

Genetic Assessment Of Soybean Genotypes For Yield And Yield Related Components

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ABSTRACT

Glycine max L. commonly known as Soybean is a highly valued leguminous crop due to its protein and oil contents which make it economically valuable in food, feed and industry. The current experiment, titled as “Genetic Evaluation of Soybean Yield and Yield Associated Traits” has been performed for the purpose of estimating genetic variation among soybean genotypes on the basis of agromorphological, yield associated, seed quality and root system architecture (RSA) traits. Thirty genotypes of soybeans along with two check varieties, NARC-21 and Ajmeri were studied in Oilseed Research Program, National Agricultural Research Center (NARC), Islamabad. Augmented design was used for preliminary screening while promising genotypes were further investigated through Randomized Complete Block Design (RCBD). Seven RSA traits were recorded through digital root imaging in three replications such as root tips, branch points, total root length, root volume, network area, average diameter and surface area. Genetic variation was observed in the stem height and 100-seed weight while some flowering and vegetative traits were found not significantly different. Phenotypic characterization of the roots exhibited variations in total root length, root volume, root network area, and root surface area, proving the efficacy of RSA in genetic studies. Significant correlation was also found in the selected root characteristics, especially between root tip and total root length. Seed quality analysis showed variations in the protein, lipid, and fiber composition; NARC-21 possessed the maximum amount of protein (32.89%), GP-121 the maximum lipid (23.40%), and GP-26 the maximum fiber (7.00%). On the basis of combined evaluation, twelve best genotypes were identified, namely GP-5, GP-12, GP-20, GP-

23, GP-26, GP-37, GP-54, GP-96, GP-120, GP-121, GP-123, and GP-151. These results highlight the existence of useful genetic variability in the tested germplasm.

Keywords: Soybean, Genetic Variability, Yield-Related Traits, Root System Architecture, Root Phenotyping, Seed Quality, Protein, Oil, Germplasm, Plant Breeding

Introduction

The soybean (*Glycine max* L.) is an agriculturally and nutritionally significant oilseed legume crop grown for protein, oil, feed, and industrial uses. Soybeans are referred to as nutritionally significant crops since the soybean seeds are composed of considerable quantities of protein and oil (Hammer, 2009). The soybean is a member of the family Fabaceae, which is a 40-chromosome crop ($2n=40$). This is owing to the nutritional significance of the soybean, making it an important part of our diet and agriculture. The uploaded study points out the fact that soybeans contain about 19.5% oil and 43.3% protein (Chawan et al., 2023).

Soybean productivity is greatly affected by the presence of genotype variation as well as by genotype environment interactions. Yield is a polygenic trait with many component traits, which include flowering habit, height, branching, nodes, pod numbers, seed weight, and other morphological traits (Chen et al., 2022). Thus, yield itself is often not enough as a criterion for breeding programs. Information about the evaluation of yield components may help to determine good genetic materials, as well as traits that can be used in breeding. In the source, results of the previous genetic analysis showed great variation between soybean genotypes in regard to some quantitative traits. Traits like plant height, branches, nodes, pod clusters, flowering date, seed weight, and seed yield could be considered valuable as a selection criterion (Belyshkina et al., 2023).

The use and exploitation of genetic diversity are thus crucial elements in soybean improvement. A wide variety of genotypes allows breeders to choose parents with desirable agronomic traits as well as improve cultivars. Investigations presented in the uploaded thesis reveal a significant morphological diversity in soybean germplasms. In this case, the study of 45 soybean genotypes based on morphological traits and cluster analysis showed that there are several groups of soybean germplasms which shows that there is genetic diversity to be exploited. Likewise, the study of huge soybean germplasms has revealed some clusters with high heritability and genetic gain (Board & Kahlon, 2011).

The yield-associated characters are very significant because they offer indirect means of selecting for better grain yield. According to research presented in the source, there is a positive relationship between soybean yield and other traits like biological yield, harvest index, number of branches, biomass, grain weight, protein and oil content. For instance, in one study, 18 soybean genotypes were found to have biological yield correlated at 0.96 with seed yield, and harvest index and number of branches at 0.92 correlated with seed yield (Chawan et al., 2023).

Environmental adaptation is yet another significant factor in the breeding of soybean crops. Soybean crops are sensitive to temperature, light cycles, and water requirements, and unsuitable environmental conditions may affect significantly their

growth, flowering, pod formation, and eventual yield. According to the source, temperature, light cycles, and water availability are some of the significant factors that determine adaptation based on the different climates. Water availability is specifically crucial when the crop flowers and pods. Failure to provide enough water at such development stages causes significant loss of yields. Thus, it becomes significant to identify genotypes with suitable adaptation qualities.

Root system architecture has been found to be another significant factor in soybean productivity and adaptation. The roots form a vital link between the plant and the soil and are tasked with taking up water and other nutrients needed by the plant for its growth and development. In the case of soybean, there is the presence of a taproot system with branching, while the process of nodulation allows the biological fixation of nitrogen due to the existence of *Rhizobium* bacteria. Differences in the root architecture of different genotypes could affect water and nutrient uptake, thus affecting productivity and stress tolerance (Kachare et al., 2020). Another area where soybean improvement can be linked with Pakistan is the production of soybeans, which can result in reduced dependency on imported edible oil crops and improve food and nutritional security in Pakistan. This source states that a number of soybean varieties like FS-85, AJK-1, NARC-1 and NARC-2 have been developed/introduced with varied goals in mind under various conditions in Pakistan. High yielding and adaptable genotypes with other traits like disease resistance have been given preference in breeding programs. Hence, evaluation of both local and exotic germplasm in Pakistan is essential for finding high-yielding and adaptable genotypes (Hossain et al., 2024)

In this regard, the current study explores soybean genetic diversity based on the analysis of agro-morphological, yield, quality, and root system architecture characteristics. The original experiment included the screening of 30 soybean genotypes along with two checks (Ajmeri and NARC-21) using the augmented design approach. Later on, 12 promising genotypes were screened by the randomized complete block design approach, whereas root system architecture was analyzed using the completely randomized design approach.

In this way, there is a chance to detect valuable genetic material and determine the relationships between different traits responsible for soybean yield.

The study, thus, pays its attention to the genetic analysis of soybean yield and yield-related traits by studying their morphology, yield-related traits, and the root system architecture. As it has been mentioned above, the initial research highlighted the following three goals: analysis of genetic variability in root system architecture and yield-related traits, determination of associations between yield and yield-related traits, and detection of valuable genotypes for higher yield (Ghanbari et al., 2018).

Problem Statement

Soybean yield improvement still remains one of the challenges facing soybean production in terms of achieving greater yield stability. It is known that yield is a polygenic trait determined by a number of morphological, developmental, physiological, and genetic characteristics. There may be significant variations among various soybean genotypes; however, systematic evaluation of those is required to select better genotypes for breeding purposes. In case there is no sufficient evaluation

of yield components, it is likely to be hard for breeders to find those traits that can serve as a criterion for indirect selection of higher yielding soybean cultivars (Wijaya et al., 2022).

In Pakistan, finding adapted and high yielding soybean germplasm is very important since soybean can be used for oil production, as well as protein and diversification of crops. However, performance of genotypes may be determined by environmental conditions, including temperature, photoperiod, and moisture conditions. As the results of the uploaded research show, the Rabi season in Islamabad is not quite suitable for obtaining soybean yields; therefore, there were no significant differences for some agro-morphological characteristics (Joshi et al., 2018).

Other limitations include the fact that traditional breeding of soybeans has concentrated on above ground morphological features and yield, whereas root architecture has not been given adequate attention. Root architecture affects the ability of plants to acquire nutrients and water, and may be associated with stress tolerance and yield. Ignoring root architecture may lead to overlooking some genetic variations that could provide valuable information (Liao et al., 2022).

The main problem addressed by this research is therefore the identification and evaluation of genetic variations among different genotypes of soybeans for yield, morphological features related to yield, and root architecture in order to understand associations which will enable selection of superior genotypes for future breeding of soybeans (Miah et al., 2024).

Research Objectives

For the study of genetic diversity in terms of yield, yield-related morphological characters, and root architecture of soybean genotypes

For studying the relationship between grain yield and the key yield-related traits of soybean genotypes

To find out superior soybean genotypes with better yield and other agronomic features for future breeding programs

Research Questions

How much variability in genetic composition is there in terms of yield and yield related morphological characters amongst the soybean genotypes being analyzed?

What morphological and yield-related traits are related to the yield of the soybean?

Which soybean genotypes demonstrate superior performance and therefore have potential value for future breeding programmes?

Significance of the Study

This work is highly important due to the fact that it offers a combined assessment of the soybean germplasm based on their agro-morphology, yield-related characteristics, quality attributes and root architecture traits. Instead of taking into account the yield trait separately, several other traits that could contribute to finding superior genotypes are investigated. The inclusion of both morphological and root phenotyping approaches allows for a more thorough assessment of genotype performance (Lesk & Anderson, 2021).

Considering plant breeding, the finding of diverse and superior soybean genotypes

will allow for having valuable parent plants to use in future hybridization programs. In this regard, the original work conducted a screening of 30 genotypes with two checks and selected further 12 genotypes which performed better (Relano et al., 2023). This research is also very valuable in terms of the improvement of efficiency of selection. Information about the connections between yield and yield components can give breeders an understanding of which particular features are the best candidates for indirect selection. Based on previous studies cited in the source, there are positive correlations between soybean seed yield and biological yield, harvest index, and number of branches, and thus yield-associated features can be potentially valuable in breeding programs (Reddy, 2023).

The addition of root system architecture makes the research even more important. Features of roots like total root length, root volume, network area, surface area, root tips, and branch points can give information about the plant's ability to take up water and nutrients. In the current research, the analysis of roots showed that there was a high variance in some RSA traits like total root length, total volume, network area, and surface area. This information may be valuable for breeding programs focusing on water-limited environments (Gnyandev et al., 2023).

Literature Review

Soybean (*Glycine max* L.) is an important leguminous crop owing to its rich content of proteins and oils as well as multiple uses in human foodstuffs, feed for livestock and industrial purposes. As reported by Pagano & Miransari (2016), soybean crop is widely cultivated on the planet Earth, and its productivity may be affected significantly by various biotic and abiotic stresses. Rising requirements for soybean products have made it increasingly necessary to breed new cultivars that will feature higher yield, better adaptation and good nutritional features. Hence, genetic improvement of soybeans requires the identification and exploitation of genetic variability inherent in soybean germplasm.

As described by Board & Kahlon (2011), there are multiple interacting factors that control yield formation in soybeans, namely plant growth, reproduction, as well as biomass and seed yield accumulation. Yield is thus treated as a complex quantitative trait whose formation is influenced by both genetic and environmental factors. Evaluation of yield-related traits like plant height, number of branches, number of pods, flowering habit and seed weight may thus become helpful in breeding new superior genotypes. Evaluation of those traits in genetically diverse germplasm is thus important for soybean breeding.

Ahmad et al. (2019) studied genetic variability among genotypes of soybeans for their productivity and quality traits in agro-climatic environment of Rawalpindi, Pakistan. This research is especially important for soybean improvement in local agro-climatic environments as differences noted due to genetic variability in one production environment are not always evident in other environments. The researchers have shown how important it is to carry out evaluation of the germplasm in order to select productive and high-quality soybean varieties.

Joshi et al. (2018) studied genetic parameters of soybean germplasm according to yield and yield components. As the results of their work show, it is possible to use genetic variation in such characters as flowering time, plant height, number of pods,

weight and productivity of seeds for selection. The reason is that if a trait has great genetic variability and heritability, then the breeding process is likely to produce positive results. Therefore, multiple yield-related traits should be considered in addition to grain yield.

Furthermore, Falk (2020) carried out another study on soybean root architecture by applying genotypic, phenotypic and form-based clustering. This research adds weight to the understanding that root architecture is one of the sources of genetic diversity which can be used for improving crops. Some root features including root number, length, branching, and form might affect plants' capability to explore soil resources. In other words, the presence of both root and agro-morphological characteristics can allow us to assess soybeans better.

Another research was conducted by Liu et al. (2021), where the authors assessed root system architecture traits in diverse soybean genotypes in a semi-hydroponic system. This work justifies the use of phenotyping techniques in order to detect differences in root features in different soybean genotypes. Phenotyping roots is very useful since it gives additional information which is not possible to obtain via traditional above-ground measurements and can be used for identifying genotypes with advantageous root architectures.

Gnyandev et al. (2023) investigated the effect of harvesting stage on soybean seed yield and quality, further confirming that agronomic and environmental management can have an effect on the expression of the soybean traits. This implies that variation in traits between different genotypes may be viewed in relation to the conditions of the production environment and the agronomic conditions under which crops are managed. Genetic improvement will require repeated testing in suitable environments to separate the effects of environment and genetics.

The research by Qin et al. (2023) shows that plant architectural traits could be improved through genetic improvement of soybean for better performance under certain production environments. In their study of PH13, there were improvements made in the shade-related traits and yield of soybean under high density planting at high latitudes. This is evidence that genetic improvement is not only focused on yield and other yield components but includes architectural traits as well.

Research Methodology

Experimental Site

The experiment was carried out at the Oilseed Research Program, National Agricultural Research Centre (NARC), Islamabad, Pakistan, in the Rabi season 2023. The location of the experimental site was around 33.67° N latitude and 73.13° E longitude, having the altitude of around 507 m above sea level. The field experiment was performed in order to evaluate the genetic variability in soybean genotypes regarding yield and morphological traits related to yield.

Experimental Material

The test materials included 30 soybean (*Glycine max* L.) varieties consisting of both local and exotic genotypes from the PGRI gene bank. Two soybean varieties Ajmeri and NARC-21 were taken as check varieties. The germplasm was first tested for its agro-morphological and root system architectural traits to select promising genotypes

for further study.

Experimental Design and Planting

The field experiment was laid out on an augmented block design that comprised of three blocks. Thirty genotypes were included in the experiment and were randomly placed in three blocks, with ten genotypes and two check varieties in each block. Two check varieties, namely Ajmeri and NARC-21, were repeated in all the blocks, but each test genotype was only used once. Thus, the comparison of many germplasm entries was made possible in the design by including repeated check varieties for adjusting the block effect.



Figure: scanned picture of NARC 21 germplasm of soybean.

The soybean germplasms were grown at the NARC Oilseed Research Program in Rabi 2023. It is reported in the thesis that the plant to plant spacing is approximately 3-4 cm, and row spacing is reported as 45 cm in the experimental-design description. In the other description in the thesis, 75 cm row spacing and 4 cm plant to plant spacing are reported. Since there is inconsistency regarding the values in the original thesis, the paper sticks to the experimental design information without considering any value for spacing.

Agro-Morphological Characterization

Data was collected in different growth stages of the crops in order to determine the genetic variations between the soybean genotypes. Parameters such as days to germination, days to flowering initiation, days to 50 percent flowering, days to flowering end, flower color, plant height, number of branches, number of nodes, number of pods, stem height, stem diameter, 100 seeds weight, grain yield, and 5 plants weight were measured.

Days to Germination

The days to germination of each genotype was counted based on the time from sowing to the onset of germination. This helped to distinguish between the different genotypes of the soybeans during their establishment phase.

Days to Flowering Initiation

Flowering initiation days were measured as the number of days from seeding to the point where about 5% of flowers had emerged within each genotypic group. This is how we measured the differences in the beginning of reproductive development.

Days to 50% Flowering

The number of days to 50 percent flowering was measured from the day of seeding until the day when 50 percent of the plants in a row had started to flower. This information gave some idea about the time of flowering and reproduction for every genotype.

Days to Flowering Completion

The number of days from sowing to flower opening was counted until all plants had opened their flowers. This parameter was used to compare the completeness of the flowering stage in the studied genotypes.

Flower Colour

The flower colour was determined by visual observation at the flowering stage. The colours observed were grouped into various categories based on morphological descriptions. The flower colours were noted down for the soybean genotypes studied under field and glasshouse conditions.

Number of Pods

The number of pods per plant was assessed at maturity stage. Number of pods was counted on five plants per each genotype randomly selected, and the observations helped to assess variations in the number of pods produced. This assessment was done for all 30 genotypes in the field, as well as for selected genotypes in further glasshouse and field experiments.

Plant Height

The plant heights were measured at maturity, and expressed in cm from the bottom of the plant to its highest point. Five plants were randomly selected from each genotype, and their means were calculated to give the average plant height for that particular genotype.

Number of Branches

The number of branches was determined for five randomly chosen plants of each genotype. Branch numbers were counted in the vegetative and mature stages, after which the average number of branches per genotype was determined.

Number of Nodes

The number of nodes was measured as nodes counted from the base of the plant to the highest node bearing a pod. Five randomly chosen plants per genotype were measured at maturity to ensure consistency in development of the plant.

Stem Girth

Stem thickness was determined in five randomly chosen plants of each genotype by the use of a digital Vernier caliper. Measurement was done in both the vegetative and mature stages in order to compare stem development in the various genotypes.

Hundred-Seed Weight

In measuring 100-seed weight, one hundred grains were arbitrarily chosen from each variety and then weighed using an electronic balance. The results were taken in grams as seed size and yield performance index.

Grain Yield

The yield of grain was determined at harvest time. For the purpose of determining grain yield, pods from five randomly chosen plants per genotype were collected and the yield was quantified in grams per plant.

Seed Quality Analysis

The quality analysis of 12 genotypes of soybean and check varieties Ajmeri and NARC-21 was performed. These analyzed traits include moisture, crude protein, crude fiber, ether extract (crude fat), ash, and carbohydrate content. After collecting the seeds, they were dried under sunlight, labeled, and preserved in polythene bags. Further, the seeds were ground into fine powder with the help of a mortar and pestle.

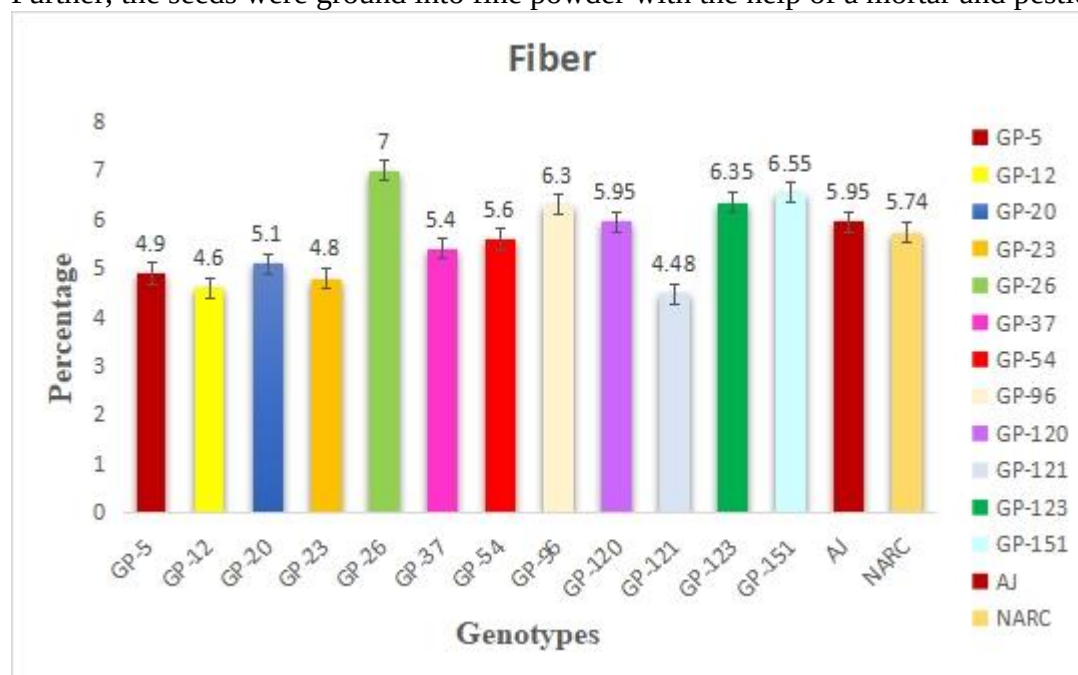


Figure : Fiber Content Analysis of the 12 Genotypes of Soybeans

Crude Protein Content

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The crude protein content determination was done through the Kjeldahl method at PMAS-Arid Agriculture University Rawalpindi. The process included digestion, distillation, and titration. In the process of digestion, one gram of the sample was added to potassium sulphate, copper sulphate, ferrous sulphate, and sulfuric acid. The digestion tubes were then heated until the mixture turned clear with a bluish-green color.



Figure Titration apparatus used in the last step of protein analysis after digestion



Figure :Potassium sulphate and Copper Sulphate, Digestion tubes

In distillation, the digested sample was distilled using the Kjeldahl method with 2% boric acid and methyl red indicator as the receiver. Before distillation, sodium hydroxide was used. The receiver solution after distillation was then titrated using 0.1 N hydrochloric acid up to the endpoint. Percentage of protein is determined using the equation in the thesis.

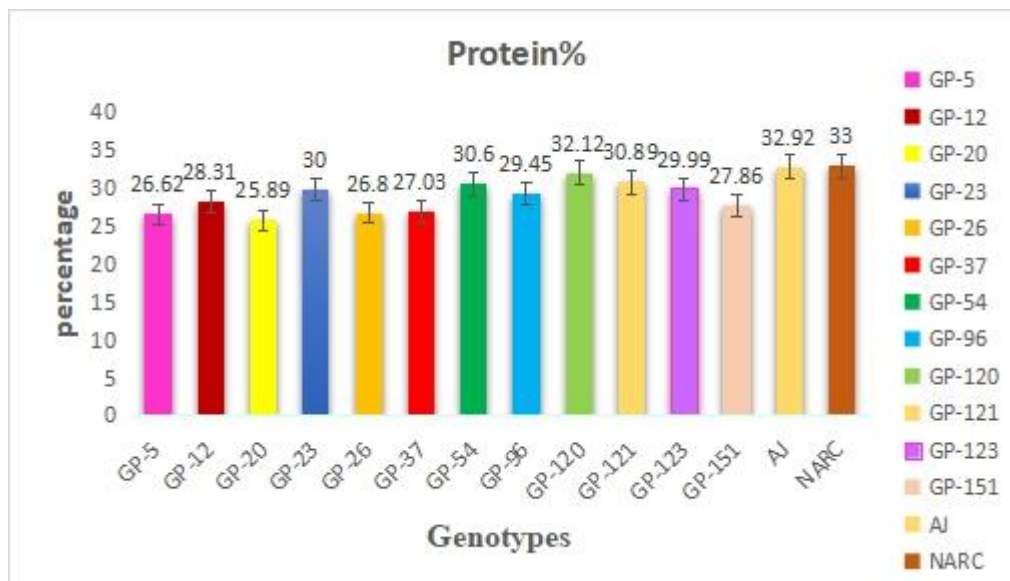


Figure: Protein Content Analysis of the 12 Genotypes of Soybeans

% Protein = $(R \times 0.014 \times 6.25 \times 100 \times 0.1) / \text{Sample weight}$

where R represents the HCl reading obtained during titration (Ahmad et al., 2019).

Ash Content

The ash content was carried out using the muffle furnace method. This involved weighing an empty crucible, adding about 5g of dried soybeans to it, and then weighing it again. It was placed in the muffle furnace at around 550°C for six hours. After that, it was cooled down in the desiccator and then weighed. The ash content was calculated based on the difference in weight.

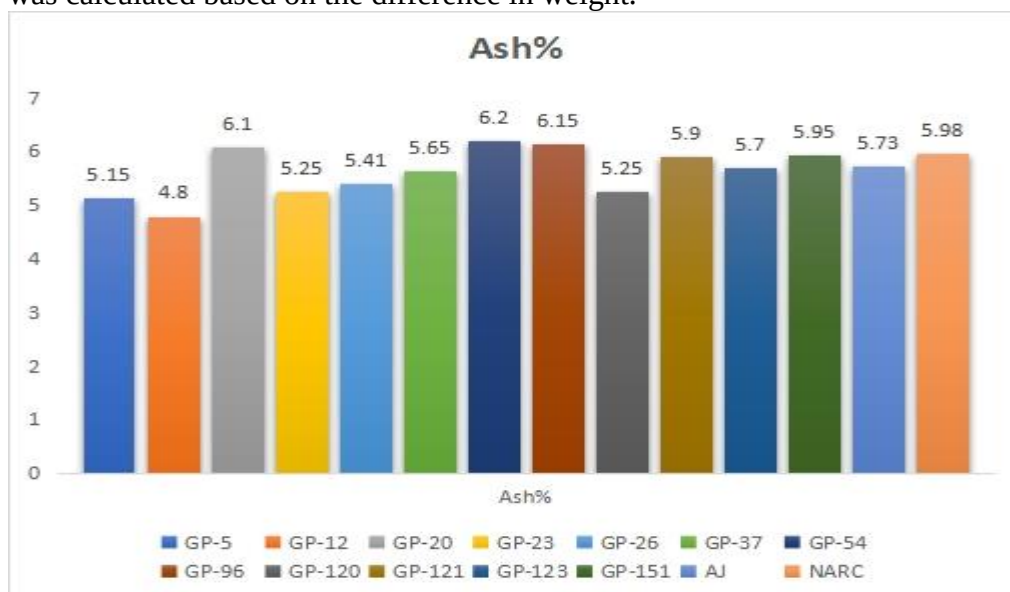


Figure : Ash Content Analysis of the 12 Genotypes of Soybeans

Crude Fibre Content

Crude fibre was quantified by acid-alkali extraction. A dried sample of fat-free soybeans was boiled in an acidic solution for about 30 minutes. This process was followed by filtration and washing with distilled water. After that, an alkaline solution was added and boiled for 30 minutes again. Filtration and washing were done once again, and drying was performed at about 105°C until constant weight was attained. Calculation of fibre was done using the formula provided in the thesis (Zobel & Waisel, 2010).

Carbohydrate Content

Carbohydrate content was estimated by difference after determining the other proximate components. The calculation used the following relationship:

% Carbohydrate = 100 – (% moisture + % fibre + % protein + % ash + % ether extract)

Thus, carbohydrate percentage represented the remaining fraction after accounting for moisture, fibre, protein, ash and ether extract.

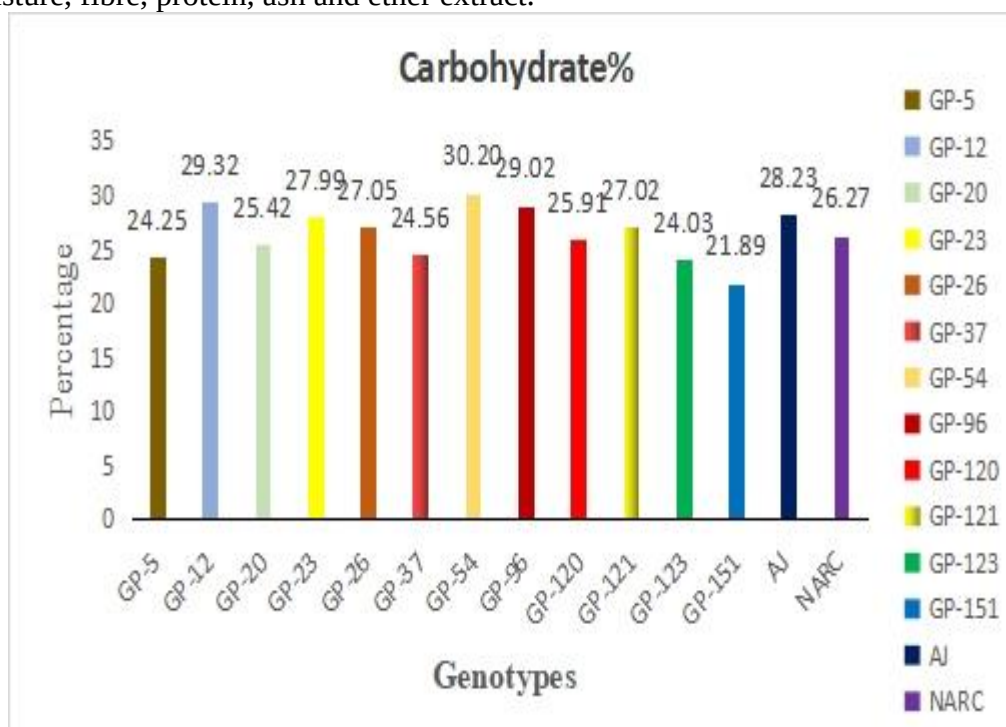


Figure: Carbohydrate Analysis of the 12 Genotypes of Soybeans

Moisture Content

Moisture content was established through an oven drying method. 10 g of ground soybeans was placed inside a labeled and previously weighed Petri dish. The ground sample was dried inside an oven maintained at a temperature of about 105 to 110 degrees centigrade for 24 hours. The dried sample was weighed and the drying and weighing were done until a constant weight was reached. The moisture content percentage was calculated by the weight difference of the samples:

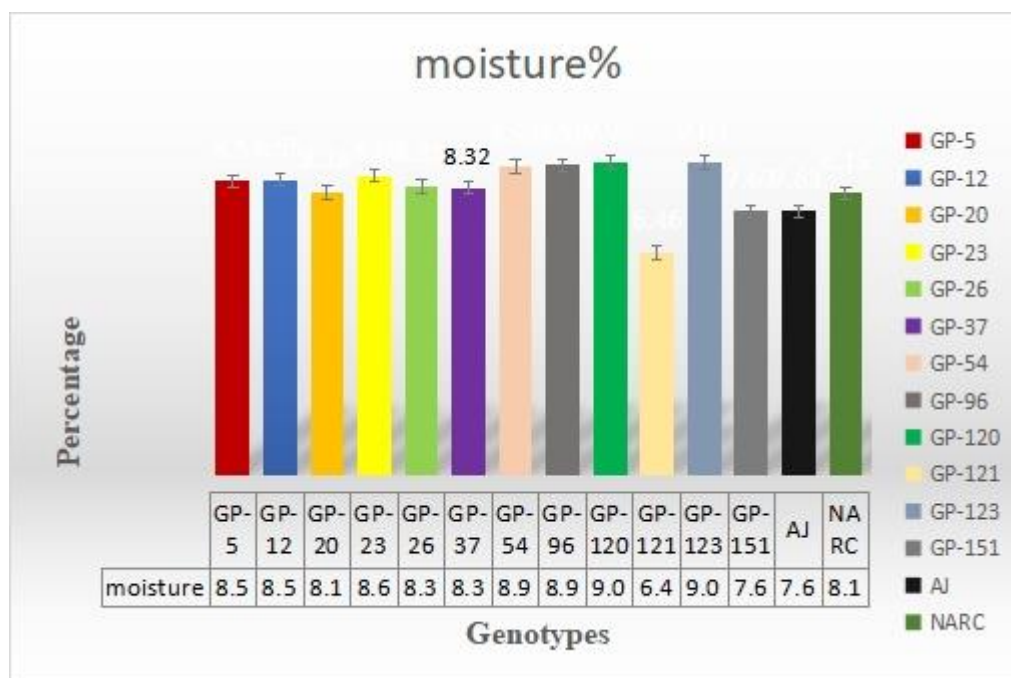


Figure : Moisture Content Analysis of the 12 Genotypes of Soybeans

$$\% \text{ Moisture} = (W_2 - W_3) \times 100 / (W_2 - W_1)$$

W_1 being the mass of the empty Petri dish, W_2 being the mass of the Petri dish with the initial sample, and W_3 being the mass of the Petri dish with the dried sample.

Ether Extract/Crude Fat

The crude fat content was estimated using Soxhlet extraction method with the use of petroleum ether. About 5 grams of dried soybean sample was put into a thimble and then petroleum ether was used to extract it for about six hours. After extraction, the solvent was removed and the flask was dried up at about 105°C.

Root System Architecture Analysis

RSA assessment was carried out in the glasshouse at the Oilseed Research Program, NARC, Islamabad. There were three replicates of 30 soybean genotypes along with two check varieties, Ajmeri and NARC-21. It is stated in the thesis that for three replicates of each genotype, three healthy seeds were sown in the pot, containing soil, sand, and peat moss (50% each). The plants were grown at about 25°C and were watered from the spray bottle.

At 21 DAS, the seedlings were harvested from the growing substrate without causing any harm to the root system. The roots were carefully cleaned with tap water in order to wash off soil and other materials that might have clung onto them. The entire root system was isolated from the shoot and was then scanned through an HP LaserJet Pro MFP M130 scanner with black paper as the background of the picture.

Root Images were analyzed by utilizing root phenotyping tools that were computerized. It is clear from the thesis that the DIRT application was used to upload

the root images and that the measurements of root system architecture were done through the Rhizovision application. Seven RSA traits were measured: Number of Root Tips (NRT), Number of Branch Points (NBP), Total Root Length (TRL, mm), Total Root Volume (TRV, mm³), Network Area (NA, mm²), Average Diameter (AD, mm) and Surface Area (SA, mm²).

Statistical Analysis

Agro-morphological data of the soybean genotypes was subjected to analysis of variance (ANOVA) to determine whether there are any significant differences amongst genotypes. Analysis of the augmented block design involved determining the effect of treatment and blocks, while for the RSA trait analysis was done using a completely randomized design (CRD) with three replicates. According to the thesis, the analysis of data followed the methodology described by Steel, Torrie and Dickey (1997).

Augmented design was first used to screen the soybean germplasm. It involved 30 genotypes and two checks where test entries appeared once and check varieties were replicated across three blocks. After the screening using agro-morphological performance and RSA, promising genotypes were selected and used in further studies using a randomized complete block design (RCBD).

Root phenotyping analysis involved use of three replications to measure the differences in RSA traits. These measurements were then compared among genotypes to determine the level of difference in root development and architecture. The thesis notes that significant differences existed especially in total root length, total root volume, network area and surface area.

Overall Methodological Framework

This is why the methodology of the study was such that 30 genotypes of soybeans together with two check genotypes were evaluated via agro-morphological field characterization, seed quality testing and root phenotyping in a glasshouse. Augmented design was employed in the first step in order to find out the promising germplasm and root system architecture was quantitatively determined by digital image analysis. Selected genotypes were then evaluated via RCBD. In this way, genetic variation of soybeans was studied with respect to aboveground morphology, yield, seed quality and root system architecture.

Results and Analysis

The results provided in this section are taken from the experiments performed in the thesis attached. The experiment was performed on 30 soybean genotypes, along with two check varieties, Ajmeri and NARC-21, through Agro-Morphological Characterization, Seed Quality Evaluation, and Root System Architecture Analysis (RSA). The analysis of Agro-Morphological data was done using Augmented Design and the RSA experiment was done under Completely Randomized Design with three replications. Twelve superior genotypes were selected for the evaluation using RCBD.

Analysis of Variance for Agro-Morphological Traits

According to the treatment adjusted analysis of variance carried out on the augmented block design, genetic variability existed for the stem height and 100-seed weight for the soybeans tested. Treatment mean squares for the stem girth, stem height and 100-seed weight were 1.43, 1.05 and significant values, respectively, whereas the other flowering and vegetative traits evaluated did not exhibit significant genetic variability. Check effect was also significant for the 100-seed weight

Block adjustment yielded a very similar pattern. Significant genotype treatment effects were found in the case of stem height and 100-seed weight. Days to flower initiation, days to 50% flower, days to flower termination, mean number of nodes, number of branches and stem girth did not show significant results. Coefficients of variations for flower traits were very low as stated in the thesis. For instance, 2.84%, 2.01% and 3.01% for flower initiation, 50% flower and flower termination respectively.

Table 4.1. Summary of genetic variation observed in agro-morphological traits

Trait	Genotypic variation	Result
Days to flowering initiation	Non-significant	Limited detectable variation
Days to 50% flowering	Non-significant	Limited detectable variation
Days to flowering completion	Non-significant	Limited detectable variation
Number of nodes	Non-significant	Limited detectable variation
Number of branches	Non-significant	Limited detectable variation
Stem girth	Non-significant	Limited detectable variation
Stem height	Significant	Genetic differences detected
100-seed weight	Significant	Genetic differences detected

Non-significance of the reaction of many characters could be due to the fact that Rabi season in Islamabad is not suitable for soybean growth, which could restrict the expression of genetic variation for many reproductive and yield-related traits.

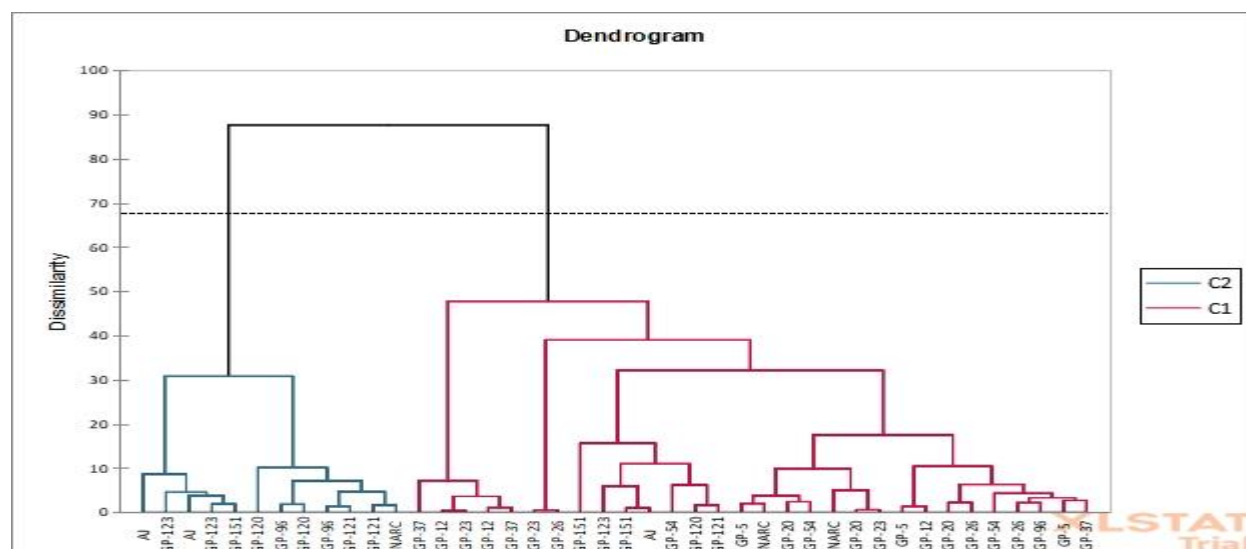


Figure : Cluster Analysis on morphological traits of soybean genotypes

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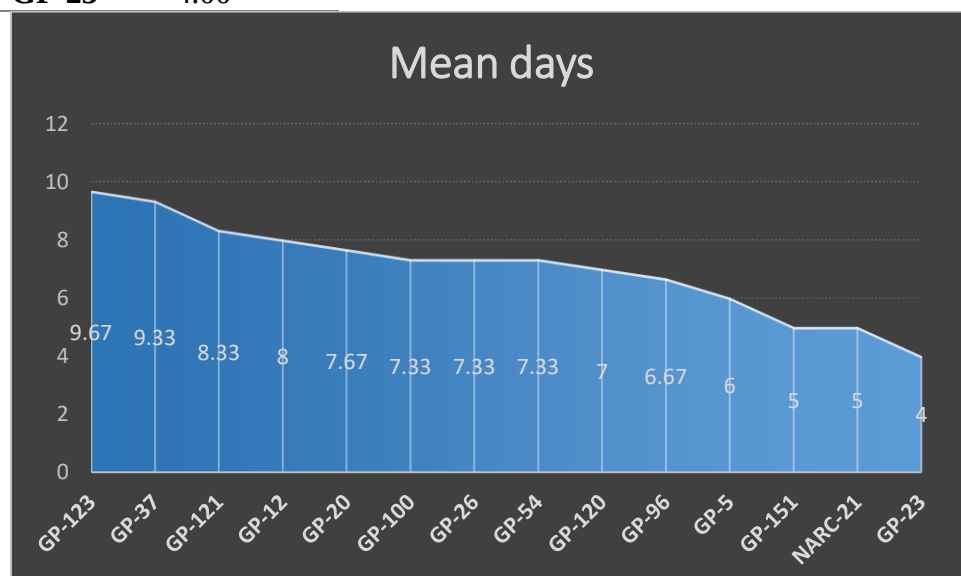
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Variation in Days to Germination

There was also variation among the evaluated genotypes with respect to days to germination. According to the results of the LSD test, GP-123 had the maximum mean value of 9.67 days, whereas GP-37 had the mean value of 9.33 days. The other two genotypes having the means of 8.33 days and 8.00 days were GP-121 and GP-12, respectively. On the contrary, the minimum mean of 4.00 days was recorded in GP-23, whereas NARC-21 check had a mean of 5.00 days.

Table 4.2. Selected means for days to germination

Genotype	Mean days
GP-123	9.67
GP-37	9.33
GP-121	8.33
GP-12	8.00
GP-20	7.67
GP-100	7.33
GP-26	7.33
GP-54	7.33
GP-120	7.00
GP-96	6.67
GP-5	6.00
GP-151	5.00
NARC-21	5.00
GP-23	4.00



Variation in Number of Branches

Significant variations were observed in terms of the mean number of branches in the chosen genotypes. GP-26 exhibited the highest mean number of branches (90.947), followed by GP-23, which had 79.133 branches, and GP-54, with 67.43 branches. GP-

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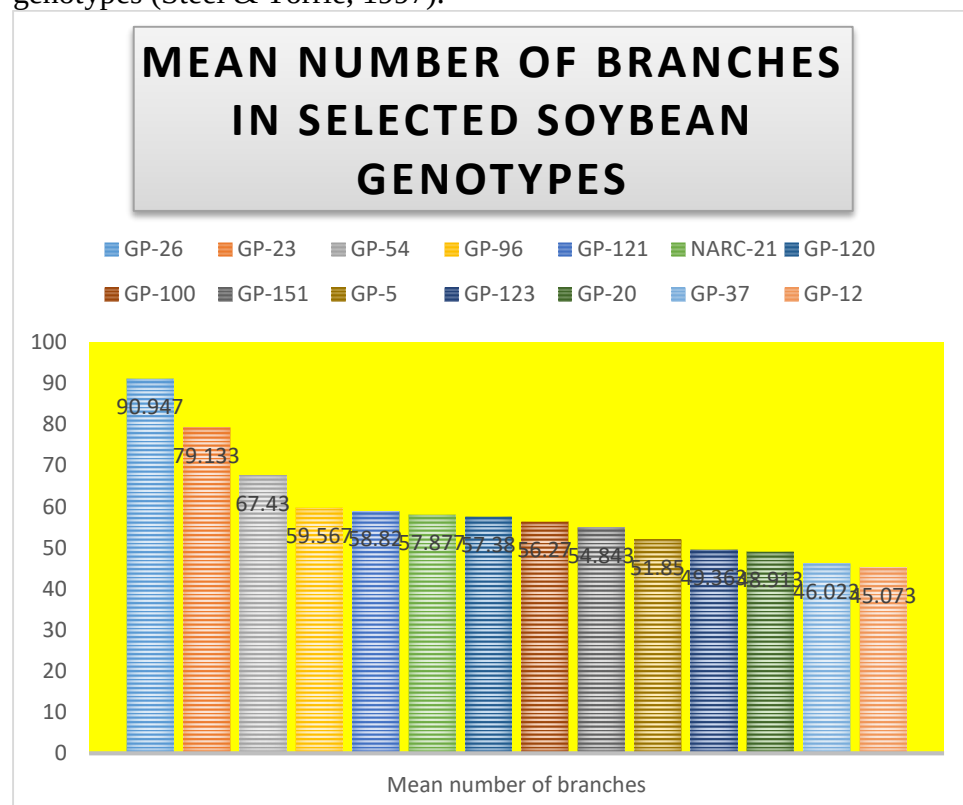
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96 and GP-121 had means of 59.567 and 58.82, respectively. The genotype that recorded the least mean was GP-12, with 45.073 branches, while GP-3.

Table 4.3. Mean number of branches in selected soybean genotypes

Genotype	Mean number of branches
GP-26	90.947
GP-23	79.133
GP-54	67.430
GP-96	59.567
GP-121	58.820
NARC-21	57.877
GP-120	57.380
GP-100	56.270
GP-151	54.843
GP-5	51.850
GP-123	49.363
GP-20	48.913
GP-37	46.023
GP-12	45.073

The big difference in numbers between GP-26 and GP-12 suggests a lot of variability in phenotypes when it comes to branching, but the augmented-design ANOVA analysis was unable to conclude that number of branches had any significance to genotypes (Steel & Torrie, 1997).



Root System Architecture

It was found that the phenotype analysis for the roots produced a much higher genetic differentiation than was found in case of many of the aboveground morphological characters. The root system architecture study involved the number of root tips, the number of branch points, total root length, total root volume, network area, mean diameter and surface area.

The analysis of variance in completely randomized design found significant differences in some of the RSA variables, especially total root length, total root volume, network area and surface area.

The thesis also reports high significance in the number of branching points, which indicates that the studied soybean genotypes exhibited significant genetic differences in developing a branched root system.

RSA trait	Genotypic response
Number of root tips	Significant variation reported
Number of branch points	Highly significant variation
Total root length	Highly significant variation
Total root volume	Highly significant variation
Network area	Highly significant variation
Average diameter	Significant variation reported
Surface area	Highly significant variation

Correlation among Root System Architecture Traits

Correlation among phenotypic traits was evaluated based on the seven RSA traits within the 30 soybean varieties. The outcomes revealed that there were both positive and negative correlations among the root traits. Network area had a positive and insignificant correlation with average diameter ($r = 0.349$). There was also a negative and insignificant correlation between total number of branches and root volume ($r = -0.29$). Root tips had a positive association with surface area ($r = 0.143$) and root volume ($r = 0.123$).

Some of the associations were very significant. There is a very significant association between average diameter and network area with $p = 0.0001$ as per the results of the thesis, while the average diameter and the number of root tips had a highly significant association with $p = 0.0002$. Similarly, the number of root tips and the total root length have a very significant association with $p = 0.0000$.

Table 4.5. Selected relationships among RSA traits

Trait relationship	Correlation/Significance reported	Interpretation
Network area $\times$ average diameter	$r = 0.349$	Positive, non-significant
Branches $\times$ root volume	$r = -0.29$	Negative, non-significant
Root tips $\times$ surface area	$r = 0.143$	Positive, non-significant
Root tips $\times$ root volume	$r = 0.123$	Positive, non-significant

		significant
Average diameter	$\times p = 0.0001$	Highly significant
network area		
Average diameter	$\times \text{root tips } p = 0.0002$	Highly significant
tips		
Root tips	$\times \text{total root length } p = 0.0000$	Highly significant
length		

However, in general, it can be stated that there was no independence between the root traits studied. Specifically, the root tip formation was highly correlated with the root length, while the average diameter was highly correlated with network area and root tips.

Seed Quality Characteristics

The seed quality analysis was done for the twelve genotypes selected along with the two check varieties, namely Ajmeri and NARC-21. The analyses were done for the characteristics related to protein, lipid/ether extract, fibre, ash, moisture, and carbohydrate. There was observed to be significant variation in the genotypes regarding their nutritional components.

The highest protein value was recorded by NARC-21 at 32.89%, while GP-20 had the lowest protein content at 25.89%. The majority of the genotypes had the protein percentage varying in the range of 25%-30%.



Figure : kjeldhal apparatus used in proximate analysis used to measure protein content

Variation was also seen in the lipid content of the selected genotypes. The highest lipid percentage was seen in GP-121 at 23.40%, while GP-5 had the lowest percentage of lipids at 18.28%. The lipid percentage in the genotypes was mostly in the range of 19%-22% (Aziz et al., 2021).

The variation in fibre content was between 4.48%-7.00%. GP-26 had the highest fibre percentage at 7.00%, while GP-121 had the lowest at 4.48%. GP-151 and GP-123 had relatively high percentages of fibre content.

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Table 4.6. Key seed-quality results

Quality trait	Highest genotype/value	Lowest genotype/value
Protein	NARC-21 — 32.89%	GP-20 — 25.89%
Lipid	GP-121 — 23.40%	GP-5 — 18.28%
Fibre	GP-26 — 7.00%	GP-121 — 4.48%

Selection of Promising Genotypes

Among the significant findings of the study was that genotypes which would qualify for further study were found. Based on the augmented design screening and the root system architecture experiment results, some genotypes which performed well enough to warrant further evaluation using RCBD were identified. Among the genotypes selected from the material include GP-5, GP-12, GP-20, GP-23, GP-26, GP-37, GP-54, GP-96, GP-120, GP-121, GP-123 and GP-151.

The selected genotypes are those whose performance was relatively good compared to the screening criteria used. The process of selection reveals the importance of the use of both agro-morphological and root system phenotyping instead of just one yield trait (Khan et al., 2019).

Overall Findings

The results indicate that there was the presence of genetic and phenotypic diversity within the soybean germplasm, though the level of variation varied considerably from one group of traits to another (Qin et al., 2023). The augmented design results showed the existence of statistically significant differences for stem height and 100-seed weight genotypes, while most of the flowering and branching trait genotypes did not show any significance within the Rabi conditions. However, root system architecture indicated considerable difference among several of the traits such as total root length and total root volume.

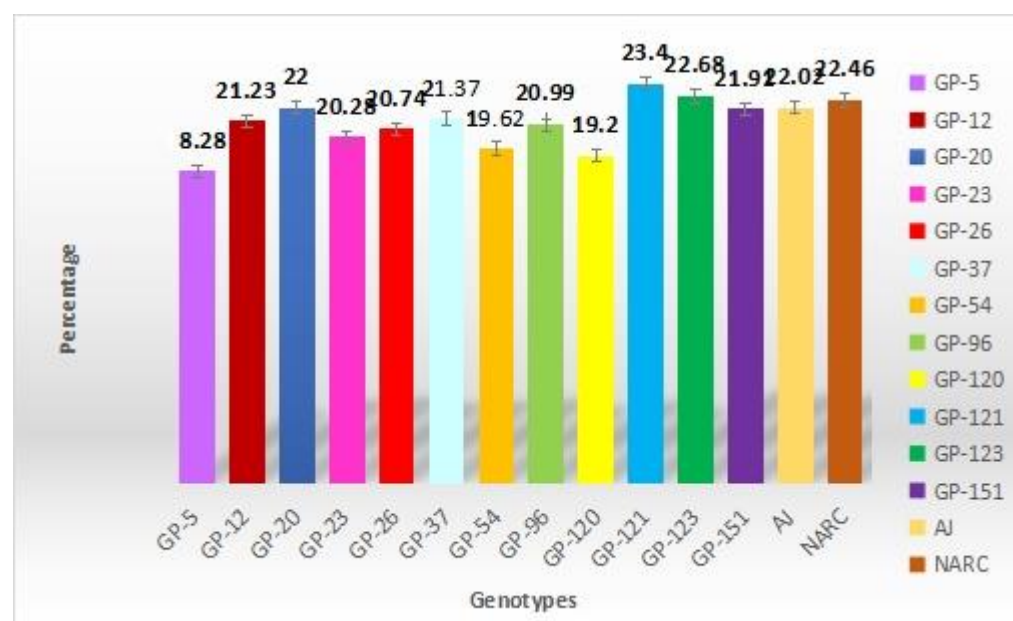


Figure: Lipid Content Analysis of the 12 Genotypes of Soybeans

Furthermore, the quality of the seed showed useful variation, where NARC-21 showed the highest protein content, GP-121 exhibited the highest lipid content and GP-26 exhibited the highest fibre content from the reported results (Zuo et al., 2023). Additionally, RSA correlation showed several traits which were associated with each other, specifically root tips, total root length, average diameter and network area. Based on these results, GP-5, GP-12, GP-20, GP-23, GP-26, GP-37, GP-54, GP-96, GP-120, GP-121, GP-123 and GP-151 were identified as potential candidates for breeding.

Discussion

In the current study, agro-morphological performance of the soybean genotypes varied, but the extent of variability was affected by the particular trait and environmental factors. Variance analysis conducted using augmented design showed significant differences among genotypes in terms of stem height and 100-seed weight, while days to start of flowering, 50 percent flowering, end of flowering, number of nodes, branches and stem girth remained non-significant. In the thesis, some of these traits were considered insignificant owing to inappropriate Rabi season conditions for soybean growth at Islamabad (Qin et al., 2023).

It is noteworthy that there is considerable variation for stem height and 100-seed weight as it plays a role in identifying genetically superior genotypes. Plant architecture variation will have its effect on the ability of plants to intercept light, biomass production, and reproduction, and 100-seed weight is a component of seed productivity. Results of the study are in line with earlier soybean diversity investigations published in the thesis. As stated by Kachare et al., there was considerable genetic diversity for morphological and yield traits in soybean genotypes (Ober et al., 2021).

Weak differentiation in the case of flowering traits does not necessarily mean that the population lacks genetic variability. Flowering is highly dependent on the environmental factors, mainly photoperiod and temperature. Thus, the Rabi environment of Islamabad could limit the manifestation of the genetic difference in the material under study. The thesis itself highlights the Rabi season as the factor limiting soybean productivity in the experiment location. Evaluation of the material in other favorable environments will be useful in isolating the genetic effect from the environmental effect.

There have been significant variations among genotypes in terms of days taken to germinate and branch number. GP-123 had the most days for germination as compared to other genotypes, whereas GP-23 took the least days for germination. GP-26 had the highest branch number, GP-23 and GP-54 had the second and third highest branch numbers, respectively, while GP-12 had the least branch number.

Germination of crops at an early stage and with consistency can have benefits as it can ensure synchronized growth and also offer competitive advantage to the plants. Likewise, another aspect related to the plant architecture of soybeans is their ability to branch, which increases the site available for reproduction. The increased branching seen in GP-26 and GP-23 thus offers a good starting point from where vigorous plant

architecture can be selected. Some of these genotypes were chosen by the thesis for testing and found to be performing well enough for further breeding evaluation (Liu et al., 2021).

The previous studies referred to in this thesis also emphasize the significance of the morphological characteristics associated with yield. Ahmad et al. (2019) found that branches, biomass, seed weight, protein, oil content, and harvest index positively correlate with soybean yield, whereas flowering time negatively correlates with the yield. Hence, the morphological differences observed in the current study could be used for indirect selection in more favorable production environments (Gabr, 2022).

One of the key outcomes of the research was the considerable genetic diversity in RSA. The trial measured the number of root tips, number of branching points, total root length, total root volume, area of network, average diameter and surface area by digital root image analysis and Rhizovision. The design of the experiment involved 30 genotypes and two controls, with 3 replications, where roots were analyzed after 21 days of seedlings growing (Rahim et al., 2024).

Differences that were found to be highly significant in terms of total root length, total root volume, area of network and surface area were also found to be significant for root tips and branch points. The significance of the result is noteworthy, especially when root characteristics are not often taken into consideration due to their relatively low visibility and the infrequency with which they feature in traditional soybean breeding programs (Karr-Lilienthal et al., 2004).

The findings support the findings by Falk (2019), which revealed genetic diversity in root number, length, weight, architecture, and angle in various soybean accessions. Likewise, Chen et al. (2022) pointed out that root architecture is an important trait in terms of acquiring water and nutrients and enhancing plant productivity, and RSA has been underinvestigated in crop breeding programs. In this regard, the considerable RSA variation found in this study can be regarded as valuable information showing the potential of using soybean roots as additional selection criteria.

Conclusion

In the current research work, the genetic diversity among soybean genotypes based on agro-morphological, yield, seed quality, and root system architecture characteristics is reported. For this purpose, thirty soybean genotypes and two check varieties NARC-21 and Ajmeri were analyzed with an augmented design while root system architecture was evaluated by taking three replications along with the help of digital root image analysis. It is reported here that considerable genetic diversity exists in the germplasm used.

Genotypic variations in stem height and 100-seed weight were significant, but some flowering and vegetative characteristics did not show significant variability. The environmental condition of the Rabi season in Islamabad seems to be restricting the expression of some genetic differences in soybean genotypes. Hence, it is concluded from the current results that the evaluation of soybean germplasm needs to be conducted in contrasting environments

RSA analysis gave very valuable information about genetic diversity. The difference was found in relation to such parameters as root length, root volume, area of the network, surface area, root tips and branching points. Relationship among selected

RSA traits was another evidence of the complex nature of root development. It makes possible to consider RSA traits as one of the criteria in future soybean selection programme.

The seed quality assessment made possible to find valuable differences as well. NARC-21 genotype had the highest content of proteins, GP-121 had the highest content of lipids, and GP-26 had the highest fibre content. Therefore, there is an opportunity to conduct selection depending on particular requirements.

Based on the results obtained, twelve promising genotypes - GP-5, GP-12, GP-20, GP-23, GP-26, GP-37, GP-54, GP-96, GP-120, GP-121, GP-123 and GP-151 - were singled out for additional evaluation. The general conclusion from the results obtained is that the integration of such traits as yield, seed quality and RSA makes it possible to improve soybeans.

Recommendations

Multi-Environment Evaluation

The twelve genotypes chosen should be evaluated under varying environments of soybean production and seasons in order to establish their stability. Specific emphasis should be made on environments having variations in temperatures, rainfalls and soil moisture content.

Evaluation under Suitable Soybean Growing Conditions

Since a number of agro-morphological traits exhibited little differentiation in Rabi season in Islamabad, it would be more appropriate to conduct future experiments on more suitable soybean growing seasons, since this would ensure that genetic differences would be expressed more reliably.

Incorporation of Root Traits into Breeding

RSA needs to be included as an additional criterion for the selection of soybean plants. Factors like total length of roots, root volume, network area, surface area, root tips, and branch points need to be considered along with other above-ground traits as RSA helps in efficient water and nutrient uptake.

Validation of Promising Genotypes

The twelve selected genotypes will have to go through RCBD trials for more than one season before making their final selection. All these factors should be considered collectively in order to select genotypes that are productive, nutritious, and adaptable.

Quality-Oriented Selection

The genotypes that have favourable protein and oil properties should be taken into account according to their specific uses. The genotype NARC-21 could be used as a source for breeding for proteins, while GP-121 could be used for oil breeding. The genotype GP-26 could be considered for higher fibre content.

Molecular Characterization

Future research should combine molecular marker-based approaches with root phenotyping and morphological studies. Combining phenotypic and molecular data

would make it easier to select genetically different parents and develop better soybean breeding lines.

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