0.164
Aspartic Acid	0.5	2.76	1.779	0.073	0.213	0.461
Cystine	0.72	8.91	5.307	0.271	2.942	1.715
Glycine	0.02	0.21	0.053	0.006	0.002	0.039
Histidine	0.06	0.96	0.17	0.025	0.025	0.16
Isoleucine	0.02	0.94	0.248	0.027	0.03	0.172
Leucine	0.26	1.74	0.764	0.038	0.056	0.238
Lysine	0.12	1.36	0.473	0.044	0.076	0.276
Methionine	0.03	0.42	0.091	0.01	0.004	0.062
Phenylalanine	0.05	0.19	0.12	0.008	0.003	0.051
Proline	0.27	1.01	0.524	0.032	0.041	0.202
Serine	0.03	0.13	0.082	0.003	0.021	0.02

Threonine	0.19	1.21	0.878	0.037	0.056	0.236
Tryptophan	0.07	0.31	0.11	0.009	0.003	0.056
Tyrosine	0.1	2.08	0.492	0.05	0.098	0.313
Valine	0.10	0.23	0.078	0.008	0.003	0.05
Glutamic acid	4.95	6.79	5.94	0.065	0.17	0.413
Asparigine	0.19	1.23	0.799	0.027	0.028	0.169
Sarcosine	1.1	11.64	5.804	0.32	4.108	2.027
Hydroxyproline	0.61	5.31	1.223	0.156	0.977	0.988
Glutamine	0.22	0.85	0.435	0.023	0.021	0.145
Norvaline	0.02	0.7	0.398	0.028	0.031	0.176

* Unit:g/100g protein

4.3.2.12. β-ODAP estimation in grasspea leaves

β-ODAP profiling has been done for selected 40 grasspea accessions using UPLC. The mean, range (minimum and maximum), standard error (SE), coefficient of variation and standard deviation were calculated for the 40 accessions and presented in the table 4.3.9. The standard chromatogram and the sample chromatogram showing β-ODAP concentration were given in the Figure 4.3.7. The accessions which are having low β-ODAP values are observed for IFLA1193 (0.03), IC470982 (0.04) and higher β-ODAP values are observed for IC18879 (0.25), IFLA479 (0.29) expressed in Unit: mg/g

Table 4.3.9. Descriptive statistics of β-ODAP values of 40 accessions (leaf) Unit: mg/g

	40 accessions
Min*	0.034
Max*	0.287
Mean	0.143
Std. error	0.009
Stand. dev	0.059

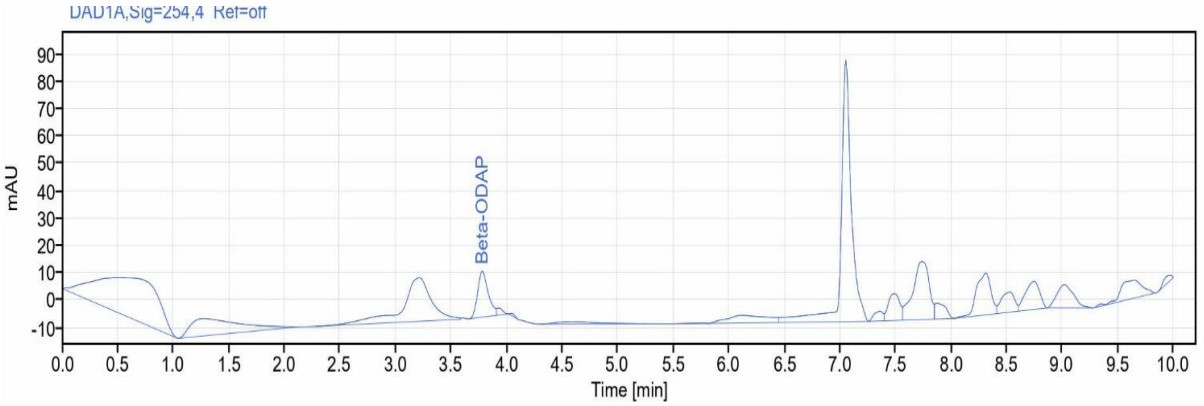

Figure 4.3.7. Chromatogram of β-ODAP profiling of seed (IFLA1864)

4.3.3. Association analysis

Correlation studies are crucial for identifying positive or negative values that imply significant or non-significant associations between the parameters. Using the Pearson correlation test, the significant correlations between the nutritional indicators were found. Pearson's $r > 0$ implies a positive correlation, whereas $r < 0$ shows a negative correlation with $p < 0.05$. An increase in one parameter causes changes in the traits of other, related parameters. Correlations among the studied traits in 40 genotypes of grasspea of proximate and β-ODAP as follows

4.3.3.1. Correlation of seed proximate compounds with β-ODAP

Correlations among the studied traits in 40 genotypes of grasspea for proximate and β-ODAP (seed) was given in the Table 4.3.10. The association analysis revealed that there is no significant positive correlation between the studied biochemical parameters with the β-ODAP content of seed. The parameter TDF (-0.33) shows significant negative correlation with the β-ODAP. The parameter starch shows significant positive and significant negative correlation with the phenol (0.38) and TDF (-0.47) respectively. For the parameter TDF, there is significant positive and significant negative correlation with the parameter sugar (0.35) and phenol (-0.36) respectively; TDF shows significant negative correlation with minerals like Fe (-0.36) and Ca (-0.45). Fe shows significant positive correlation with Zn (0.34) and Ca (0.48). The parameter Ca (0.32) and Zn (0.42) shows significant positive correlation with each other. The correlation plot is given in the Figure 4.3.8.

Figure 4.3.8. Correlation of seed proximate compounds with β-ODAP

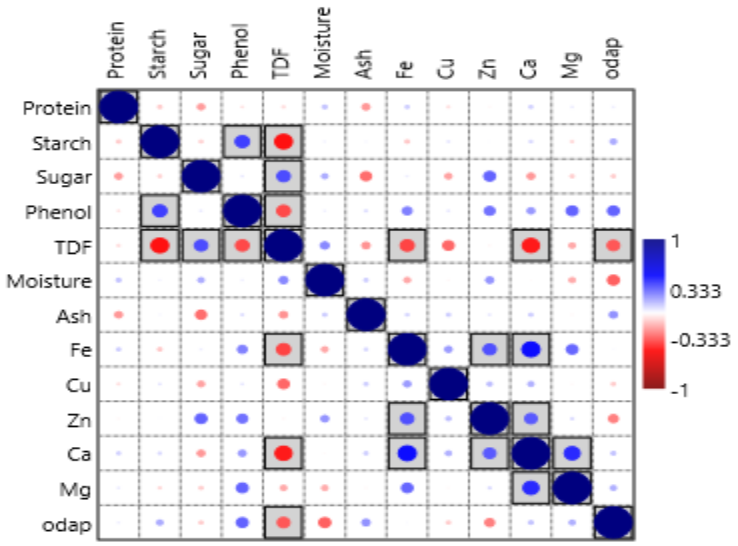

Table 4.3.10. Correlation of seed proximate compounds with β-ODAP

	Protein	Starch	Sugar	Phenol	TDF	Moisture	Ash	Fe	Cu	Zn	Ca	Mg
Starch	-0.10											
Sugar	-0.19	-0.09										
Phenol	-0.06	0.38*	0.04									
TDF	-0.07	-0.47**	0.35*	-0.36*								
Moisture	0.12	0.03	0.16	0.03	0.23							
Ash	-0.20	0.02	-0.28	0.06	-0.21	0.10						
Fe	0.10	-0.11	0.03	0.25	-0.36*	-0.17	0.08					
Cu	-0.06	0.05	-0.18	0.05	-0.30	-0.01	0.10	0.20				
Zn	-0.01	0.00	0.31	0.28	-0.02	0.21	0.04	0.34*	0.13			
Ca	0.09	0.07	-0.20	0.20	-0.45**	-0.01	0.10	0.48**	0.16	0.32*		
Mg	0.04	-0.07	-0.10	0.31	-0.17	-0.16	-0.01	0.30	-0.01	0.07	0.42**	
β-ODAP	0.04	0.16	-0.10	0.31	-0.33*	-0.31	0.21	0.01	-0.10	-0.25	0.12	0.16

Significance levels: p < .0001 '****'; p < .001 '***', p < .01 '**', p < .05 '*'

4.3.3.2. Correlation of leaf proximate compounds with β-ODAP

Correlations among the studied traits in 40 genotypes of grasspea for proximate and β-ODAP (leaf) was given in the Table 4.3.11. The association analysis reveals that the parameters sugar (0.37), TDF (0.80) and Fe (0.37) shows significant positive correlation and the parameters like Phenol (-0.33) and ash (-0.48) shows significant negative correlation with the β-ODAP content. The parameter protein shows significant negative correlation with TDF (-0.32), Fe (-0.32) and Mg (-0.34). The parameter moisture content (-0.45) shows significant negative correlation with ash. The mineral Fe content (0.38) shows significant positive correlation with Mg and Cu content (0.36) shows significant positive correlation with Zn. The correlation plot of proximate composition of leaf with β-ODAP is given in the Figure 4.3.9.

Table 4.3.11. Correlation of seed proximate compounds with β-ODAP

	Protein	**Sugar**	**Phenol**	**TDF**	**Moisture**	**Ash**	**Fe**	**Cu**	**Zn**	**Ca**	**Mg**
Sugar	-0.08										
Phenol	0.14	-0.21									
TDF	-0.32*	0.28	-0.25								
Moisture	-0.10	0.10	-0.03	0.02							
Ash	0.10	-0.27	0.23	-0.27	-0.45**						
Fe	-0.32*	0.29	-0.24	0.16	0.28	0.03					
Cu	-0.01	0.06	-0.03	0.04	0.12	-0.02	0.16				
Zn	-0.10	0.14	-0.09	0.17	-0.01	0.31	0.26	0.36*			
Ca	-0.04	0.04	-0.26	0.19	-0.17	-0.16	-0.11	0.18	-0.05		
Mg	-0.34*	-0.14	-0.07	0.10	0.16	-0.06	0.38*	-0.06	-0.03	-0.01	
β-ODAP	-0.29	0.37*	-0.33*	0.80**	0.25	-0.48**	0.37*	0.06	0.09	0.20	0.17

Significance levels: p < .0001 '****'; p < .001 '***', p < .01 '**', p < .05 '*'

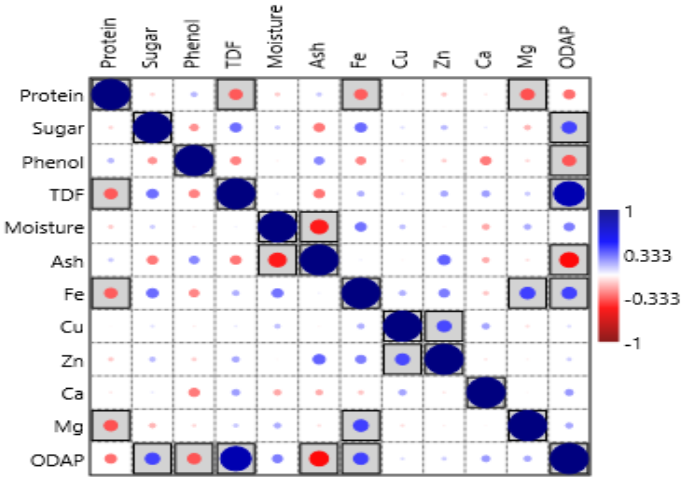

Figure 4.3.9. Correlation of seed proximate compounds with β-ODAP

4.3.3.3. Trends of biochemical composition in grasspea

The proximate composition of both seed and leaf are plotted together for better understanding of the nutritional composition of grasspea in Figure 4.3.10a and 4.3.10b. Similarly, trend curve for β-ODAP content in both seed and leaf are given in the Figure 4.3.11.

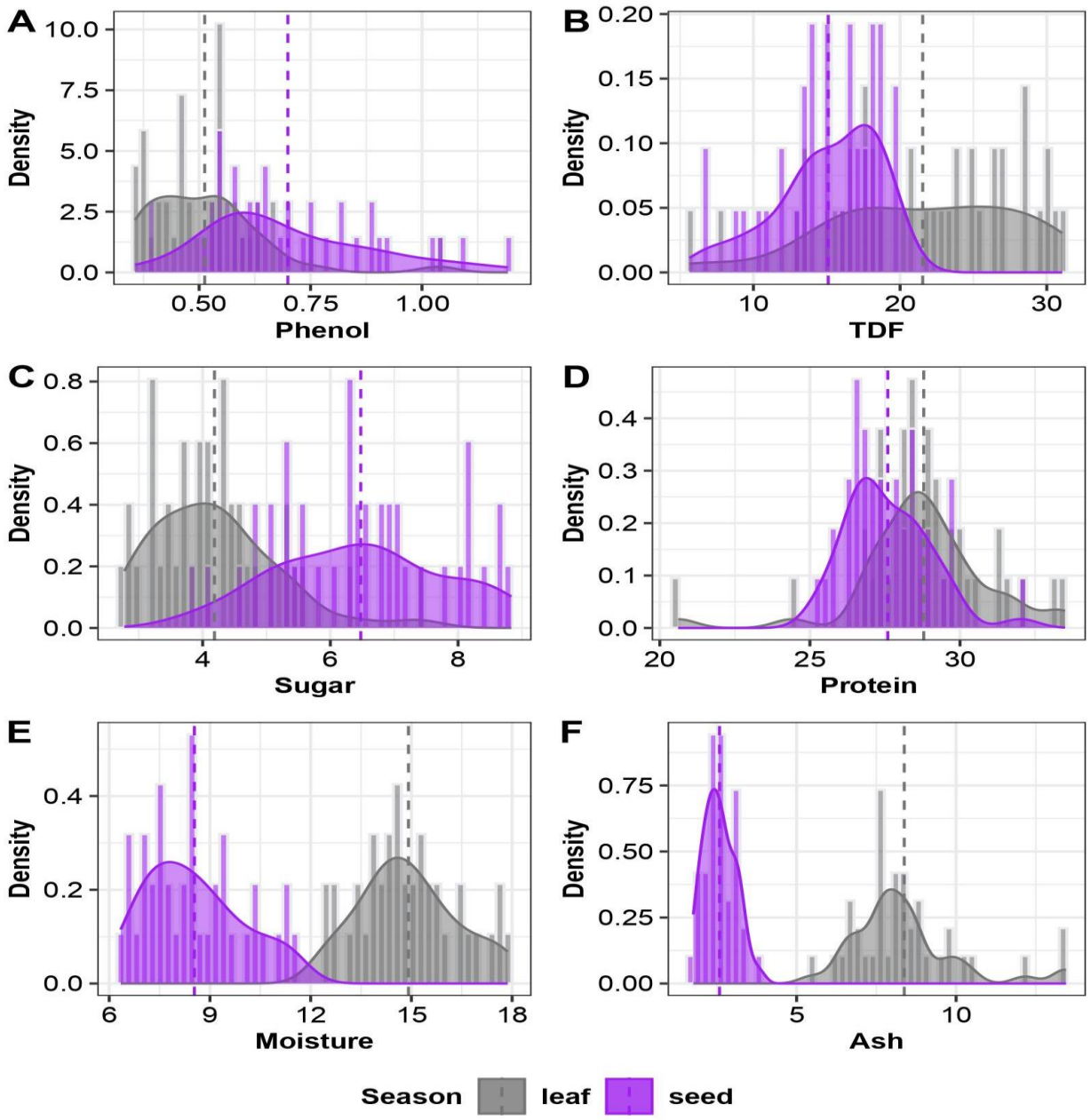

Figure 4.3.10a Trend curve of nutritional composition of grasspea

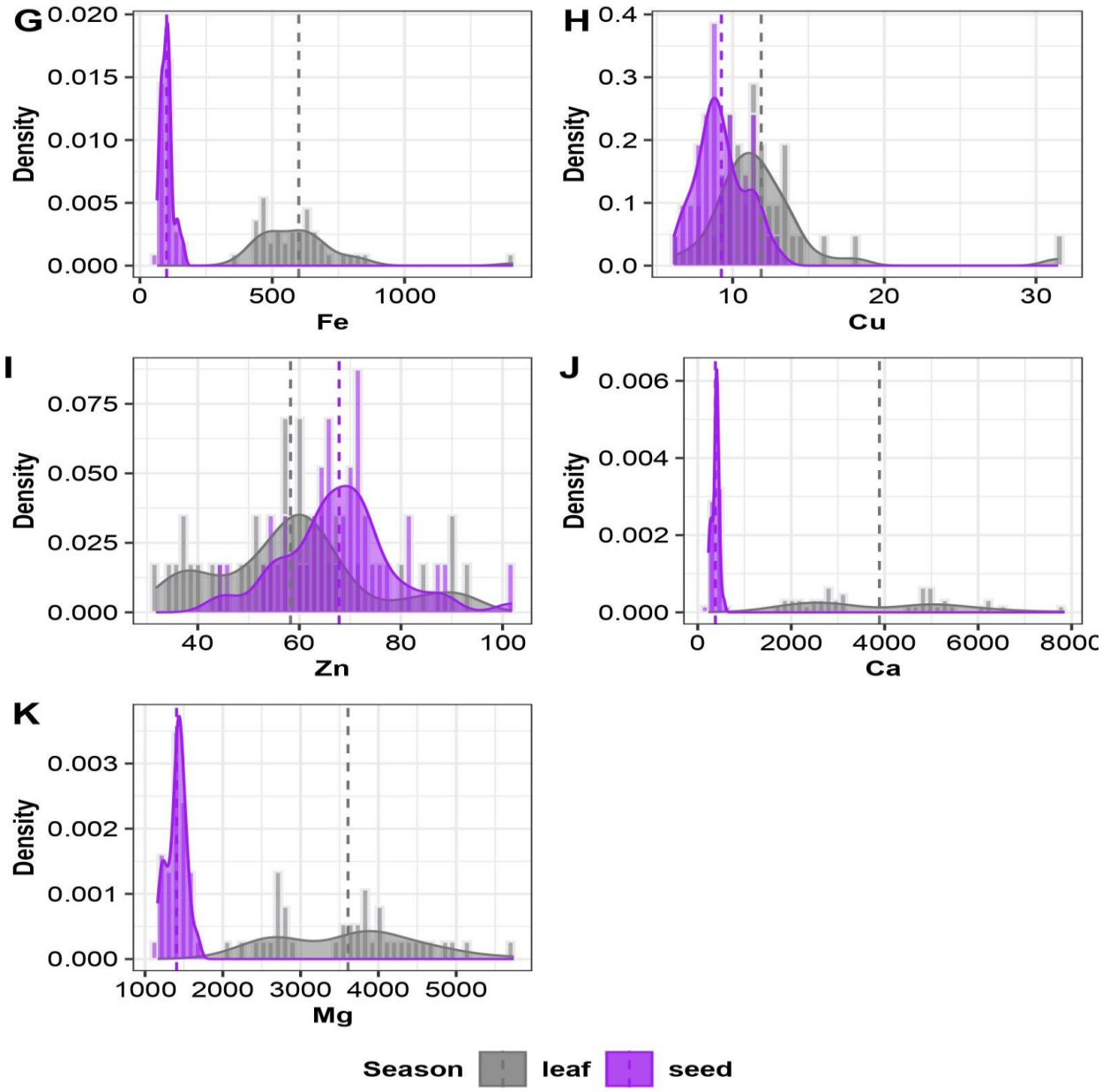

Figure 4.3.10a Trend curve of nutritional composition of grasspea (minerals)

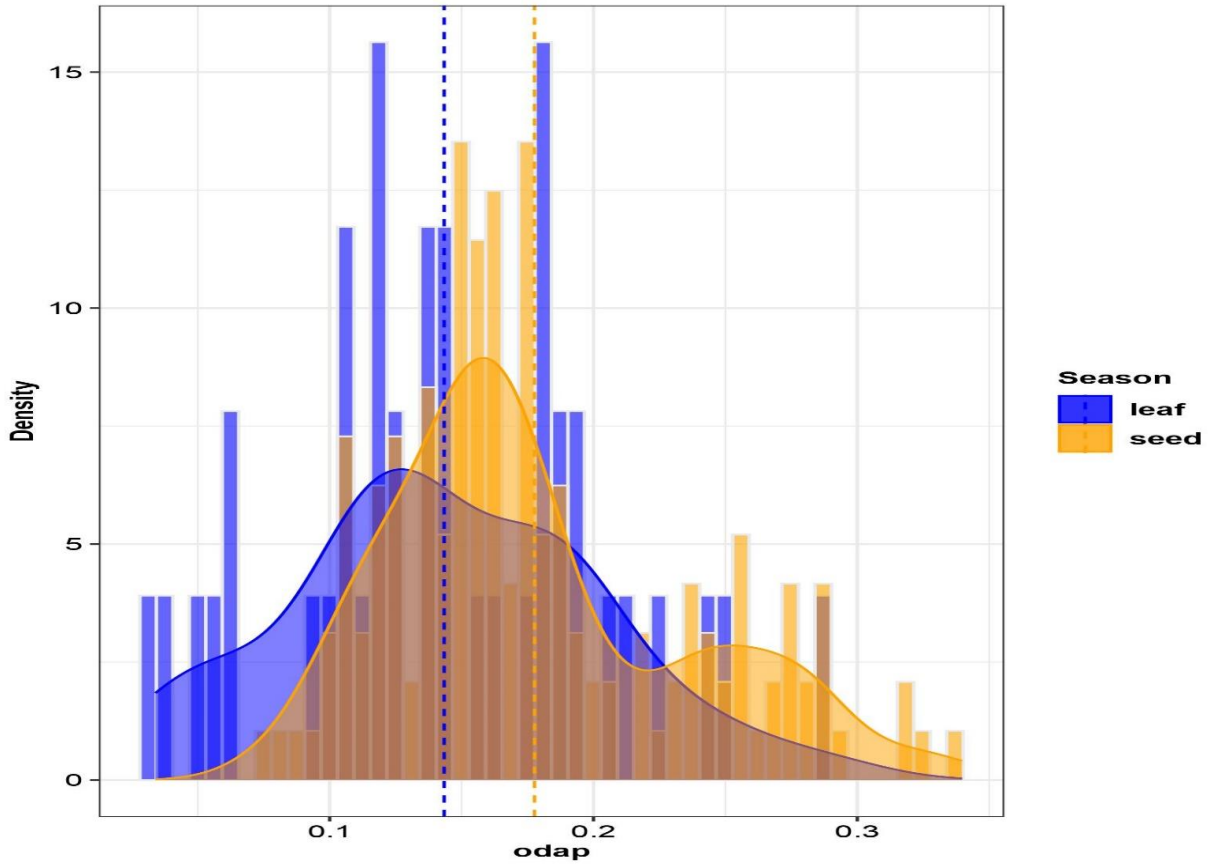

Figure 4.3.11. Trend curve of β-ODAP content of grasspea

Chapter V

DISCUSSION

5.1. Morphological characterization of Grasspea

The analysis of variance for the 14 quantitative traits revealed that high genetic diversity is present among the grasspea accessions. Whereas, the means of all the observed traits varied significantly across the accessions. Similar observations were also recorded during agro- morphological characterization of grasspea conducted by various workers (Poligano et al. 2005; Tavolette et al. 2005; Grela et al. 2005, 2012; Barpete et al. 2015; Singh et al 2017; Jeberson et al. 2018; Mahapatra et al. 2020) which revealed presence of wide range of variability in the grasspea germplasm grown by them. Such variation is valuable and can be used as the initial breeding material as it provides opportunities for selection of desired traits through phenotypic characterization for varietal development.

In grasspea, maximum diversity was observed for plant height ranged from 48.28cm to 119.45cm, 80%M ranges from 112.35 to 147.47 days, the highest NPP was 96.78 pods and the highest HSWT recorded was 14.02g. The accessions with high plant height are proportional to the high biomass of the plant. The ranges of the observed PCV are from 26.06 to 4.65%; high PCV was recorded for HSWT; moderate PCV observed for PTL, PHT, SDL, NSP, NPP, SDW; similar results observed by (Mahapatra et al. 2020). In all the traits examined, the extent of PCV surpassed the GCV, underscoring that the observed variability was not solely attributed to genetic factors but was also influenced by environmental conditions. The substantial levels of GCV and PCV suggest ample opportunities for selecting superior germplasm entries concerning these traits (Burton and Devane, 1953). However, assessing heritable portions is not solely reliant on calculating PCV and GCV; it additionally hinges on the Genetic Advance as a percentage of the mean (GAM) (Govindaraj et al. 2010). In a broad context, heritability defines the share of overall variability attributed to genetic factors, signifying the ratio of genotypic variance to the entire variance. It serves as a reliable gauge for the transfer of traits from parents to their descendants (Falconer, 1960). Moreover, it quantifies genetic progress through selection. Genetic advance represents an additional parameter, signifying the extent of genetic modification resulting from selection. The

anticipated genetic advance for a specific trait serves as a gauge of potential genetic improvement through further selection. Relying solely on heritability estimates for efficient selection is insufficient, as it doesn't accurately quantify genotypic variation. To make informed selections, one should also consider genetic advance as a percentage of the mean value. Genetic advance, the distinction between the average genotypic value of selected lines and the parental population, was calculated following the method established by Johnson et al. (1955) and presented as a percentage of the mean value. High heritability was observed for the traits HSWT (78.93%), SDL (63.76%), SDW (61.90%), PHT (66.72%), PTL (70.69%), DFF (80.39%), 50%F (84.28%), 80%M (74.89%) and PDW (72.19%). Similar results were observed by Kumari and Prasad, 2005 and Mahapatra et al. 2020. High GAM observed for the traits PHT (21.66%), PTL (26.08%), HSWT (41.25%); similar results were observed by Kumar and Dubey (2001). Traits exhibiting substantial heritability coupled with high genetic advance as a percentage of the mean (GAM) offer breeders a valuable opportunity for selecting superior genotypes from genetically diverse populations which is observed for the traits PHT, PTL and HSWT; high heritability with moderate GAM was observed for the traits like DFF, PDW, NSP, SDL and SDW. This combination suggests that these traits are less influenced by environmental factors in their expression and are likely influenced by additive gene action. Consequently, selecting for these traits becomes more feasible.

Correlation coefficients serve as valuable tools for gauging the extent of associations among various characteristics. They are particularly useful in identifying attributes closely linked to grain yield or HSWT, aiding in selection processes. Understanding correlations is vital for evaluating how selection impacts interconnected traits. The strength of these associations is denoted by 'r,' ranging from -1 to +1. An 'r' of -1 signifies a 100% negative correlation, where variables move in opposite directions; +1 indicates a 100% positive correlation, while 'r=0' implies no correlation, indicating the variables are independent of each other (Nadarajan et al. 2016). The present study revealed that the HSWT showed significant positive correlation with the traits like PTL, SDL and SDW; similar results were obtained in the earlier studies (Talukdar, 2009 and Jeberson et al. 2018). Similar results were observed by Singh et al. 2017; Therefore, selection these traits which are positively correlated with HSWT will ultimately increase the HSWT and thereby the seed yield. the traits related to flowering and maturity time, PHT, PDL, PDW, number of primary branches, NSP and NPP showed the nonsignificant association with HSWT.

The contribution of these characters was further analyzed by computing their direct and indirect

effects on HSWT. Path coefficient analysis represents a standardized partial regression coefficient that divides the correlation coefficient into direct and indirect influence measurements. In essence, it quantifies both the direct and indirect impacts of various independent characteristics on a dependent trait. Path coefficient was worked out based on the HSWT as a dependent variable and all the other 13 characters as independent variables and the residual effect was (0.56). The traits like 50%F, 80%F showed very high direct effects on the HSWT. SDW and 80%M showed high direct effects; PTL showed moderate direct effects and PHT, NPB and NSP showed low direct effects to the HSWT whereas remaining traits like DFF, PDL, PDW, NPP and SDL showed low direct effects; similar results were obtained by Tyagi et al. 2011; Singh et al. 2012 in lentil.

The qualitative traits viz., flower color, growth habit, leaf size, seed size and seed coat color showed variability between the accessions. In the present study, the leaf size was recorded which varies from small to medium and also larger leaf size also observed and it is one of the traits considered in importance with fodder and forage, green leafy vegetable. The different seed shapes observed in the accessions are rhomboid, square, obtriangular and spherical. The shape of seed predominantly varies within the accession and among the accessions and there is medium constriction between seeds of pods. The various colors observed in the accessions under study widens the opportunity of pollinators like honey bee and also makes the possibility of cross pollination up to 28% (Rahman et al. 1995) percent in grasspea and thereby making this crop as an often-cross pollinated crop.

The hierarchical cluster analysis based on 14 quantitative trait and 20 qualitative traits grouped all the 168 accessions into four major clusters at the distance of 35. Likewise, previous researchers were also used K means clustering to group the accessions and to select desired multiple traits in the genotypes (Poligano et al. 2005; Grela et al. 2010 and 2012; Barpete et al. 2015).

In the present study, PCA was employed to examine the 14 quantitative characters that played an important role in establishment of phenotypic variability among the accessions. The first five principal components contributed 70.63% to the total genetic variability exhibited by the germplasm. The variability on the first principal component (28.14 %) was accounted by high positive loading for the traits 50%F (0.99), 80%F (0.99), 80%M (0.97) and DFF (0.94). Our results were similar to previously reported results by various workers while studying in grasspea germplasm. Gixhari et al. (2016) reported that first three principal components contributed to

85.97% of variability that contributed by four factors viz., plant height, days to first flowering, days to maturity and petiole length. Our study indicated that greater amount of genetic diversity was present between the accession of grasspea. Thus, these accessions have the inordinate potential to exploit in crop improvement programme both for varietal development and genetic base broadening to fight against abiotic and biotic stresses.

5.2. Molecular characterization by SSR markers

Out of all the available molecular markers, simple sequence repeat makers are frequently used by earlier workers for genetic diversity assessment due to their ability to detect codominance, reproducibility, distribution throughout the genomic region, polymorphism, noninfluence by environment, not crop stage bound (Kumar et al. 2019; Lioi et al. 2010; Wang et al. 2015). According to Agrama et al. (2009), the knowledge of genetic variations in germplasm has been widely acknowledged in the selection of genotypes for yield and yield-attributing traits, resistance to biotic and abiotic challenges, as well as for nutritional improvement of crop species. According to Sokal and Sneath (1963) and Murty and Arunachalam (1966), multivariate analysis utilizing statistical distance offers great potential for evaluating genetic diversity in germplasm.

Conventional CTAB extraction approach proved to be ineffective and unsatisfactory. Given the need for quality genomic DNA for diverse use of molecular markers, an improved extraction method was sought. The study employed kit method and diverse chemical combinations with CTAB for DNA isolation (Sahu et al. 2012). The four different methods of DNA isolation used in the experiment are given as follows.

CTAB 1: Leaves were grinded with liquid nitrogen with 2% CTAB, 1% β-mercaptoethanol, followed with C: I wash and RNAase purification; precipitation with C_3H_8O overnight with DNA extraction the next day. ***CTAB 2:*** The above-mentioned procedure with 2% CTAB, 1% β-mercaptoethanol C,10% PVP with twice the C: I wash and P: C: I wash. ***Kit Method:*** DNA extracted using the kit (FAVORGEN).

CTAB 3: The modified protocol for the isolation of genomic DNA was given as follows. Extraction buffer (pH 8) was made of 120mM Tris-HCl*/#, 1M NaCl* 50mM EDTA*/#. Besides these required 1% PVP40 solution, 0.5 % β – mercaptoethanol#, 2% Triton – X-100*/#$, 3 M Sodium acetate# and Washing solution of 70% (v/v) ethanol. TE buffer*/# for DNA suspension which contains 10mM Tris-HCl (pH 8) and 1mM EDTA. [Triton buffer (2% Triton – X-100 + 1%

PVP40 + 1% β – mercaptoethanol); CTAB buffer (3% CTAB + 1% β – mercaptoethanol); *- Himedia; #- Gbioscciences; $- Sigma). The procedure is given in the Figure 3.3. The incorporation of diverse buffers improved the effectiveness of the adapted CTAB3 method, yielding DNA of exceptional quality. This enhancement considerably extends the DNA's storage potential, outperforming previous methods by addressing technical challenges. In comparison to methods like CTAB 1/2/Kit, the DNA obtained through the CTAB3 method displays remarkable stability, remaining viable for over a year when stored at 4°C which starkly contrasts with earlier methods with reduced maintenance costs and make it as the preferred option for DNA extraction in grasspea.

In the present study, 20 polymorphic primers generated polymorphic information content (PIC) value was ranged from 0.02 to 0.67 with an average of 0.34. The maximum PIC was observed for primer LTR12 (0.67) and the minimum for LTR62 (0.02). The PIC value of primers indicated greater diversity within and between studied grasspea accessions and also revealed that selected primers are very informative and can be utilized in the further studies (Kumar et al. 2019; Lioi et al. 2010; Wang et al. 2015). Our results were similar to Wang et al. 2015, they used 30 SSR markers to examine the genetic diversity between the grasspea and its related species. The study included 283 individuals from wild and domesticated populations in Africa, Europe, Asia, and ICARDA. The allele number per loci ranged from 3 to 14. They observed the average polymorphism information content (PIC) of 0.4817 and the average gene diversity index was 0. 5340.

In hierarchical clustering, analysis of 168 accessions using SSR markers of grasspea were grouped into four major clusters (cluster I, cluster II, cluster III and cluster IV). Cluster I is the second largest and is composed of two subclusters, having 55 genotypes. Cluster II is the largest cluster and again subclustered into two clusters, comprising total of 87 genotypes. Cluster III again subclustered as two cluster and each subcluster has eight and 10 genotypes accounting for total of 18 genotypes in this cluster. Cluster IV is the smallest cluster having eight genotypes into it. These results were in consistent with the grouping of accession by population structure studies. These results were consistent with earlier workers reports (Lioi et al. 2010; Wang et al. 2015).

Based on the model-based clustering, 168 accessions got distributed into four populations. In this study, mean Fst value was 0.122 which indicate moderate genetic differentiation. AMOVA of 168

genotypes of grasspea was executed to investigate the genetic diversity distribution between and within the populations. The analysis revealed maximum diversity within individuals was 48%, diversity among individuals was 40% and diversity among populations was 12%.

A principal coordinate analysis (PCoA) was performed based on genetic distance to describe proportions of genetic variance. The first three PCoA axes explained 13.85%, 9.20%, 8.83%of variation, respectively. The PCoA also grouped 168 accessions of grasspea into four clusters, which compliments the results of cluster analysis. Similarly, Gixhari et al. (2016) also reported that the results of PCA were comparable to the cluster analysis. The first two components explained 39.14% and 76.77% of the total variation and effectively differentiated the 14 grasspea accessions.

5.3. Biochemical constituent's estimation of grasspea

Food legumes have been known as vital sources of numerous minerals in Indian diets (Gopalan et al. 1978). Wild legumes consumed by various tribes have a great potential for use as food and fodder (Maikhuri et al. 1996; Arinathan et al. 2009; Kala et al. 2010).

Grasspea (*L. sativus* L.) is the multifaceted legume with high nutritional profile in both the seed and leaf. The parts of southeast Asia, Bangladesh and in India, the young leaves and branches of grasspea is being used as a green leafy vegetable apart from being used as fodder. The current study, investigates the nutritional properties of both seed and leaf for proximate parameters which includes estimation of minerals by AAS and aminoacid, β-ODAP estimation by UPLC.

5.3.1. Biochemical composition of seeds:

The range of variations in protein content exhibited by accessions was ranged between 25.23 % (Sel190) to 32.02 % (BANG31), with best performing check Ratan 27.62 %. Similar results obtained by Urga et al. 1995 and it is about 22.6-28.1% and another report by Kartika et al. 2018 showed protein content of about 28.82-30.72% and other similar results by Barpete et al. 2012; Tamburino et al. 2012; Girma and Korbu, 2012 and Kumari and Kumar Jha, 2018. It exhibited significant variations among the accessions in starch content ranging from 26.16 (BANG285) to 39.00 g/100g (IC0634654) with value of best performing checks Ratan 32.02/100g. Previous studies reported starch content about (32.0-43.9%) (Urga et al. 1995); 41% starch (Akalu et al. F1998). The TSS content ranged from 3.87 g/100 g (IC421914) to 8.83 g/100 g (IC208430) which

is higher than the value of best performing check Narayangon 6.02 g/100g. The total phenolic content varied between 0.39 g/100g (BANG292) to 1.19 g/100g (IFLA1870) with the best performing check Prateek has lower phenol content 0.45 g/100g than the genotypes. The TDF ranged from 6.75 % (IFLA3001, IC421914) to 19.91% % (IC208430) with the best performing check ratan having 13.21%; previous reports suggested that up to 17% (Akalu et al. 1998). The moisture content ranged from 6.34 % (BANG291) to 11.52 % (BANG227) with a mean value of 8.54 % (Table 4.3.3). The best performing check Narayangon has lower moisture content 6.25% than the genotypes. The ash content ranged from 1.76 % (BANG227) to 3.81% (BANG208), mean value of 2.58 % (Table 4.3.3), with best performing Narayangon (3.11%). Urga et al. 1995 got similar kind of results total protein (22.6-28.1%), ash (1.0-2.1%), phosphorous (380.4-511.6 mg/100g), starch (32.0-43.9%), iron (6.6-18.4 mg/100g), calcium (131.6-200.1 mg/100g), and β-ODAP (172-353 md/100g) in the grasspea varieties were observed and concluded that Crude fiber (7.2-8.3%), total sugars (5.1-6.2%).

The Fe content of grasspea genotypes ranged from 64.92 (BANG218) to 164.35ppm (BANG206) with best performing check is Ratan 103.38ppm; Fe content about 184ppm was observed by Urga et al.1995; Grela et al. 2010). The Cu content ranged from 6.45 (IFLA2924) to 12.97ppm (BANG291) with best performing check is mahateora 10.32ppm. The Zn content ranged from 44.01 (BANG292) to 101.86ppm (Bio520) and best performing check is ratan 87.86 ppm. The Ca content of grasspea genotypes ranged from 230.50 (BANG285) to 557.70ppm (BANG208) and best performing check is mahateora 378.36 ppm; Urga et al. 1995 obtained Ca content up to 2000ppm. The Mg content ranged from 1162.83 (BANG285) to 1677.59ppm (BANG218) and the best performing check is ratan 1445.12 ppm; similar results were found by Grela et al. 2010 and Gupta et al. 2021.

Aminoacid profiling reveals that sarcosine accounts for highest (11.64 g/100g protein) followed by cysteine (8.91 g/100g protein), glutamic acid (6.79 g/100g protein) and valine accounts for the lowest (0.23 g/100g protein)

β-ODAP profiling has been done for grasspea using UPLC for the all the accessions included in the study irrespective of the cluster. The accessions which are having low β-ODAP content in their seeds include IC525182 (0.074), IC0634755 (0.078), IFLA2924 (0.088) and the accessions having high β-ODAP in the seeds include IFLA1926 (0.327) and IFLA2765 (0.340) expressed in Unit:

mg/g. The method of extraction and the solvents used are concluded from two different paper works and makes it the economically feasible one.

5.3.1.1. Correlation of seed proximate compounds with β-ODAP

The association analysis revealed that there is no significant positive correlation between the studied biochemical parameters with the β-ODAP content of seed. The parameter TDF (-0.33) shows significant negative correlation with the β-ODAP. The parameter starch shows significant positive and significant negative correlation with the phenol and TDF respectively. For the parameter TDF, there is significant positive and significant negative correlation with the parameter sugar and phenol respectively; TDF shows significant negative correlation with minerals like Fe and Ca. Fe content shows significant positive correlation with Zn and Ca. The parameter Ca and Zn shows significant positive correlation with each other.

5.3.2. Biochemical composition of leaf:

The biochemical composition of leaves is the first report ever for these many parameters. The range of variations in protein content exhibited by accessions was ranged between 20.62 % (IFLA276) to 33.50 % (IFLA1193), with best performing check was Prateek 29.33 %. The TSS content ranged from 2.77 g/100 g (IFLA2475) to 7.36 g/100 g (IFLA1715) with a best performing check was ratan 3.35 g/100g. The total phenolic content varied between 0.36 g/100g (IC349809) to 1.04 g/100g (BANG224) with best performing check Ratan has lower phenol content 0.32 g/100g than the genotypes. The TDF content ranged from 15.25 % (IFLA2475) to 31.05 % (BANG198) with best performing check having higher TDF was Narayangon 24.32%. The moisture content ranged from 12.42 % (IC567350) to 17.87 % (BANG198) with the best performing check ratan 12.39 %. The ash content ranged from 5.45 % (BANG208) to 13.45% (BANG22) with best performing ratan (7.29 %).

The Fe content of grasspea genotypes ranged from 338.71 (IC470982) to 795.21 ppm (IFLA479) with best performing check was Ratan having 436.61ppm. The Cu content ranged from 6.12 (BANG224) to 23.45 (IFL2765) with best performing check was ratan 9.76ppm. The Zn content ranged from 31.77 (IC574469) to 90.22 ppm (IC208430) with best performing check was Narayangon 68.12 ppm. The Ca content ranged from 607.61 (IC567350) to 2849.27 (IC208437) with best performing check was Narayangon 1065.32 ppm. The Mg content of grasspea genotypes

ranged from 1291.42 (IFLA1193) to 3739.89 (IC0634654) with best performing check was ratan having 1767.32 ppm.

Amino acid profiling has been done for selected 40 grasspea accessions using UPLC. 23 total amino acids have been identified in the studies which includes three non-protein aminoacids. In the aminoacid profiling of leaves, sarcosine accounts for highest (14.6 g/100g protein) followed by cysteine (7.85 g/100g protein), glutamic acid (6.82 g/100g protein) and the lowest observed is valine (0.3 g/100g protein).

β-ODAP profiling has been done for selected 40 grasspea accessions using UPLC. The accessions which are having low β-ODAP values are observed for IFLA1193 (0.03), IC470982 (0.04) and higher β-ODAP values are observed for IC18879 (0.25), IFLA479 (0.29).

5.3.2.1. Correlation of leaf proximate compounds with ODAP

Correlations among the studied traits in 40 genotypes of grasspea for proximate and β-ODAP (leaf) and the association analysis reveals that the parameters sugar, TDF and Fe shows significant positive correlation and the parameters like Phenol and ash shows significant negative correlation with the β-ODAP content. The parameter protein shows significant negative correlation with TDF, Fe and Mg. The parameter moisture content shows significant negative correlation with ash. The mineral Fe content shows significant positive correlation with Mg and Cu content shows significant positive correlation with Zn.

The trend curve shows the how the nutritional composition of grasspea is distributed in seed and leaf simultaneously and this concludes the distribution pattern of nutrients in the entire plant. The prominent difference in the curves of ash, Ca, Mg, Fe indicates that the contents difference might be due to biomass difference and the individual proximate compounds in each. The trend curve of β-ODAP between seeds and leaves indicate that the leaves have low β-ODAP content than the seeds in an average.

CHAPTER VI
SUMMARY AND CONCLUSION

Legumes are essential dietary source for both people and animals. They are the major sources of protein for the vegetarian people around the world. Although, we have reached the food sufficiency, the serious issues of current concern like yield plateau in major crops, pest resurgence, climate change, malnutrition and hidden hunger there is a need to look for alternate climate smart crop species for sustenance and sustainable living and food production in the world. The viable solution for this situation is the use of crop wild relatives or underutilized crop for the sustainable agriculture. Grasspea (*L. sativus* L.) is one such legume where its high nutritional profile in their seed and leaves, pharmaceutical application with the extraction of secondary metabolites, industrial uses, as food (pulse and green leafy vegetable) and fodder purposes had been diminished with the presence of neurotoxin β-ODAP which causes lower limb paralysis. But there was no study proved that β-ODAP was the sole cause of the lathyrism and nowadays with the improvement in the sequencing and breeding technologies, there is an availability of germplasm or varieties of grasspea which are having low β-ODAP this was accomplished by breeders all over the world working with grasspea crop. Out of 184 species of genus Lathyrus, nine species are present in India. The present study defined and accomplished with the aim to characterize the grasspea germplasm for its agro-morphological, molecular and biochemical traits and help to trace out the evidences to bring back this golden legume "grasspea" into the Indian agriculture.

The experiment was carried out in *Rabi* 2019-2020 and 2020-2021 for entire germplasm accessions of 168 numbers along with four checks Ratan, Prateek, Mahateora and Narayangon for agro-morphological characterization at the experimental fields of Pusa research farm, ICAR-NBPGR, New Delhi. Molecular characterization was conducted in the Division of Genomic Resource, ICAR-NBPGR, New Delhi and the biochemical parameters were evaluated in the Division of Germplasm Evaluation, ICAR-NBPGR, New Delhi. The results obtained are summarized as below:

Agro-morphological characterization of grasspea

- The germplasm which was taken under study have exhibited high agro-morpho variability in plant height up to 119.45cm (BANG285) which is directly linked with the plant biomass which in turn the important trait for fodder and forage purposes.
- ANOVA revealed that highly significant variability was present among the 168 accessions of grasspea studied for the quantitative traits like plant height, petiole length, early maturing lines i.e., 80%M, seed length and hundred seed weight.
- Qualitative traits like seedling pigmentation, flower color, seed shape and seed coat color were showing variation within accessions.
- The variability studies revealed that PCV was higher than the GCV in all the 14 traits taken. The high heritability along with high GAM observed for the traits PHT, PTL and HSWT; high heritability with moderate GAM was observed for the traits DFF, PDW, NSP, SDL and SDW. These traits are governed by additive gene action and selection for these traits will be done easily. Hundred seed weight showed significant positive correlation with the trait's PTL, SDL and SDW. With path analysis, it was observed that the traits like 50%F, 80%F, 80%M and SDW showed high direct effect and the trait PTL showed moderate directs to the hundred seed weight. So, the selection of these traits would be considered mindful in breeding programmes.
- The correlation study revealed that the HSWT showed significant positive correlation with the traits like PTL, SDL and SDW. Therefore, selection these traits which are positively correlated with HSWT will ultimately increase the HSWT and thereby the seed yield.
- Traits that demonstrated notably high positive correlations with HSWT based on correlation analysis such as PTL, SDW and SDL and the traits that exhibited very high and direct and indirect effects on HSWT through path analysis, hold promise for inclusion in upcoming plant breeding programs.
- The cluster analysis based on 14 quantitative traits grouped all the 168 accessions of grasspea into four major clusters, at the distance of 10 grouped into 35 viz., Cluster I, II, III, IV and V

- PCA analysis was employed to examine the 14 quantitative characters that played an important role in establishment of phenotypic variability among the accessions. The first five principal components contributed 70.63% to the total genetic variability exhibited by the germplasm. The variability on the first principal component (28.14 %) was accounted by high positive loading for the traits 50%F, 80%F, 80%M and DFF.
- Agro-morphological characterization identified promising accessions for important traits like hundred seed weight – IFLA1439 (14.02g), IFLA432 (13.38g), IC634674 (13.384g) and IFLA143 (12.32g); early flowering accessions- BANG31 (64.41days), IC525179 (63.34days), BANG208 (69.28days), BANG285, IC0634660, IC0634661 (69.31days); higher plant height- BANG285 (119.45cm); number of pods per plant- IFLA276 (87.08 pods), IC489623 (86.27pods); days to 80% maturity- BANG31 (112.35days).

Molecular characterization by SSR markers

- The modified DNA protocol as mentioned Figure 3.3 displays remarkable stability, remaining viable for over a year when stored at 4°C which starkly contrasts with earlier methods with reduced maintenance costs and make it as the preferred option for DNA extraction in grasspea.
- In the present study, 20 polymorphic primers generated polymorphic information content (PIC) value was ranged from 0.02 to 0.67 with an average of 0.34. The maximum PIC was observed for primer LTR12 (0.67) and the minimum for LTR62 (0.02). The PIC value of primers indicated greater diversity within and between studied grasspea accessions and also revealed that selected primers are very informative and can be utilized in the further studies.
- In hierarchical clustering, analysis of 168 accessions using SSR markers of grasspea were grouped into four major clusters (cluster I, cluster II and cluster III).
- A principal coordinate analysis (PCoA) reveals that the first three PCoA axes explained 13.85%, 9.20%, 8.83% of variation, respectively. The PCoA also grouped 168 accessions of grasspea into four clusters, which compliments the results of cluster analysis.

Biochemical characterization of Grasspea

Seed:

- The accessions which are showing high nutritional composition in seed are given as follows: protein content - 32.02 % (BANG31), with best performing check Ratan 27.62 %; starch content- 39.00 g/100g (IC0634654) with value of best performing checks Ratan 32.02/100g; TSS content- 8.83 g/100 g (IC208430) with best performing check Narayangon 6.02 g/100g. The total phenolic content - 0.39 g/100g (BANG292) with the best performing check Prateek has lower phenol content 0.45 g/100g than the genotypes; TDF - 19.91% % (IC208430) with the best performing check ratan having 13.21%
- The mineral composition like Fe content - 164.35ppm (BANG206) with best performing check is Ratan 103.38ppm; Cu content - 12.97ppm (BANG291) with best performing check is mahateora 10.32ppm; Zn content - 101.86ppm (Bio520) and best performing check is ratan 87.86 ppm; Ca content- 557.70ppm (BANG208) and best performing check is mahateora 378.36 ppm and Mg content- 1677.59ppm (BANG218) and the best performing check is ratan 1445.12 ppm.
- Aminoacid profiling reveals that sarcosine accounts for highest (11.64 g/100g protein) followed by cysteine (8.91 g/100g protein), glutamic acid (6.79 g/100g protein) and valine accounts for the lowest (0.23 g/100g protein)
- The method of extraction and the solvents used in the β-ODAP analysis makes it the economically feasible in comparison with the existing methods available.
- The accessions which are having low β-ODAP content in their seeds include IC525182 (0.074), IC0634755 (0.078), IFLA2924 (0.088) and the accessions having high β-ODAP in the seeds include IFLA1926 (0.327) and IFLA2765 (0.340) expressed in Unit: mg/g. The method of extraction and the solvents used are concluded from two different paper works and makes it the economically feasible one.
- The association analysis revealed that there is no significant positive correlation between the studied biochemical parameters with the β-ODAP content of seed. The parameter TDF (-0.33) shows significant negative correlation with the β-ODAP content.

Leaf:

- This is the first report of exhaustive study on the nutritional composition of grasspea.
- The accessions which are showing high nutritional composition in leaf are given as follows: protein content- 33.50 % (IFLA1193), with best performing check was Prateek 29.33 %; TSS content - 7.36 g/100 g (IFLA1715) with a best performing check was ratan 3.35 g/100g; total phenolic content - 0.36 g/100g (IC349809) with best performing check Ratan has lower phenol content 0.32 g/100g than the genotypes. The TDF content - 31.05 % (BANG198) with best performing check having higher TDF was Narayangon 24.32%.
- The mineral composition like Fe content- 795.21 ppm (IFLA479) with best performing check was Ratan having 436.61ppm; Cu content- 23.45 (IFL2765) with best performing check was ratan 9.76ppm; Zn content- 90.22 ppm (IC208430) with best performing check was Narayangon 68.12 ppm.; Ca content- 2849.27ppm (IC208437) with best performing check was Narayangon 1065.32 ppm and Mg content- 3739.89 (IC0634654) with best performing check was ratan having 1767.32 ppm.
- In the aminoacid profiling of leaves, sarcosine accounts for highest (14.6 g/100g protein) followed by cysteine (7.85 g/100g protein), glutamic acid (6.82 g/100g protein) and the lowest observed is valine (0.3 g/100g protein)
- The accessions which are having low β-ODAP values are observed for IFLA1193 (0.03), IC470982 (0.04) and higher β-ODAP values are observed for IC18879 (0.25), IFLA479 (0.29). This indicates that the β-ODAP content in leaf was lower than the seed and it can be recommended as green leafy vegetable.
- The association analysis reveals that the parameters sugar, TDF and Fe shows significant positive correlation and the parameters like Phenol and ash shows significant negative correlation with the β-ODAP content.

With the all the aspects of the objective of current research work, the following elite accessions which are having superior agro-morphological traits, nutritional composition with low β-ODAP values are found and these accessions can be further validated and used in the crop improvement programmes for both seed and as green leafy vegetable. The accessions include

- ✓ IC208430 having early days to first flowering, maturity, higher number of pods per plant, high range of protein and minerals with low β-ODAP in both seed and leaf
- ✓ IC0634654 having early days to first flowering, maturity, higher plant height, hundred seed weight, high range of protein and minerals with low β-ODAP in both seed and leaf
- ✓ IC0634662 having early days to first flowering, maturity, higher number of pods per plant, number of seeds per pod, hundred seed weight, high range of protein and minerals with low β-ODAP in both seed and leaf
- ✓ IC0634674 having early days to first flowering, maturity, higher number of pods per plant, number of seeds per pod, plant height, hundred seed weight, high range of protein and minerals with low β-ODAP in both seed and leaf
- ✓ BANG285, BANG31 having early days to first flowering, maturity, higher number of pods per plant, hundred seed weight, high range of protein and minerals with low β-ODAP in both seed and leaf
- ✓ IFLA143, IFLA1193 having early days to first flowering, maturity, higher number of pods per plant, hundred seed weight, high range of protein and minerals with low β-ODAP in both seed and leaf

Conclusion and Future aspects

Grasspea is the multifaceted, climate smart legume high nutritional composition finds its application in pharmaceutical and industrial sectors. It has morphological adaptations such as winged pods, stem, hardy penetrating root system makes them as a suitable source of underutilized plant tolerant to biotic and abiotic stresses and can be grown with minimal inputs. The current study with the objectives covering the major three aspects such as agro-morphological, molecular, biochemical characterization yields out the list of accessions which are mentioned above as important note for grasspea breeding programmes. The variability studies revealed that traits like plant height, petiole length and hundred seed weight are having high heritability along with high GAM. These traits are governed by additive gene action and selection for these traits will be done easily. Agro-morphological characterization identified promising accessions for important traits like hundred seed weight – IFLA1439 (14.02g), IFLA432 (13.38g), IC634674 (13.384g) and IFLA143 (12.32g); early flowering accessions- BANG31 (64.41days), IC525179 (63.34days),

BANG208 (69.28days), BANG285, IC0634660, IC0634661 (69.31days); higher plant height- BANG285 (119.45cm); number of pods per plant- IFLA276 (87.08 pods), IC489623 (86.27pods); days to 80% maturity- BANG31 (112.35days). The modified DNA protocol displays remarkable stability and make it as the preferred option for DNA extraction in grasspea. The 20 polymorphic SSR markers generated polymorphic information content (PIC) value was ranged from 0.02 to 0.67 with an average of 0.34. The PIC value of primers indicated greater diversity within and between studied grasspea accessions and also revealed that selected primers are very informative and can be utilized in the further studies.

The biochemical characterization revealed the elite accessions which are having early days to first flowering, maturity, higher number of pods per plant, number of seeds per pod, plant height, hundred seed weight, high range of protein and minerals with low β-ODAP in both seed and leaf. The elite accessions include IC208430, IC0634654, IC0634662, IC0634674, BANG285, BANG31, IFLA143, IFLA1193. The method of extraction and the solvents used in the β-ODAP estimation in the present study makes it the economically feasible in comparison with the existing methods available. The higher sarcosine in aminoacid estimation validates that this can be derived from seeds and leaves which can be used in treating gums as a component of toothpaste as per the literature studies. These elite outperforming accessions can be used as the donors for future breeding work.

CHAPTER VII
ABSTRACT

Grasspea is a versatile cool season legume crop with exemplary nutritional profile is a spectacular choice as climate smart species to overcome malnutrition in the arid and semi-arid regions of the world. The present experiment was conducted at Pusa research farm, ICAR- NBPGR, New Delhi during the two consecutive years of rabi season (2019-20 and 2020-21). The experimental material consists of 168 germplasm along with four checks namely, Ratan, Prateek, Mahateora and Narayangon and the design is augmented block design. Agro-morphological characterization for 14 quantitative and 20 qualitative traits, identified promising accessions for important traits like hundred seed weight – IFLA1439 (14.02g), IFLA432 (13.38g), IC634674 (13.384g) and IFLA143 (12.32g); early flowering accessions- BANG31 (64.41days), IC525179 (63.34days), BANG208 (69.28days), BANG285, IC0634660, IC0634661 (69.31days); higher plant height- BANG285 (119.45cm); number of pods per plant- IFLA276 (87.08 pods), IC489623 (86.27pods); days to 80% maturity- BANG31 (112.35days). These outperforming accessions can be used in the further grasspea breeding programmes for the desired traits. The 20 polymorphic SSR markers generated polymorphic information content (PIC) value was ranged from 0.02 to 0.67 with an average of 0.34. The PIC value of primers indicated greater diversity within and between studied grasspea accessions and also revealed that selected primers are very informative and can be utilized in the further studies. The biochemical characterization which includes both seed and leaf for proximate parameters like protein, starch, sugar, phenol, moisture, ash, TDF, minerals like Fe, Cu, Zn, Ca and Mg along with aminoacid profiling and β-ODAP estimation using UPLC revealed the elite accessions which are having superior agro-morphological traits like early days to first flowering, maturity, higher number of pods per plant, number of seeds per pod, plant height, hundred seed weight along with high range of protein and minerals with low β-ODAP in both seed and leaf. The elite accessions include IC208430, IC0634654, IC0634662, IC0634674, BANG285, BANG31, IFLA143, IFLA1193. These elite outperforming accessions can be used as the donors for future breeding work.

खेसारी दाल में मॉर्फो-पोषण विविधता पर अध्ययन (लैथिरस सैटिवस एल.)
संक्षेप

खेसारी दाल एक बहुमुखी ठंडे मौसम की फलीदार फसल है, जिसमें अनुकरणीय पोषण प्रोफ़ाइल है, जो दुनिया के शुष्क और अर्ध-शुष्क क्षेत्रों में पोषण की कमी दूर करने के लिए जलवायु स्मार्ट प्रजाति के रूप में एक शानदार विकल्प है। वर्तमान प्रयोग रबी सीज़न (2019-20 और 2020-21) के लगातार दो वर्षों के दौरान पूसा अनुसंधान फार्म, आईसीएआर- एनबीपीजीआर, नई दिल्ली में आयोजित किया गया था। प्रायोगिक सामग्री में 168 जर्मप्लाज्म के साथ-साथ चार चेक, रतन, प्रतीक, महाटेओरा और नारायणगोन शामिल हैं और डिज़ाइन संवर्धित ब्लॉक डिज़ाइन है। 14 मात्रात्मक और 20 गुणात्मक लक्षणों के लिए कृषि-रूपात्मक लक्षण वर्णन, सौ बीज वजन जैसे महत्वपूर्ण लक्षणों के लिए आशाजनक परिग्रहण की पहचान की गई - IFLA1439 (14.02g), IFLA432 (13.38g), IC634674 (13.384g) और IFLA143 (12.32g); प्रारंभिक पुष्पन परिग्रहण- BANG31 (64.41 दिन), IC525179 (63.34 दिन), BANG208 (69.28 दिन), BANG285, IC0634660, IC0634661 (69.31 दिन); उच्च पौधे की ऊंचाई- BANG285 (119.45 सेमी); प्रति पौधा फलियों की संख्या- IFLA276 (87.08 फली), IC489623 (86.27 फली); दिन से 80% परिपक्वता तक - BANG31 (112.35 दिन)। इन बेहतर प्रदर्शन करने वाले उपकरणों का उपयोग वांछित गुणों के लिए खेसारी के आगे के प्रजनन कार्यक्रमों में किया जा सकता है। 20 बहुरूपी एसएसआर मार्करों द्वारा उत्पन्न बहुरूपी सूचना सामग्री (पीआईसी) का मान 0.34 के औसत के साथ 0.02 से 0.67 तक था। प्राइमरों के पीआईसी मूल्य ने अध्ययन किए गए खेसारी के मैदानों के भीतर और उनके बीच अधिक विविधता का संकेत दिया और यह भी खुलासा किया कि चयनित प्राइमर बहुत जानकारीपूर्ण हैं और आगे के अध्ययनों में इसका उपयोग किया जा सकता है। जैव रासायनिक लक्षण वर्णन जिसमें प्रोटीन, स्टार्च, चीनी, फिनोल, नमी, राख, टीडीएफ, Fe, Cu, Zn, Ca और Mg जैसे खनिजों के साथ-साथ अमीनो एसिड प्रोफाइलिंग और UPLC का उपयोग करके β-ODAP अनुमान के लिए बीज और पत्ती दोनों शामिल हैं। विशिष्ट परिग्रहण जिनमें बेहतर कृषि-रूपात्मक विशेषताएं हैं जैसे शुरुआती दिन से लेकर पहले फूल आने तक, परिपक्वता, प्रति पौधा फलियों की अधिक संख्या, प्रति फली में बीजों की संख्या, पौधे की ऊंचाई, सौ बीज का वजन, कम ओडीएपी के साथ प्रोटीन और खनिजों की उच्च श्रेणी। बीज और पत्ती दोनों में. विशिष्ट पहुंच में IC208430, IC0634654, IC0634662, IC0634674, BANG285, BANG31, IFLA143, IFLA1193 शामिल हैं। बेहतर प्रदर्शन करने वाले इन विशिष्ट परिग्रहणों का उपयोग भविष्य में प्रजनन कार्य के लिए दाताओं के रूप में किया जा सकता है।

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ABBREVIATIONS

%	Per cent
°C	Degree celsius
AMOVA	Analysis of molecular variance
ANOVA	Analysis of variance
BC	Before Christ
BCE	Before the Common Era
Ca	Calcium
cm	Centimeter
CTAB	CetylTrimethylAmmonium Bromide
Cu	Copper
CV	Coefficient of Variation
DNA	DeoxyRibonucleic Acid
EDTA	EthyleneDiamineTetraacetic Acid
EST	Expressed Sequence Tags
et al	and others
F	Forward Primer
Fe	Iron
g	Gram
GA	Genetic Advance
GAM	Genetic Advance as percent of Mean
GCV	Genotypic Coefficient of Variation
GENSTAT	General statistics
GOI	Government of India
ha	hectare
Ho	Heterozygosity
HPLC	High Performance Liquid Chromatography
IARI	Indian Agricultural Research Institute
IC	Indigenous Collection
ICAR	Indian Council of Agricultural Research

ICARDA	International Center for Agricultural Research in the Dry Areas
ITPGRFA	International Treaty on Plant Genetic Resources for food and Agriculture
LTR	Lathyrus
M	Molar
MA&FW	Ministry of Agriculture and and Farmers Welfare
MAS	Marker-assisted Selection
Max.	Maximum
mg	Milligram
Mg	Magnesium
Min.	Minimum
ml	Milliliter
mm	Millimeter
mM	Millimolar
NBPGR	National Bureau of Plant Genetic Resources
NBPGR	National Bureau of Plant Genetic Resources
NIRS	Near infra-red spectroscopy
No.	Number
O.D.	Optical Density
PCA	Principal Component Analysis
PCoA	Principal Coordinate Analysis
PCR	Polymerase Chain Reaction
PCV	Phenotypic Coefficient of Variation
pH	Potential of Hydrogen
PIC	Polymorphic Information content
pmol	picomoles
ppm	Parts per million
PVP	Polyvinylpyrrolidone
R	Reverse Primer
RNA	RiboNucleic Acid
rpm	Revolutions Per Minute

SE	Standard Error
SSR	Simple Sequence Repeats
TDF	Total Dietary Fibre
TE	Tris-EDTA buffer
TE	Tris-EDTA
TRIS	tris(hydroxymethyl)aminomethane
TSS	Total Soluble Sugar
UPGMA	Unweighted pair-group average
UPLC	Ultra-performance liquid chromatography
V/V	Volume/Volume
viz.,	Namely
Zn	Zinc
β-ODAP	β-N-oxalyl-l-α,β-diaminopropionic acid
µg	Microgram

ANNEXURE

Table 1. First season data for 14 quantitative traits

Block	Treatment	PHT	PTL	DFF	50%F	80%F	80%M	PDL	PDW	NPB	NPP	NSP	SDL	SDW	HSWT
Block I	G1	63.4	3.2	80	85	101	132	3.8	1.13	5	60.5	4	7.76	6.35	14.6
Block I	G2	69.4	3.2	80	85	101	132	4.1	0.97	6.5	86.5	4	6.73	5.72	11.96
Block I	G3	66.5	3.5	69	75	91	122	4.2	1.07	6.5	60.5	4	6.57	5.77	11.58
Block I	G4	91.5	4	81	88	104	135	4.1	1.07	7	58.5	3.7	7.32	5.84	11.88
Block I	G5	83.5	4.1	82	89	105	136	3.6	1.07	7	83.5	3.7	7.51	6.67	13.8
Block I	G6	84.2	5	84	90	106	137	4.2	1	7.5	78.5	3	8.16	6.45	15.08
Block I	G7	73.1	4	82	88	104	135	3.7	1	7.5	67.5	3.7	6.86	5.88	13.18
Block I	G8	69.3	4.5	75	80	96	127	5.4	1	7.5	71.5	4	6.29	5.74	9.76
Block I	G9	73.5	2.5	81	86	102	133	3.6	1.03	7.5	66.5	4.7	5.95	5.31	10.46
Block I	G10	73.5	2.5	82	90	106	137	4.2	1.03	7.5	69.5	3.7	6.81	6.22	10.78
Block I	G169	76.5	3.5	79	85	101	132	4.2	1.07	6.5	60.5	4	6.57	5.77	9.58
Block I	G11	69.5	2	77	83	99	130	4.6	0.93	7.5	75.5	4.7	7.6	6.09	10.4
Block I	G12	60.5	2.5	76	81	97	128	3.9	0.93	7.5	73.1	3.7	6.36	4.95	11.78
Block I	G13	75.9	2	80	85	101	132	4.5	1.07	7.5	74.5	4	6.78	5.63	10.52
Block I	G14	79.4	3.2	82	88	104	135	4.5	1	7.5	61.5	4.3	7.12	6.89	12.08
Block I	G15	76.3	3.2	80	87	103	134	3.9	0.97	7.5	40.5	4	7.52	6.44	13.66
Block I	G16	71.6	2.5	82	93	109	140	3.7	1.03	7	69.5	4	7.24	6.19	12.62
Block I	G170	79.3	4.5	71	80	96	127	4.4	1	7.5	71.5	4	6.29	5.74	10.76

Block I	G17	69.2	2.3	82	92	108	139	5.4	1.1	7	79.5	3.7	5.22	6	15.22
Block I	G18	69.5	2.3	81	90	106	137	3.6	0.97	7	65.5	3.7	7.57	5.98	11.4
Block I	G19	86.7	3.5	82	89	105	136	4.2	1.03	7	52.5	5	6.3	5.22	8.9
Block I	G20	60.7	2.5	75	81	97	129	4.1	0.93	7	68.5	4.5	6.85	5.79	8.46
Block I	G21	77.1	2	78	85	101	133	4.1	1.03	7	66.5	4	6.2	5.53	10
Block I	G171	76.6	3.6	78	84	100	132	3.7	0.93	7	68.5	3	6.64	5.73	9.6
Block I	G22	64.7	2	76	81	97	129	3.7	0.93	7	62.5	4.3	4.79	4.21	9.08
Block I	G23	45	2.5	80	88	104	136	3.9	1	7	72.5	4.3	6.57	5.62	8.06
Block I	G24	55.6	2.5	80	88	104	136	3.7	0.83	7	75.5	4.3	6.39	5.41	8.06
Block I	G25	66.6	3.6	78	84	100	132	3.7	0.93	7	68.5	3	6.64	5.73	11.6
Block I	G26	69.4	1.8	75	84	100	132	4.2	1.03	7	71.5	4	5.75	4.88	6.76
Block I	G27	58.2	1.3	75	84	100	132	3.6	0.83	7	75.5	4	5.72	5.18	7.8
Block I	G28	68.2	2	75	84	100	132	4.2	1	7	62.5	4.3	6.4	5.46	9.78
Block I	G29	58.2	2	80	88	104	136	4.1	0.97	7	75.5	4.3	6.6	6.21	8.78
Block I	G30	82.7	2.5	77	82	99	130	3.8	1.03	7	64.5	3	6.95	5.99	10.06
Block I	G31	69.8	2	82	89	106	137	4.1	0.97	7	66.5	4	7.27	6.23	11.06
Block I	G32	47.9	2.5	77	82	99	130	4.1	0.93	7	65.5	3.7	7.37	6.39	10.88
Block I	G33	70.5	2.9	72	78	95	126	4.1	1.03	7	71.7	4.3	8.39	6.49	11.68
Block I	G172	74.6	3	78	90	107	138	3.9	1.07	7	68.5	3	6.76	5.2	10.16
Block I	G34	71.5	3	82	93	110	141	3.8	0.97	7	66.5	3	7.3	5.69	16.12
BLOCK II	G35	62.5	1.5	69	75	92	123	4.5	1	7	65.5	4	8.09	6.39	12.42

BLOCK II	G36	67.8	3	82	89	106	137	4.4	1.07	7	47.5	4	6.82	5.87	15.18
BLOCK II	G37	74.6	3	82	90	107	138	3.9	1.07	7	68.5	3	6.76	5.2	13.16
BLOCK II	G172	80.4	2.4	75	81	98	132	4.6	1	6.5	43	4.3	6.21	5.47	11.2
BLOCK II	G38	64.9	2	82	91	108	139	4.3	0.93	7	68.5	4	7.59	6.33	14.12
BLOCK II	G39	61.9	3	82	90	107	138	3.7	1.07	7.5	72.5	3	7.58	6.59	13.3
BLOCK II	G40	59.9	3	82	90	107	139	3.9	1	7.5	65.5	3	7.85	6.29	12.94
BLOCK II	G41	78.5	2.3	82	88	105	137	3.9	1.03	7.5	62.5	3	6.78	6.09	9.34
BLOCK II	G42	55.5	3	80	85	102	134	3.7	1.03	7.5	46.5	4	7.4	6.61	12.3
BLOCK II	G43	72.2	1.8	82	87	104	136	4.5	1.03	7	65.5	3.7	7.89	6.87	13.86
BLOCK II	G44	77.6	2	77	81	98	130	3.9	1.03	7	69.5	3.7	7.47	6.22	13.8
BLOCK II	G45	60.6	2.3	82	88	105	137	3.8	1.23	6	74	3.3	5.7	5.85	7.42
BLOCK II	G46	60.9	3	82	89	106	138	3.6	1.03	6	70	4	6.5	6.01	10.18
BLOCK II	G170	74.6	3	76	80	97	129	3.9	0.97	6	77	3.7	6.71	7.22	10.82
BLOCK II	G47	71.2	3	82	90	107	139	3.9	0.83	6.5	73	3.7	6.5	6.1	10.74
BLOCK II	G48	78.7	2	82	90	107	139	4.1	0.83	6.5	77	3	6.76	5.61	10.96
BLOCK II	G49	67.2	3	76	81	98	130	3.9	1.2	6.5	64	3.7	5.93	5.32	8.2
BLOCK II	G50	78.9	2.4	77	83	100	132	4.5	1.03	6.5	77	4	5.74	5.09	8.58
BLOCK II	G51	69.1	3	77	83	100	132	3.7	1	6.5	66	4.7	5.29	5.09	7.7
BLOCK II	G52	56.2	2.8	80	88	105	137	3.9	1.03	4.5	68	4.3	5.84	5.51	7.84
BLOCK II	G53	63.4	2.5	82	90	107	139	4.5	0.97	6.5	58.2	5	6.32	5.31	7.02
BLOCK II	G169	66.2	2.8	84	93	110	142	3.9	1.03	6.5	68	4.3	5.84	5.51	7.84

BLOCK II	G54	56.8	2.5	82	90	107	139	4.1	0.83	6.5	68	4	6.41	5.55	8.2
BLOCK II	G55	81.2	2.4	82	91	108	140	3.8	0.87	6.5	67	3.7	6.71	5.59	9.86
BLOCK II	G56	85.4	2.5	77	83	100	132	3.8	1.03	6.5	49	3.7	5.77	5.59	10.12
BLOCK II	G57	86.5	2	78	84	101	133	4.1	1.03	6.5	70	4	6.61	5.8	10.44
BLOCK II	G58	74.1	2	72	78	95	127	3.8	1.03	6.5	70	4.3	6.73	6.01	10.86
BLOCK II	G59	83.2	2	78	85	102	134	3.9	1.07	6.5	74	3.7	6.49	5.92	11.18
BLOCK II	G60	81.4	3	77	84	101	133	3.8	1.17	6.5	67	4	6.57	5.61	9.92
BLOCK II	G61	77.9	2	77	84	101	133	4.2	1.07	6.5	64	4	6.33	6	11.18
BLOCK II	G171	87.4	2.5	77	82	99	131	3.8	1.03	6.5	49	3.7	5.77	5.59	10.12
BLOCK II	G62	87.5	3.1	75	80	97	129	4.2	1.03	6	48	4.3	6.37	5.51	11.58
BLOCK II	G63	69.5	2.5	80	88	105	137	4.5	0.9	6	67	3.7	6.3	5.47	9.6
BLOCK II	G64	59.2	3	75	81	98	130	4.5	0.83	6	71	3.3	6.33	5.3	9.34
BLOCK II	G65	79.2	2.5	75	81	98	130	3.8	1.03	6	63	4	6.18	5.77	9.82
BLOCK II	G66	68.8	2.3	75	80	97	129	5.4	1	6	89	3	6.69	5.74	8.22
BLOCK II	G67	75.2	2.5	76	83	100	132	4.6	0.87	6	63	3.7	5.89	5.28	9.22
BLOCK II	G68	64.2	3	82	88	105	137	3.8	0.93	6	61	4	6.42	5.07	10.24
BLOCK II	G69	92.3	3	84	94	111	143	4.5	0.97	6	86	3.3	7.54	6.41	14.74
Block III	G70	79.5	2.9	82	94	111	143	3.7	1.23	6	81	4.7	7.03	6.62	10.56
Block III	G71	70.4	2.7	82	94	111	143	4.5	1	6	70	4	6.68	5.29	10.74
Block III	G72	63.9	2.8	77	86	103	135	3.9	0.93	6	74	4.3	6.3	5.34	9.54
Block III	G73	69.7	2.2	78	88	105	137	3.9	1.23	6	69	4	6.43	5.42	9.84

Block III	G74	66.7	2.2	82	88	105	139	3.8	0.77	6	78	4	6.25	5.44	10.06
Block III	G75	53.7	3.1	82	89	106	140	3.9	1.03	6	66.54	4	6.16	5.36	10
Block III	G171	73.6	2	75	81	98	127	4.4	1.03	7	66.8	4.7	5.47	4.65	8.81
Block III	G76	66.7	2.4	82	89	106	140	3.7	1	6.5	77	4	7.05	6	13.24
Block III	G77	66.7	2.5	82	90	107	141	4.5	0.93	6.5	81	4	6.96	6.18	11.76
Block III	G78	50.5	3.1	82	90	107	141	4.1	1.07	6.5	67	4.3	4.88	4.22	9.16
Block III	G79	52.5	2.2	70	77	94	128	5.4	0.87	6.5	54	4	5.49	4.89	8.36
Block III	G80	68.7	3	78	84	101	135	5.4	0.97	6.5	70	3.7	5.52	4.68	8.44
Block III	G81	56.7	2.5	76	81	98	132	4.5	0.93	7	70	4.7	5.83	5.01	8.24
Block III	G82	70.9	2	78	84	101	135	4.1	0.93	7	74	4	5.32	4.7	8.42
Block III	G83	56.2	2.2	75	81	98	132	4.5	0.97	7	67	3.7	6.38	4.98	9.42
Block III	G84	49.3	2	78	84	101	135	3.9	0.93	7	64	4	6.5	4.61	8.4
Block III	G85	63.9	2.1	80	86	103	137	3.7	0.9	7	48	2.7	5.44	4.56	8.82
Block III	G170	71.2	2	78	85	102	136	3.8	0.97	7	68	3.7	6.66	6.17	9.44
Block III	G86	51.2	2.4	82	89	106	140	5.4	1.07	7	67	4	6.12	5.2	8.52
Block III	G87	66.9	2.2	82	90	107	141	4.1	1.07	7	71	4.7	5.05	4.73	7.46
Block III	G88	65.4	2.4	82	90	107	141	3.8	1.03	7	63	4	4.93	4.5	7.28
Block III	G89	71.8	2.1	82	90	107	141	4.5	1	7	89	3.3	5.31	4.54	6.68
Block III	G90	60.6	3.1	82	90	107	141	4.3	1.03	7	63	3.7	4.57	4.22	13.52
Block III	G91	71.2	3.2	78	84	101	135	3.8	1.03	6.5	61	5.3	5.73	4.92	7.6
Block III	G92	65.7	2.1	82	88	105	139	4.1	1.03	6.5	86	4.5	5.56	4.76	8.34

Block III	G93	69.2	1.3	82	88	105	139	3.8	1.07	6.5	81	4.3	5.99	5.31	8.58
Block III	G94	72.2	3.2	82	89	106	140	3.9	1	6.5	70	4	5.98	5.3	8.76
Block III	G169	73.9	2.3	81	90	107	136	4.5	1.03	6.5	77	3.7	5.43	4.55	6.72
Block III	G95	81.2	2.3	75	81	98	132	3.8	1.03	6.5	74	3	6.15	5.41	9.66
Block III	G96	45.7	2.5	81	89	106	140	3.9	1	6.5	69	3.7	5.68	4.82	8.66
Block III	G97	61.2	2.5	82	91	108	142	3.8	0.97	6.5	72	4.3	5.75	4.35	7.74
Block III	G98	57.8	2.2	82	92	109	138	4.5	1.03	6.5	78	4	4.96	4.02	7.1
Block III	G99	58	2	77	84	101	130	3.7	0.93	6.5	68	3	5.7	4.63	7.24
Block III	G100	71.9	2.3	81	90	107	136	4.5	1.03	6.5	77	3.7	5.43	4.55	6.72
Block III	G101	64.2	2.5	80	88	105	134	3.8	1	6.5	64	4	5.77	5.19	6.8
Block III	G172	67.3	2.1	78	87	104	134	4.3	1.07	6.5	75.5	3.7	6.45	5.57	7.34
Block III	G102	65.9	2.2	76	82	99	128	3.7	0.83	7	64.8	2.3	5.68	4.91	8.5
Block III	G103	69.8	2	78	85	102	131	3.7	1.03	7	74.8	4.3	6	4.81	8.9
Block III	G104	73.7	2.2	78	85	102	131	4.5	1.1	7	77.8	4.3	6.83	5.49	8.22
BLOCK IV	G105	74.6	2.1	75	81	98	127	4.5	1	7	70.8	4.3	6.17	5.47	7.38
BLOCK IV	G169	60	2.5	84	90	107	140	4.5	1	6.5	64	4.7	6.04	6.11	8.11
BLOCK IV	G106	71.3	1.4	75	81	98	127	3.7	1.03	7	73.8	4.3	5.4	5.13	6.48
BLOCK IV	G107	76.7	2.1	78	84	101	130	3.8	1.07	7	77.8	4.7	5.38	5	6.6
BLOCK IV	G108	76.2	2.3	79	84	101	130	4.5	1.17	7	34.8	4	4.65	4.92	4.9
BLOCK IV	G109	83.6	2	79	83	100	129	3.7	1.03	7	77.8	4.3	4.41	4.23	6.12
BLOCK IV	G110	73.6	2	75	81	98	127	4.2	1.03	7	66.8	4.7	5.47	4.65	6.18

BLOCK IV	G111	79.2	2.1	75	81	98	127	4.4	1.07	6.5	62.5	4	4.87	4.38	5.58
BLOCK IV	G112	57.1	2.4	84	92	109	138	4.2	0.8	6.5	72.5	3.3	6.61	5.98	9.54
BLOCK IV	G113	48	2.5	63	70	87	116	4.5	0.83	6.5	75.5	3.7	6.85	5.82	9.03
BLOCK IV	G171	62	2.4	78	93	110	143	4.1	0.97	6.5	66	3	8.31	4.37	6.19
BLOCK IV	G114	58	2.3	73	81	98	127	3.7	0.97	6.5	68.5	4	7.83	6.14	9.21
BLOCK IV	G115	52	2.3	84	92	109	139	4.1	1.03	6.5	71.5	4	8.62	6.48	6.21
BLOCK IV	G116	57.3	2.1	84	92	109	139	4.5	1.07	6.5	75.5	3.7	6.45	5.57	7.34
BLOCK IV	G117	47.2	2.1	83	91	108	138	3.8	1	6.5	62.5	4	6.2	5.81	6.32
BLOCK IV	G118	62.11	2	84	91	108	138	3.7	1.03	6.5	75.5	4.3	5.8	5.25	7.11
BLOCK IV	G119	51.22	1.8	84	91	108	138	3.9	1.03	6.5	66.8	4	7.49	5.65	7.56
BLOCK IV	G120	53.21	2.1	70	76	93	123	4.2	0.83	6.5	68.8	3.3	8.56	5.4	5.38
BLOCK IV	G170	71	2.4	74	86	104	136	4.5	1	6	70	4.7	6.21	6.43	8.56
BLOCK IV	G121	66.8	2.1	84	90	107	137	4.2	0.97	6.5	67.8	4.7	6.49	5.29	5.99
BLOCK IV	G122	74	2.5	84	90	107	137	3.8	0.97	6.5	73.8	4	7.08	5.46	6.74
BLOCK IV	G123	65.47	2.5	86	95	112	142	3.7	0.83	6	68.8	4.3	6.86	6.67	11.22
BLOCK IV	G124	78	2.5	84	95	112	142	4.5	0.93	6	67.8	4	8.06	5.93	8.63
BLOCK IV	G125	59.2	2.2	87	95	112	142	4.5	0.87	6	49.8	4	7.8	5.49	7.25
BLOCK IV	G126	48.8	2	86	91	108	138	4.2	0.97	6	70.8	3.3	7.24	6.25	6.98
BLOCK IV	G127	61.3	2.4	86	91	108	138	4.5	0.93	6	70.8	3.3	7.49	6.18	6.14
BLOCK IV	G128	59	2.5	73	80	97	127	4.2	0.87	6	74.8	5	7.66	7.17	6.47
BLOCK IV	G172	72.7	1.2	77	86	103	136	4.2	1.03	6	70.5	3.7	5.2	4.98	8.33

BLOCK IV	G129	66	3	63	70	87	117	3.8	0.93	6	67.8	5	8.01	6	5.26
BLOCK IV	G130	67	2.5	85	92	109	139	3.9	1	6	64.8	4.3	6.49	5.44	7.41
BLOCK IV	G131	65	2.4	65	71	88	118	4.2	0.93	6	48.8	4.7	7.21	5.93	8.26
BLOCK IV	G132	77	2.4	70	77	94	127	4.4	1.03	6	47.5	4.3	6.02	5.47	8.35
BLOCK IV	G133	56	2.4	70	77	94	127	3.8	1	6	68.5	4.3	4.58	4.42	7.21
BLOCK IV	G134	58	1.4	70	79	96	129	4.5	1.03	6.5	68.5	4.3	6.84	5.84	5.62
BLOCK IV	G135	57	2	87	93	110	143	5.4	1.03	6.5	72.5	4.3	6.9	5.44	9.14
BLOCK IV	G136	50	2.5	84	90	107	140	4.5	1	6.5	64	4.7	6.04	6.11	8.11
BLOCK IV	G137	50	2.5	70	77	94	127	3.7	1.03	6.5	74	4	5.67	4.49	8.54
BLOCK IV	G138	58	2.5	70	76	93	126	4.5	1.03	6.5	77	4.3	5.66	5.11	9.11
Block V	G139	47	2.3	63	69	86	119	4.4	0.97	6.5	70	4	6.3	5.88	9.45
Block V	G169	71.35	3.15	81.5	89	105.5	137	3.65	1.05	6.5	64.25	4.17	6.2	5.64	8.71
Block V	G140	59	2.5	63	68	85	118	4.1	1.07	6.5	73	3.3	6.21	6.94	7.11
Block V	G141	116	2.2	70	76	93	126	3.9	1.07	6.5	77	4	7.15	5.6	6.84
Block V	G142	48	2.4	70	77	94	127	3.7	0.97	6.5	64	3.7	6.48	6.02	9.12
Block V	G143	49	2.1	84	94	111	144	3.8	1.03	6.5	77	3.7	7.86	5.84	6.46
Block V	G144	52	2.4	84	95	112	145	4.1	0.97	6.5	66	3	8.31	4.37	6.19
Block V	G145	53	1.5	86	94	111	144	4.6	0.77	6.5	68	3.3	6.85	5.92	6.99
Block V	G146	53	1.5	84	91	108	141	4.1	0.93	6	67	5	8.38	6.47	7.01
Block V	G147	57	2.8	84	94	111	144	4.2	0.93	6	73.2	3.7	7.42	5.57	7.03
Block V	G148	61	1.6	84	91	108	141	4.2	0.97	6	68	5	7.85	4.07	6.59

Block V	G149	78	2.3	84	91	108	141	3.7	1.03	6	67	3.7	6.84	6.54	5.66
Block V	G172	72.9	2.5	77	82.5	99.5	132.5	3.75	0.97	6.5	72.5	3.67	6.69	6.7	10.13
Block V	G150	62	2.4	73	80	97	130	4.2	1.03	6	49	3.7	8.21	6.03	6.78
Block V	G151	57	2.1	84	92	109	142	4.1	0.97	6	70	4	7.48	6.56	6.59
Block V	G152	51	2.4	84	93	111	143	4.5	1	6	70	4.7	6.21	6.43	7.56
Block V	G153	48	1.5	84	91	109	141	4.5	1.03	6	74	4.3	7.82	5.5	5.69
Block V	G154	54	2.8	70	77	95	127	4.1	1.03	6	66	4.3	8.33	6.82	6.22
Block V	G155	48	1.6	70	78	94	128	3.8	0.93	6	68.5	5	7.86	7.08	6.87
Block V	G156	60	2.3	84	91	108	141	4.2	1.03	6	71	3.7	6.9	5.72	8.95
Block V	G157	61	2.8	84	92	109	142	4.6	1	6.5	65.6	2.4	5.7	5.59	9.57
Block V	G158	65	2.1	84	91	108	141	4	1.03	6	71	3.7	6.09	7.06	9.44
Block V	G159	53	2	84	91	108	141	4.5	1.1	6	64	4	6.01	5.89	8.47
Block V	G160	62	1.3	84	92	109	142	4.1	0.93	6	44.5	4.7	5.99	6.31	7.55
Block V	G170	72.9	2.5	77	82.5	99.5	132.5	3.75	0.97	6.5	72.5	3.67	6.69	6.7	10.13
Block V	G161	47	3.1	87	97	114	147	4.1	1	6	70	5	7.09	6.18	8.02
Block V	G162	51	2	84	90	107	140	4.4	1.1	6	69.5	3.7	6.23	5.17	9.47
Block V	G163	60	2	84	91	108	141	4.1	0.93	6	76	4	6.26	5.45	10.01
Block V	G164	62.7	1.2	82	88	105	138	3.9	1.03	6	70.5	3.7	5.2	4.98	8.45
Block V	G171	59	2.05	84	91	108	141	4.26	1.07	6	67.5	3.83	6.05	6.48	8.96
Block V	G165	67.3	1.4	87	95	112	145	4.1	1.07	6	65.5	4	5.97	5.66	7.88
Block V	G166	73.1	1.4	84	92	109	142	4.2	1	6	79.5	5	6.22	5.32	6.22

Block V	G167	70.8	2	87	96	113	146	4.6	1.03	6	79	5.3	6.59	5.06	7.58
Block V	G168	56	2.4	87	95	112	145	5.4	1.03	6	67	4.7	6.4	5.76	7.44

G169- Ratan
G170- Prateek
G171- Mahateora
G172- Narayangon

Note

PHT - Plant height (cm)

PTL - Petiole length(cm)

DFF- Days to first flowering

50%F- Days to 50% flowering

80%F- Days to 80% flowering

80%M- Days to 80% maturity

NPB- Number of primary branches

NPP- Number of pods per plant

SDL- Seed length (mm)

SDW- Seed width (mm)

HSWT- Hundred seed weight (g)

Table 2. Second season data of 14 quantitative traits

Block	Treatment	PHT	PTL	DFF	50%F	80%F	80%M	PDL	PDW	NPB	NPP	NSP	SDL	SDW	HSWT
Block I	G1	66.6	2	82	87	103	134	3.53	1.03	7	63	4	7.54	6.13	13.86
Block I	G2	72.6	3.2	85	93	109	140	3.9	1.03	7	89	3.7	6.51	5.5	11.22
Block I	G3	69.7	3.2	82	88	104	135	3.2	1.03	7	63	4	6.35	5.55	10.84
Block I	G4	94.7	2.2	85	92	108	139	3.8	0.83	7.5	61	3.3	7.1	5.62	11.14
Block I	G5	86.7	2.5	85	92	108	139	3.27	0.97	7.5	86	3.7	7.29	6.45	13.06
Block I	G6	87.4	2.3	86	95	111	142	3.5	0.97	7.5	81	4	7.94	6.23	14.34
Block I	G7	76.3	2.2	85	92	108	139	4.2	0.83	7.5	61	4.7	7.1	5.62	12.44
Block I	G8	72.5	3.2	82	87	103	134	3.53	0.93	7.5	70	4	6.64	5.67	9.02
Block I	G9	76.7	2.3	68	74	90	121	3.43	1.11	7.5	74	3.3	6.07	5.53	9.72
Block I	G10	76.7	3.5	68	73	89	120	3.67	1	7.5	69	3.7	5.73	5.1	10.04
Block I	G169	80.5	3.4	82	89	105	136	3.23	1.65	7.5	72	5.2	6.59	6.01	8.84
Block I	G11	73.5	3.2	82	87	103	134	3.57	1.03	7.5	78	5	7.38	5.88	9.66
Block I	G12	64.5	3.4	68	73	89	120	4.2	1.13	7.5	75.6	4.7	6.14	4.74	11.04
Block I	G13	79.9	3	82	87	103	134	4.24	0.87	7.5	77	4	6.56	5.42	9.78
Block I	G14	83.4	2	62	68	84	115	3.57	0.97	7.5	64	4.3	6.9	6.68	11.34
Block I	G15	80.3	3.4	63	69	85	116	3.37	1.03	7.5	46.5	3.8	7.3	6.23	12.92
Block I	G16	75.6	3.2	85	92	108	139	3.3	0.93	7.5	72	3.7	7.02	5.98	11.88
Block I	G170	83.3	2.5	86	95	111	142	3.57	0.87	7.5	81	4.3	5.02	5.79	10.02
Block I	G17	73.2	2	72	88	104	135	3.57	0.93	7.5	64	4	6.9	6.68	14.48
Block I	G18	73.5	2.2	86	95	111	143	3.47	0.93	7.5	67	3.6	7.37	5.75	10.66

Block I	G19	90.7	2.4	68	73	89	121	3.63	0.93	7.5	54	3.7	6.1	4.99	8.16
Block I	G20	64.7	2.3	62	67	83	115	3.2	0.97	7.5	70	4	6.65	5.56	7.72
Block I	G21	81.1	2.1	82	87	103	135	3.2	1.03	7.5	68	3.3	6	5.3	9.26
Block I	G171	80.6	2	84	89	105	137	3.43	1.07	7.5	64	4.3	4.59	4.2	8.86
Block I	G22	68.7	1.8	71	76	92	124	3.3	0.97	7.5	74	3.7	6.37	5.39	8.34
Block I	G23	45.5	2.1	61	69	85	117	3.57	1.03	7.5	77	4.3	6.19	5.18	7.32
Block I	G24	59.6	2.5	77	84	100	132	3.27	1.03	7.5	64	4.3	6.22	5.23	7.32
Block I	G25	71.6	2.1	82	88	104	136	3.3	1.03	7.5	70	4.7	6.44	5.5	10.86
Block I	G26	74.4	2.5	57	62	78	110	4.5	0.83	7.5	73	3.3	5.55	4.65	6.02
Block I	G27	63.2	2.5	68	74	91	122	3.43	0.97	7.5	77	4	5.54	4.95	7.06
Block I	G28	73.2	2.5	68	74	91	122	3.27	1.07	7.5	64	4.3	6.22	5.23	9.04
Block I	G29	63.2	2.2	68	74	91	122	3.27	1.03	7.5	77	4.7	6.42	5.98	8.04
Block I	G30	87.7	2	68	74	91	122	3.37	0.47	7.5	66	4	6.77	5.76	9.32
Block I	G31	72.8	2.1	77	87	104	136	3.8	0.97	7.5	67	3.3	7.67	6.05	10.32
Block I	G32	74.8	2.4	71	77	94	125	3.2	1.03	7.5	68	3.7	7.09	6	10.14
Block I	G33	52.9	2.5	63	69	86	117	3.33	0.97	7.5	67	4	7.19	6.16	10.94
Block I	G172	75.5	3	62	68	85	116	3.3	0.44	7.5	73.2	4	8.21	6.25	9.42
Block I	G34	79.6	2.5	61	66	83	114	3.23	0.93	7.5	68	5	7.12	5.45	15.38
BLOCK II	G35	76.5	2.4	85	94	111	142	3.2	0.93	7.5	67	3.7	7.91	6.15	11.68
BLOCK II	G36	67.5	2.4	68	75	92	123	3.47	0.97	7.5	49	4.7	6.64	5.63	14.44
BLOCK II	G37	82.4	2.1	84	92	109	143	4.4	1.03	6.5	79.5	4.3	6.04	5.26	12.42

BLOCK II	G172	79.6	2.4	85	90	107	139	3.27	1.07	7.5	70	4.7	6.58	4.96	10.46
BLOCK II	G38	69.9	1.4	85	90	107	139	4.4	1.03	7.5	70	3.3	7.41	6.09	13.38
BLOCK II	G39	66.9	2.4	82	87	104	136	3.56	0.97	7.5	74	4.7	7.4	6.35	12.56
BLOCK II	G40	64.9	2.1	85	93	110	142	4.5	1	7.5	67	4	7.67	6.05	12.2
BLOCK II	G41	83.5	1.5	85	94	111	143	4.2	1.03	7.5	64	4.3	6.6	5.85	8.6
BLOCK II	G42	60.5	2.5	68	75	92	124	4.2	1.03	7.5	48	3.7	7.22	6.37	11.56
BLOCK II	G43	77.2	2.2	85	90	107	139	3.53	0.93	7.5	67	4	7.71	6.63	13.12
BLOCK II	G44	82.6	3.1	85	90	107	139	3.6	1.1	7.5	71	4.3	7.29	5.98	13.06
BLOCK II	G45	65.6	3	82	87	104	136	3.47	1	6.5	75.5	4	5.52	5.66	6.68
BLOCK II	G46	65.9	2.5	85	90	107	139	3.33	1.03	6.5	71.5	3.7	6.32	5.82	9.44
BLOCK II	G170	79.6	2	85	90	107	139	3.57	1.03	6.5	74.5	4	6.32	5.91	10.08
BLOCK II	G47	76.2	2.1	81	89	106	138	3.37	0.97	6.5	78.5	3.3	5.56	4.9	10
BLOCK II	G48	83.7	2.2	62	67	84	116	3.9	0.83	6.5	78.5	4	6.58	5.42	10.22
BLOCK II	G49	72.2	2	61	66	83	115	3.8	1.23	6.5	65.5	3.3	5.75	5.13	7.46
BLOCK II	G50	80.9	2.1	61	66	83	115	3.37	0.97	6.5	78.5	3.3	5.56	4.9	7.84
BLOCK II	G51	71.1	2.4	82	87	104	136	3.87	1.07	6.5	67.5	5	5.11	4.9	6.96
BLOCK II	G52	58.2	2.2	62	67	84	116	3.3	0.83	6.5	69.5	5	5.66	5.32	7.1
BLOCK II	G53	65.4	2.1	80	85	102	134	3.47	1.2	6.5	68.5	4	6.14	5.12	6.28
BLOCK II	G169	68.2	3.1	82	86	103	135	3.27	1.07	6.5	69.5	3.7	6.23	5.36	7.1
BLOCK II	G54	58.8	3.2	85	90	107	139	3.43	1	6.5	68.5	4.7	6.53	5.4	7.46
BLOCK II	G55	83.2	2.1	82	86	103	135	3.33	0.97	6.5	50.5	4.7	5.59	5.4	9.12

BLOCK II	G56	87.4	2.5	82	87	104	136	3.53	0.87	6.5	68.5	4	6.36	5.42	9.38
BLOCK II	G57	88.5	1.3	62	68	85	117	3.33	0.56	6.5	71.5	4.3	6.43	5.61	9.7
BLOCK II	G58	76.1	3.2	82	87	104	136	3.3	0.97	6.5	71.5	4	6.52	5.82	10.12
BLOCK II	G59	85.2	2.3	82	87	104	136	3.3	0.83	6.5	75.5	4	6.28	5.73	10.44
BLOCK II	G60	83.4	2.5	82	87	104	136	3.53	0.87	6.5	68.5	4.7	6.36	5.42	9.18
BLOCK II	G61	79.9	2.5	62	67	84	116	3.67	1.03	6.5	65.5	3.3	6.12	5.81	10.44
BLOCK II	G171	89.4	2.2	82	87	104	136	3.7	0.97	6.5	49.5	3.3	6.16	5.32	9.38
BLOCK II	G62	89.5	2	85	89	106	138	3.4	1.03	6.5	68.5	5	6.09	5.28	10.84
BLOCK II	G63	71.5	2.3	82	88	105	137	3.8	0.97	6.5	72.5	3.3	6.12	5.11	8.86
BLOCK II	G64	61.2	2.5	85	94	111	143	3.5	1.17	6.5	64.5	4	5.97	5.58	8.6
BLOCK II	G65	81.2	1.4	71	78	95	127	3.7	1.03	6.5	82.5	3.7	6.82	6.44	9.08
BLOCK II	G66	70.8	2.2	68	74	91	123	4.4	1.07	6.5	90.5	3.7	6.48	5.55	7.48
BLOCK II	G67	77.2	2	82	88	105	137	3.27	1.03	6.5	64.5	4.2	5.68	5.1	8.48
BLOCK II	G68	66.2	2.2	85	94	111	145	4.2	0.9	6.5	62.5	3.3	6.21	4.89	9.5
BLOCK II	G69	94.3	2.1	81	89	106	140	3.43	0.83	6.5	87.5	3.7	7.33	6.23	14
Block III	G70	81.5	1.4	61	67	84	118	3.7	1.03	6.5	82.5	5	6.82	6.44	9.82
Block III	G71	72.4	2.1	85	95	112	146	3.53	1	6.5	71.5	3.7	6.47	5.11	10
Block III	G72	65.9	2.3	82	90	107	141	3.77	0.87	6.5	75.5	5	6.09	5.16	8.8
Block III	G73	71.7	2	62	67	84	118	3.6	0.93	6.5	70.5	3.7	6.22	5.24	9.1
Block III	G74	68.7	2.1	62	68	85	119	4.4	1.23	6.5	79.5	3.7	6.04	5.26	9.32
Block III	G75	55.7	2.4	63	69	86	120	3.33	1	6.5	70.5	4	5.95	5.18	9.26

Block III	G171	78.7	2.5	88	93	110	140	3.53	0.83	7	64	4	4.68	4.23	8.07
Block III	G76	68.7	2.5	68	74	91	125	3.37	0.93	7	78.5	4	6.84	5.82	12.5
Block III	G77	68.7	2	81	90	107	141	3.8	1	7	82.5	4	6.75	5.96	11.02
Block III	G78	52.5	2.1	68	74	91	125	3.63	1.03	7	68.5	4	4.67	4	8.42
Block III	G79	54.5	2.1	68	74	91	125	3.33	1.07	7	55.5	4	5.28	4.67	7.62
Block III	G80	70.7	2.5	68	74	91	125	3.57	1	7	71.5	4	5.31	4.46	7.7
Block III	G81	69.3	2.4	79	89	106	139	3.37	1.17	7	64	3.7	6.01	5.64	7.5
Block III	G82	58.7	2.5	82	88	105	139	3.83	1.27	7	71.5	3.7	5.62	4.79	7.68
Block III	G83	72.9	2.2	81	89	106	140	3.8	1.07	7	75.5	4.3	5.11	4.48	8.68
Block III	G84	58.2	2	61	67	84	118	3.2	0.87	7	68.5	3.3	6.17	4.76	7.66
Block III	G85	51.3	2.4	82	89	106	140	3.27	1.14	7	65.5	4	6.29	4.39	8.08
Block III	G170	73.2	2.5	82	90	107	141	4.2	1.03	7	49.5	4.7	5.23	4.34	8.7
Block III	G86	53.2	3	82	92	109	143	3.53	1.08	7	68.5	4.3	5.91	4.98	7.78
Block III	G87	68.9	2.5	85	94	111	145	3.77	1.1	7	72.5	4	4.84	4.51	6.72
Block III	G88	67.4	2.4	82	92	109	143	3.37	0.97	7	64.5	4.3	4.72	4.28	6.54
Block III	G89	65.9	2.1	78	84	101	135	3.33	1.07	7	55.5	4	5.28	4.67	5.94
Block III	G90	73.8	2.4	82	92	109	138	4.4	0.97	7	90.5	4	5.1	4.32	12.78
Block III	G91	62.6	2.4	82	92	109	138	3.5	0.93	7	64.5	4	4.36	4	6.86
Block III	G92	73.2	1.4	82	92	109	138	3.8	0.93	7	62.5	3.3	5.52	4.7	7.6
Block III	G93	67.7	2	82	93	110	139	3.87	0.97	7	87.5	3.7	5.35	4.54	7.84
Block III	G94	71.2	2.5	82	92	109	138	3.43	0.93	7	82.5	3.7	5.78	5.09	8.02

Block III	G169	74.2	2.5	82	92	109	138	3.57	0.9	7	71.5	4.7	5.77	5.08	5.98
Block III	G95	75.9	2.5	82	92	109	138	3.8	1.07	7	75.5	4	5.94	5.19	8.92
Block III	G96	83.2	2.3	82	92	109	138	3.43	1.07	7	70.5	3.7	5.47	4.6	7.92
Block III	G97	47.7	2.5	82	92	109	138	3.57	1.03	7	73.5	4	5.56	4.13	7
Block III	G98	63.2	1.4	78	87	104	133	3.8	0.93	7	62.5	3.3	5.52	4.7	6.36
Block III	G99	59.8	2.2	82	92	109	138	3.5	1	7	79.5	2.7	4.77	3.8	6.5
Block III	G100	60	2.4	82	92	109	138	3.2	1.03	7	69.5	4	5.51	4.41	5.98
Block III	G101	73.9	2.1	82	92	109	138	3.23	1.03	7	78.5	4.7	5.24	4.4	6.06
Block III	G172	66.2	2.4	68	75	92	121	3.2	1.03	7	65.5	4	5.58	5.04	6.6
Block III	G102	67.9	1.5	68	75	92	121	3.56	1.03	7	66.3	3.3	5.49	4.76	7.76
Block III	G103	71.8	1.5	68	75	92	121	3.57	1	7	76.3	3.7	5.81	4.66	8.16
Block III	G104	75.7	2.8	68	75	92	121	3.6	1.03	7	79.3	5.3	6.64	5.34	7.48
BLOCK IV	G105	76.6	2.4	68	74	91	121	3.37	1	7	72.3	5	5.98	5.32	6.64
BLOCK IV	G169	75.6	2.4	85	93	110	140	3.67	0.97	7	75.3	4.7	5.21	4.98	7.37
BLOCK IV	G106	73.3	1.4	82	91	108	138	3.7	1.03	7	79.3	4	5.19	4.85	5.74
BLOCK IV	G107	66.2	2.1	73	80	97	130	3.7	0.97	6.5	50.3	4.3	7.02	5.76	5.86
BLOCK IV	G108	78.2	2	82	90	107	137	3.7	0.93	7	66.3	3	4.46	3.77	4.16
BLOCK IV	G109	85.6	2.5	82	89	106	136	3.37	1.03	7	79.3	3.7	4.22	4.08	5.38
BLOCK IV	G110	75.6	2.5	70	76	93	123	3.33	1	7	68.3	4.3	5.28	4.5	5.44
BLOCK IV	G111	81.2	2.5	68	73	90	120	3.53	0.64	7	64	4	4.68	4.23	4.84
BLOCK IV	G112	59.1	2.3	85	90	107	137	3.7	1.03	7	74	3	6.42	5.83	8.8

BLOCK IV	G113	50	2.5	61	68	85	115	3.23	0.97	7	77	3.7	6.66	5.67	8.29
BLOCK IV	G171	60	2.2	71	79	96	126	3.33	1.03	7	70	4	7.64	5.99	5.45
BLOCK IV	G114	54	2.4	82	90	107	137	3.33	1.03	7	73	2.3	8.43	6.31	8.47
BLOCK IV	G115	59.3	2.1	82	90	107	137	3.37	1.07	7	77	4.3	6.26	5.4	5.47
BLOCK IV	G116	49.2	2.4	81	89	106	136	3.37	1.17	7	64	3.7	6.01	5.64	6.6
BLOCK IV	G117	64.11	1.5	82	89	106	136	3.3	1.03	7	77	4.3	5.61	5.08	5.58
BLOCK IV	G118	53.22	1.5	82	89	106	136	3.37	1.03	7	68.3	4.3	7.3	5.48	6.37
BLOCK IV	G119	55.21	2.8	68	74	91	121	3.8	1.07	7	70.3	4.7	8.37	5.23	6.82
BLOCK IV	G120	43.3	2.3	82	93	110	143	3.8	0.93	7	65.5	3.3	8.12	4.2	4.64
BLOCK IV	G170	68.8	1.6	82	88	105	138	3.6	0.8	7	69.3	4	6.3	5.12	7.82
BLOCK IV	G121	76	2.3	82	88	105	138	3.7	0.83	7	75.3	4.3	6.89	5.29	5.25
BLOCK IV	G122	67.47	2.4	85	93	110	143	3.57	0.97	6.5	70.3	4.7	6.67	6.5	6
BLOCK IV	G123	84.2	2.1	82	93	110	143	3.23	1.03	6.5	69.3	4	7.87	5.76	10.48
BLOCK IV	G124	65.4	2.4	85	93	110	143	3.27	1.07	6.5	51.3	3.3	7.61	5.32	7.89
BLOCK IV	G125	55	1.5	85	89	106	139	3.53	1.03	6.5	72.3	3.7	7.05	6.08	6.51
BLOCK IV	G126	67.5	2.8	85	89	106	139	3.53	1.03	6.5	72.3	4	7.3	6.01	6.24
BLOCK IV	G127	65.2	1.6	71	78	95	128	3.6	1.03	6.5	76.3	4	7.47	6.74	5.4
BLOCK IV	G128	66.3	2.1	82	89	106	139	3.43	1.03	6.5	72.5	5	6.69	5.52	5.73
BLOCK IV	G172	72.2	2.3	61	68	85	118	3.43	0.83	6.5	69.3	3.7	7.82	5.83	7.59
BLOCK IV	G129	72.3	2.8	85	90	107	140	3.3	0.97	6.5	66.3	4.7	6.3	5.27	4.52
BLOCK IV	G130	70.3	2.1	63	69	86	119	3.7	0.97	6.5	50.3	4.3	7.02	5.76	6.67

BLOCK IV	G131	82.3	2	68	75	92	125	3.4	0.83	6.5	49	4.7	5.83	5.3	7.52
BLOCK IV	G132	61.3	1.3	68	75	92	125	3.33	0.93	6.5	70	3.3	4.39	4.25	7.61
BLOCK IV	G133	63.3	3.1	68	77	94	127	3.37	1.11	7	70	4.7	6.65	5.67	6.47
BLOCK IV	G134	62.3	2	85	91	108	141	3.3	1	7	74	4	6.71	5.27	4.88
BLOCK IV	G135	55.3	2	82	88	105	138	3.27	1.42	7	67	4.3	5.85	5.94	8.4
BLOCK IV	G136	65	1.8	80	89	106	139	3.57	1.03	6.5	68.5	3.7	6.38	4.86	7.37
BLOCK IV	G137	55.3	1.2	68	75	92	125	3.33	1.13	7	64	4	5.48	4.32	7.8
BLOCK IV	G138	52.3	2.1	68	74	92	124	3.4	0.87	7	65.5	4.3	5.47	4.94	8.37
Block V	G139	67.3	3.1	61	67	85	117	3.67	0.97	7	75.5	4	6.11	5.71	8.71
Block V	G169	73.9	2.3	81	90	107	136	3.5	1.03	6.5	77	3.7	5.43	4.55	7.97
Block V	G140	64.3	3.2	61	66	84	116	3.27	0.93	7	78.5	4	6.02	6.77	6.37
Block V	G141	121.3	2.1	68	74	90	124	3.37	0.87	7	71.5	3.3	6.96	5.43	6.1
Block V	G142	53.3	1.3	68	75	92	125	3.2	0.93	7	74.5	4	6.29	5.85	8.38
Block V	G143	49.3	3.2	82	92	109	142	3.33	0.93	7	78.5	3.3	7.67	5.67	5.72
Block V	G144	57.3	2.3	82	93	110	143	3.8	0.93	7	65.5	3.3	8.12	4.4	5.45
Block V	G145	58.3	2.5	85	92	109	142	3.57	0.97	7	78.5	5	6.66	5.75	6.25
Block V	G146	48.3	1.5	82	89	106	139	3.4	1.03	6.5	75.6	5	8.17	6.3	6.27
Block V	G147	72.7	2	82	92	109	142	3.33	1.07	6.5	81.5	4	7.21	5.4	6.29
Block V	G148	74.6	3	76	80	97	129	3.9	0.97	6	77	3.7	6.71	6.88	5.85
Block V	G149	83.3	1.3	82	89	106	139	3.43	1.03	6.5	70	4.7	7.64	3.9	4.92
Block V	G172	62.7	2	82	89	106	139	3.4	1.03	6.5	65.5	4.7	6.63	6.37	9.39

Block V	G150	67.3	2	71	78	95	128	3.27	1.03	6.5	79.5	4	8	5.83	6.04
Block V	G151	73.1	1.2	82	90	107	140	3.23	0.83	6.5	79	4.3	7.27	6.36	5.85
Block V	G152	70.8	1.4	82	91	108	141	3.4	0.97	6.5	67	4	6	6.23	6.82
Block V	G153	77.2	1.4	82	89	106	139	3.87	1.07	6.5	75.5	4	7.61	5.3	4.95
Block V	G154	73.6	2	75	81	98	127	4.7	1.03	7	66.8	4.7	5.47	4.65	5.48
Block V	G155	77.2	2	68	75	92	125	3.57	0.47	6.5	67.5	3.3	8.12	6.62	6.13
Block V	G156	52	2.4	68	76	93	126	3.7	0.97	6.5	70	3.3	7.65	6.88	8.21
Block V	G157	56	2.1	82	89	106	139	3.43	1.03	6.5	72.5	5	6.69	5.52	8.83
Block V	G158	48	1.3	82	89	106	139	3.3	0.97	6.5	70.6	3.3	5.49	5.39	8.7
Block V	G159	49	1.2	82	89	106	139	3.33	0.44	6.5	72.5	4	5.88	6.86	7.73
Block V	G160	73	1.4	82	89	106	139	3.5	0.93	6.5	65.5	3.7	5.8	5.69	6.81
Block V	G170	61	1.2	82	90	107	140	4.2	0.93	6.5	46	3.7	5.78	6.11	9.39
Block V	G161	70	2.1	85	95	112	145	3.23	0.97	6.5	71.5	3	6.88	5.98	7.28
Block V	G162	80.7	2.4	82	88	105	138	3.4	1.03	6.5	71	3.3	6.02	4.97	8.73
Block V	G163	67.3	2.1	78	87	104	134	3.6	1.07	6.5	75.5	3.7	6.45	5.57	9.27
Block V	G164	59	2.4	82	89	106	139	3.37	1.03	6.5	77.5	5	6.05	5.25	7.71
Block V	G171	68	2.5	80	86	103	136	3.8	0.97	6.5	72	3.7	4.99	4.78	8.22
Block V	G165	70.7	2.1	85	93	110	143	3.27	1	6.5	65.5	5	5.76	5.46	7.14
Block V	G166	75.3	1.2	82	90	107	140	3.73	1.03	6.5	51	3.7	6.01	5.12	5.48
Block V	G167	81.1	1.8	85	94	111	144	3.57	1.03	6.5	68.5	3.7	6.38	4.86	12.87
Block V	G168	78.8	2.3	85	93	110	143	3.77	0.93	6.5	69.5	4	6.19	5.56	11.12

G169- Ratan

G170- Prateek
G171- Mahateora
G172- Narayangon

Note

PHT - Plant height (cm)	80%F- Days to 80% flowering	SDL- Seed length (mm)
PTL - Petiole length(cm)	80%M- Days to 80% maturity	SDW- Seed width (mm)
DFF- Days to first flowering	NPB- Number of primary branches	HSWT- Hundred seed weight (g)
50%F- Days to 50% flowering	NPP- Number of pods per plant	

Table 3. Scoring data of 20 Qualitative table

Accessions	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
IFLA 143	1	3	2	5	1	5	3	5	2	2	0	3	3	4	2	1	0	1	0	1
IFLA276	1	3	10	3	0	5	3	5	2	2	0	5	3	5	2	1	0	3	0	1
IFLA1765	2	3	10	7	0	7	3	5	2	2	0	3	3	4	4	1	0	3	0	1
IFLA1870	1	3	10	5	1	5	3	5	3	2	0	3	4	5	4	1	1	3	1	1
IFLA2026	1	3	10	5	0	5	3	5	3	2	0	3	3	4	4	1	0	3	0	1
IFLA151	1	3	1	3	0	5	0	5	3	2	0	3	3	5	4	1	1	1	1	1
IFLA2282	1	3	10	5	0	5	3	5	3	2	3	5	3	5	2	1	0	3	0	1
IFLA2475	2	3	10	5	0	7	3	5	3	2	0	3	3	5	4	1	2	3	1	1
IFLA274	1	3	10	5	0	5	3	5	3	2	3	5	3	6	2	1	2	3	1	1
IFLA2765	2	3	10	5	0	5	3	5	3	2	0	5	3	5	4	1	2	3	1	1
IFLA2668	2	3	10	7	0	7	3	7	2	2	0	3	4	4	4	1	0	3	0	1
IFLA2736	2	3	10	5	0	7	3	5	2	2	3	3	3	4	4	1	1	3	1	1
IFLA2668	2	3	10	5	0	7	3	5	2	2	0	3	3	4	4	1	0	3	0	1
IFLA2624	1	3	10	7	0	5	3	5	2	2	0	3	3	4	4	1	0	4	0	1
IFLA172	1	3	2	7	0	5	3	5	2	2	3	3	4	4	9	1	2	1	1	1
IFLA1720	1	3	10	5	0	5	3	5	2	2	3	3	3	4	9	1	2	3	1	1
IFLA128	1	3	2	5	0	5	3	5	3	2	0	3	4	4	4	1	0	1	0	1
IFLA2636	2	3	10	5	0	5	3	5	2	2	3	3	3	5	2	0	0	3	0	1
BANG211	2	3	10	5	0	3	3	5	2	2	0	3	3	5	9	1	2	3	1	1

BANG184	2	3	10	5	0	5	3	5	2	2	3	3	3	4	9	1	2	3	1	1
BANG261	2	3	10	5	0	7	3	5	2	2	3	3	4	4	9	1	1	3	1	1
BANG267	2	3	10	7	0	3	3	5	3	2	3	3	3	5	2	0	0	3	0	1
BANG308	2	3	10	5	0	7	3	5	2	2	0	3	3	5	9	1	2	3	1	1
BANG218	2	3	10	5	0	5	3	5	2	2	3	5	3	4	9	1	2	3	1	1
BANG168	2	3	10	5	0	3	3	5	2	2	3	3	4	3	4	1	1	3	1	1
BANG224	2	3	10	5	0	7	3	5	3	2	3	3	3	5	4	1	0	3	0	1
BANG262	2	3	10	5	0	7	3	5	3	2	3	3	3	5	1	1	2	3	1	1
BANG266	2	3	10	5	0	3	3	5	3	2	0	3	3	4	4	1	2	3	1	1
BANG227	2	3	10	5	0	3	3	5	2	2	3	3	3	5	2	1	1	3	1	1
BK154	2	3	10	7	0	7	3	5	3	2	3	3	4	4	4	1	2	3	1	1
BK148	2	3	10	5	1	7	3	5	3	2	3	3	3	5	1	1	2	3	1	1
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IFLA2750	1	3	10	5	0	3	3	5	3	2	0	3	3	4	9	1	2	4	1	1
IFLA1626	2	3	2	5	0	3	3	5	3	2	3	3	3	5	2	1	1	1	1	1
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IFLA432	1	3	2	5	0	5	3	5	2	2	3	3	3	5	1	1	2	1	1	1
IFLA2341	1	3	2	3	0	5	3	5	3	2	3	3	4	3	2	1	0	1	0	1
IFLA2025	1	3	2	3	0	3	3	5	2	2	0	3	3	5	2	1	0	3	0	1
IFLA1864	1	3	10	5	0	5	3	5	3	2	3	3	3	5	1	1	2	1	1	1
IFLA2687	1	3	10	3	1	5	3	5	3	2	3	5	3	5	2	1	1	5	1	1

IFLA126	1	3	10	3	1	3	3	5	3	2	3	3	4	4	4	1	2	4	1	1
IFLA1813	1	3	2	5	0	5	3	5	3	2	3	5	3	5	1	1	2	1	1	1
IFLA1715	1	3	2	5	0	5	3	5	3	2	3	3	4	3	2	1	0	1	0	1
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IFLA144	1	3	10	5	0	5	3	5	3	2	3	3	3	5	4	1	1	5	1	1
IFLA127	1	3	10	5	0	7	3	5	3	2	0	5	3	5	2	1	0	3	0	1
IFLA2126	1	3	10	3	0	7	3	5	3	2	3	5	3	5	1	1	2	3	1	1
IFLA1846	1	3	10	5	0	5	3	5	3	2	0	3	4	3	9	1	2	3	1	1
BANG208	2	3	10	5	1	7	3	5	3	4	0	3	3	4	2	1	0	3	0	1
BANG206	2	3	10	5	0	7	3	5	3	2	3	5	3	4	9	1	2	3	1	1
BANG257	2	3	10	5	1	5	3	5	3	1	3	5	3	5	1	1	2	3	1	1
BANG268	2	3	10	5	1	5	3	5	3	2	3	3	3	5	9	1	2	3	1	1
BANG263	2	3	10	3	0	3	3	5	2	2	3	3	3	4	4	1	2	3	1	1
BANG286	2	3	10	5	0	3	3	5	2	2	3	3	3	5	1	1	2	3	1	1
BANG284	2	3	10	5	0	3	3	5	2	2	3	3	3	4	9	1	2	3	1	1
BK551	2	3	10	3	0	7	3	5	2	2	3	3	3	4	9	1	2	3	1	1
BK401	2	3	10	5	1	7	3	5	3	2	3	3	3	5	1	1	2	3	1	1
BK3111	2	3	10	7	1	7	3	5	3	2	3	3	4	3	2	1	0	3	0	1
BK101	2	3	10	7	1	7	3	5	3	2	3	3	3	4	4	1	2	3	1	1
BK361	2	3	10	5	0	7	3	5	3	2	3	3	4	3	2	1	0	3	0	1
BK541	2	3	10	5	1	7	3	5	3	2	0	3	3	5	9	1	2	3	1	1

BK301	2	3	10	7	1	7	3	5	3	2	3	3	3	5	4	1	2	4	1	1
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IFLA220	2	3	10	5	1	7	3	5	3	2	0	3	3	3	9	1	1	4	1	1
IFLA160	2	3	10	5	1	5	3	5	2	2	3	3	3	5	9	1	2	5	1	1
IFLA2861	2	3	10	5	1	5	3	5	3	2	3	3	3	4	4	1	1	4	1	1
IFLA2460	2	3	10	5	1	7	3	5	3	2	3	3	4	5	1	1	2	3	1	1
BIO520	2	3	10	5	1	5	3	5	3	2	3	5	3	4	9	1	2	3	1	1
IFLA123	1	3	10	5	1	5	3	5	2	2	3	3	3	4	4	1	1	1	1	1
IFLA118	1	3	10	5	0	7	3	5	2	2	0	7	3	5	2	1	0	5	0	1
IFLA1826	1	3	10	5	0	7	3	5	3	2	3	3	4	4	9	1	1	3	1	1
IFLA2441	1	3	10	5	0	5	3	5	3	2	0	5	3	4	9	1	2	3	1	1
IFLA3001	2	3	10	5	1	3	3	5	3	2	0	5	3	5	9	1	2	3	1	1
SeI160	2	3	10	3	1	3	3	5	3	2	3	5	3	5	9	1	2	5	1	1
IFLA2660	2	3	10	7	0	3	3	5	3	2	3	5	3	5	4	1	1	5	1	1
IFLA1436	1	3	10	7	0	5	3	5	3	2	3	3	3	4	4	1	0	1	0	1
IFLA1613	2	3	2	7	0	7	3	5	2	2	3	3	3	5	9	1	2	1	1	1
BANG306	2	3	10	7	0	5	3	5	2	1	3	3	3	6	9	1	2	4	1	1
BANG310	2	3	10	7	0	5	3	5	2	2	3	3	3	5	1	1	2	3	1	1
BANG186	2	3	10	7	0	5	3	5	3	2	0	3	4	4	4	1	1	3	1	1
BANG246	2	3	10	7	1	5	3	5	3	1	0	3	4	4	9	1	2	3	1	1
BANG182	2	3	10	7	0	5	3	5	3	2	3	5	4	4	9	1	2	3	1	1

BANG267	2	3	10	7	0	7	3	5	2	2	0	5	4	3	9	1	2	3	1	1
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IC148352	2	3	10	5	1	7	3	5	3	2	3	3	3	5	9	1	1	3	1	1
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IC148356	3	3	10	5	1	7	3	5	3	2	0	3	4	4	9	1	1	3	1	1
IC486623	3	3	10	3	1	7	3	5	3	2	0	3	4	4	4	1	1	4	1	1
IC574470	3	3	10	5	1	7	3	5	3	2	3	3	3	5	9	1	2	3	1	1
IC208426	3	3	10	5	1	7	3	5	3	1	3	3	3	5	1	1	2	4	1	1
IC208430	3	3	10	3	1	7	3	5	3	1	3	3	3	5	4	1	2	3	1	1
IC208433	3	3	10	7	1	7	3	5	3	2	0	5	3	5	9	1	2	3	1	1
IC208434	3	3	10	5	1	7	3	5	3	4	3	3	4	3	2	1	0	3	0	1
IC208436	3	3	10	5	1	7	3	5	3	4	0	3	3	4	9	1	2	3	1	1
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IC415678	3	3	10	5	1	5	3	5	3	2	0	3	3	4	4	1	1	3	1	1
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IC366712	4	3	10	5	1	5	3	5	3	2	0	3	3	4	9	1	1	3	1	1

IC470682	4	3	10	3	1	7	3	5	3	1	0	5	4	3	9	1	2	3	1	1
IC427676	4	3	10	5	1	5	3	5	3	1	0	3	4	4	9	1	2	3	1	1
IC427710	4	3	10	5	1	5	3	5	3	1	3	3	4	5	9	1	2	5	1	1
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IC347378	3	3	10	3	1	5	3	5	3	2	0	3	4	5	9	1	1	3	1	1
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BANG21	1	3	10	3	0	3	3	5	3	1	3	3	4	5	4	1	2	3	1	1
BANG26	1	3	10	5	0	5	3	5	3	1	3	3	3	5	9	1	2	3	1	1
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BANG30	2	3	10	5	0	5	3	5	3	2	3	5	4	4	4	1	2	3	1	1
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BANG5	2	3	10	5	0	5	3	5	3	2	3	3	3	4	4	1	1	3	1	1
BANG13	2	3	10	5	1	5	3	5	3	2	3	3	3	4	4	1	1	3	1	1
BANG22	2	3	10	5	0	5	3	5	3	2	3	3	3	5	9	1	1	3	1	1
BANG32	2	3	10	3	0	3	3	5	3	2	0	3	4	3	9	1	2	3	1	1
BANG33	2	3	10	5	1	5	3	5	3	2	0	3	4	3	4	1	1	3	1	1
BANG38	2	3	10	5	0	7	3	5	3	2	0	3	4	3	9	1	2	3	1	1
BANG41	2	3	10	5	0	5	3	5	3	2	0	3	4	3	4	1	1	3	1	1
BANG43	2	3	10	5	0	5	3	5	2	2	0	3	4	3	9	1	2	3	1	1
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IC0634658	3	3	10	3	1	5	3	5	3	2	3	3	3	3	4	1	0	3	1	1
IC0634656	3	3	10	3	1	5	3	5	3	1	3	3	4	3	9	1	2	3	1	1
IC0634660	3	3	10	5	1	5	3	5	3	1	0	3	3	4	1	1	1	1	1	1
IC0634661	3	3	10	5	1	5	3	5	3	2	0	3	4	3	1	1	2	1	1	1
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IC0634664	3	3	10	3	1	3	3	5	3	2	3	3	3	5	1	1	2	1	1	1
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IC0634671	3	3	10	5	1	7	0	5	3	2	3	3	3	4	9	1	2	3	1	1
IC0634672	3	3	10	5	1	3	0	5	3	1	0	3	3	5	4	1	2	3	1	1

IC0634673	3	3	10	7	1	7	3	5	3	2	3	3	4	5	4	1	1	3	1	1
IC0634674	3	3	10	7	1	7	3	5	3	2	3	3	4	4	4	1	1	3	1	1
Ratan	3	2	3	5	1	5	3	5	3	2	3	3	3	4	9	1	2	3	1	1
Prateek	3	3	7	5	1	5	0	5	3	2	3	3	3	4	4	1	2	3	1	1
Mahateora	3	3	10	5	1	5	3	5	3	2	3	3	4	4	9	1	2	3	1	1
Narayangon	3	3	10	5	1	3	3	5	3	2	3	3	3	4	9	1	2	3	1	1

Note:

A	Seedling pigmentation	K	Pod dehiscence
B	Growth habit	L	Constriction between pods
C	Flower color	M	Seed size
D	Plant vigour	N	Seed shape
E	Plant pigmentation	O	Seed coat color
F	Leaf size	P	Seed coat surface
G	Pod pubescence	Q	Seed coat pattern
H	Pod wingedness	R	Seed coat pattern color
I	Immature pod color	S	Seed ornamentation
J	Pod shape	T	Cotyledon color