ATATÜRK ÜNİVERSİTESİ YAYINLARI
ATATURK UNIVERSITY PUBLICATIONS

Research Article Araştırma Makalesi DOI: 10.5281/zenodo.17976793

Integrating Image-Based Phenotyping: A Comprehensive Analysis Using PCA, Cluster Analysis, and Path Coefficient Analysis

Görüntü Tabanlı Fenotiplemenin Entegrasyonu: PCA, Kümeleme Analizi ve Yol Katsayısı Analizi Kullanılarak Kapsamlı Bir Analiz

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Geliş Tarihi/Received	30.09.2025
Revizyon Talebi/Revision Requested	13.11.2025
Son Revizyon/Last Revision	25.11.2025
Kabul Tarihi/Accepted	28.11.2025
Yayın Tarihi/Publication Date	26.12.2025

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Cite this article: Ashiq, M. W., Sheraz, M., Shahnawaz, M., Zahoor, S., Aziz, A., Khan, T. N., Razzaq, S., Khan, M. T. F., Malik, M. Y., Ahmad, M., & Muzamil, M. (2025). Integrating image-based phenotyping: A comprehensive analysis using PCA, cluster analysis, and path coefficient analysis. *Journal of Ecological Harmony*, 1(2), 51-60.

ABSTRACT

Field pea (Pisum sativum L.) is a significant legume crop in Pakistan, and the development of high-yielding varieties is essential for enhancing productivity. This study aimed to evaluate the morphological diversity of 23 pea genotypes using both image-based phenotyping and field-based traits. A total of 21 traits were analyzed. Principal Component Analysis (PCA) revealed a strong positive correlation among pod area, pod perimeter, pod length, pod weight, pod aspect ratio, number of seeds per pod, seed weight per pod, and number of nodes, with pod area showing the highest eigenvalue of 9.715. Cluster analysis identified four major clusters and six sub-clusters, highlighting significant genetic diversity in the germplasm. Correlation analysis revealed a highly significant positive correlation between pod weight and pod factor from density (r = 0.91), seed weight per pod (r = 0.88), and number of seeds per pod (r = 0.85). Path coefficient analysis indicated that pod area had the highest direct positive effect on pod weight (0.010), while pod length exhibited a negative direct effect (-0.002). Genetic variance estimates ranged from 41,108,350 for pod area to 0.00 for pod roundness, with phenotypic variance ranging from 50,332,040 for pod area to 0.00 for pod roundness. Broad-sense heritability was high for traits such as pod weight (0.80), pod area (0.82), seed weight per pod (0.81), and number of pods per plant (0.81). These findings highlight the strong genetic influence on pea yield-related traits and provide a foundation for future breeding programs aimed at improving productivity.

Keywords: Cluster, Correlation, High-throughput phenotyping, PCA, Pea

ÖZ

Bezelye (*Pisum sativum* L.), Pakistan'da önemli bir baklagil ürünüdür ve verimliliği artırmak için yüksek verimli çeşitlerin geliştirilmesi şarttır. Bu çalışma, hem görüntü tabanlı fenotipleme hem de saha tabanlı özellikler kullanılarak 23 bezelye genotipinin morfolojik çeşitliliğini değerlendirmeyi amaçlamıştır. Toplam 21 özellik analiz edilmiştir. Ana Bileşen Analizi (PCA), bakla alanı, bakla çevresi, bakla uzunluğu, bakla ağırlığı, bakla en boy oranı, bakla başına tohum sayısı, bakla başına tohum ağırlığı ve düğüm sayısı arasında güçlü bir pozitif korelasyon olduğunu ortaya koymuştur; bakla alanı 9,715 ile en yüksek özdeğere sahiptir. Korelasyon analizi, bakla ağırlığı ile bakla yoğunluğu faktörü (r = 0,91), bakla başına tohum ağırlığı (r = 0,88) ve bakla başına tohum sayısı (r = 0,85) arasında oldukça anlamlı pozitif bir korelasyon olduğunu ortaya koymuştur. Yol katsayısı analizi, bakla alanının bakla ağırlığı üzerinde en yüksek doğrudan pozitif etkiye (0,010) sahip olduğunu, bakla uzunluğunun ise negatif doğrudan bir etkiye (-0,002) sahip olduğunu göstermiştir. Genetik varyans tahminleri, bakla alanı için 41.108.350'den bakla yuvarlaklığı için 0,00'a kadar değişirken, fenotipik varyans bakla alanı için 50.332.040'tan bakla yuvarlaklığı için 0,00'a kadar değişmiştir. Geniş anlamda kalıtılabilirlik, bakla ağırlığı (0,80), bakla alanı (0,82), bakla başına tohum ağırlığı (0,81) ve bitki başına bakla sayısı (0,81) gibi özellikler için yüksek olmuştur. Bu bulgular, bezelye verimiyle ilgili özellikler üzerindeki güçlü genetik etkiyi vurgulamakta ve verimliliği artırmayı amaçlayan gelecekteki ıslah programları için bir temel oluşturmaktadır.

Anahtar Kelimeler: Kümeleme, Korelasyon, Yüksek verimli fenotipleme, PCA, Bezelye

Introduction

Pea (Pisum sativum L.) is an important annual herbaceous crop belonging to the family Leguminosae. It has a chromosome number of 2n=14 and is considered one of the oldest domesticated legumes. Legumes, with over 650 genera and 18,000 species, represent the third largest flowering plant family (Lewis et al., 2005). Among these, Pisum sativum is a self-pollinated food crop (Fontes et al., 2014), contributing significantly to global food security. According to the Food and Agriculture Organization (FAO), the top nine pea-producing countries in 2021 were Russia, Canada, China, Ukraine, India, France, the USA, Australia, and Germany. In Pakistan, the cultivated area for dry peas was 28005 hectares, while 54177 hectares was dedicated to green peas in the same year (FAO, 2023).

Advancements in technology, such as computer-aided image analysis, have facilitated the efficient analysis of seed and pod features in peas. Digital imaging, for instance, allows for high-throughput (HTP) phenotyping, enabling rapid and precise assessment of traits that contribute to yield. This method is particularly useful in discriminating varieties based on morphological traits (Dell'Aquila, 2006; Daniel et al., 2012). HTP has the potential to accelerate plant breeding initiatives by enabling the development of crops that are more suited to changing environmental conditions and capable of meeting the increasing global demand for food. In precision agriculture, real-time monitoring of crop health and growth is also facilitated by digital imaging, allowing for optimized resource use (Yang et al., 2019).

Recent research has focused on the acquisition, processing, and analysis of seed and pod images to understand the genetic and phenotypic diversity of peas. Studies on morphological variation have proven useful for estimating variability and genetic diversity within pea populations (Cupic et al., 2009; Gixhari et al., 2014; Sarikamiş et al., 2010; Smykal et al., 2008).

Breeding efforts have greatly improved the yield potential and regional adaptation of peas through enhanced resistance or tolerance to abiotic stresses. Yield is a complex trait controlled by numerous genes and is heavily influenced by environmental factors. Quality traits, on the other hand, are generally more stable and less influenced by the environment, making them more suitable for selection in breeding programs. These quality traits have a direct or indirect influence on yield and often serve as more reliable targets for selection. Understanding the extent of genetic diversity and the relationship between genotypes is crucial for effective breeding strategies, including the identification of duplicates, better characterization of genotypes, and selection of parents with the appropriate genetic distance for transgressive segregation (Hornokova et al., 2003; Joshi et al., 2004).

Correlation and path analysis are key tools in understanding the relationship between yield-contributing traits. Simple correlation analysis, while useful, may not fully capture the complexity of these relationships, especially when multiple variables are involved. Path analysis provides a more detailed understanding of how different traits contribute to yield, revealing direct and indirect effects of traits such as pod number, seed count, and plant height on overall yield (Mahajan et al., 2011; Williams et al., 2013). Several studies have shown that seed yield is positively correlated with traits such as the number of pods per plant, pod length, and seed weight (Assen, 2020; Bijalwan et al., 2018). Furthermore, genetic and phenotypic variances in quantitative traits, such as plant height and seed yield, are essential for selecting high-yielding genotypes (Tiwari et al., 2012). Heritability, or the proportion of phenotypic variation that is passed from parent to offspring, is another important factor in breeding. High heritability increases the potential for successful selection, making it a key consideration in breeding programs aiming to enhance yield and quality (Sharma & Bora, 2013).

This study aims to investigate the relationship between pod and seed shape parameters and yield components in Pisum sativum, with the goal of improving yield-related traits using high-throughput phenotyping methods. We will also explore the genetic diversity of these traits and utilize an integrated approach combining morphological analysis with digital imaging to better understand and select for yield-contributing traits.

Methods

Study Site and Germplasm

The study was conducted in the Department of Plant Breeding and Genetics, Bahauddin Zakariya University, Multan, Pakistan, in November 2022. A total of 23 pea genotypes were selected for the morphological analysis of various traits. These genotypes were evaluated based on their morphological traits and digital image-based phenotyping, using high-throughput digital cameras to assess genetic diversity within the germplasm.

Experimental Design

The experiment was conducted using a Randomized Complete Block Design (RCBD) with three replications. The field used for the experiment had clay-loamy soil, and the plant-to-plant and row-to-row spacing were maintained at 5 cm and 10 cm, respectively, to ensure optimal lighting and aeration. Standard agronomic practices, including timely irrigation and insecticide management, were followed to maintain plant health and prevent insect damage.

Morphological and Phenotypic Measurements

To assess genetic diversity at the field level, various morphological parameters were recorded, including seed weight per pod, number of seeds per pod, pod weight per plant, number of pods per plant, and number of nodes. These measurements were taken from five randomly selected plants for each genotype.

For digital image-based phenotyping, pod and seed shapes were evaluated using a digital photography-based phenotyping technique. Photographs of the genotypes were taken at the flowering stage, with flower pollination dates recorded to determine the time to pod maturity. Six pods and twelve seeds were selected based on visual appearance. The pods and seeds were placed horizontally on a dark sheet to ensure proper spacing, and an image studio setup was created using black paper and energy-saving lights to eliminate shadows.

Image Analysis

The analysis of pod and seed characteristics was performed using Image J software, developed by the National Institutes of Health. Image J enables precise measurement of items in two-dimensional images, ensuring reliable and standardized metrics by utilizing a global scale and size standard. The images illustrated by software are given in Figure 1. The "Analyze Particle" function was used for images of pods and seeds, with measurements for length (major axis) and width (minor axis) recorded. The results were exported to a spreadsheet for further analysis. Additionally, parameters such as area, perimeter, length, width, aspect ratio, and Factor from Density (FFD) were calculated using the following formulas:

$$\text{Aspect Ratio} = \text{Length} / \text{Width}$$

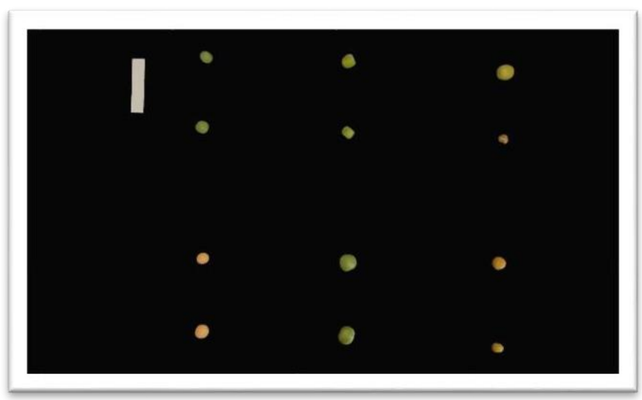
$$\text{FFD} = \text{Individual grain weight} / (\text{Length} \times \text{Width})$$

Weight of pods and seeds was measured using an analytical balance.

Original images

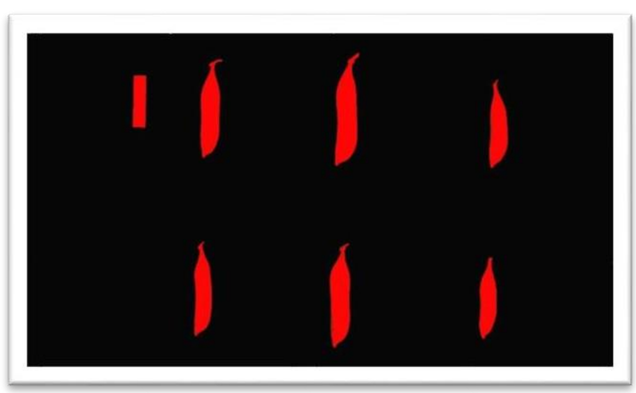
Figure 1.
Original, threshold and outline images of pod and grain of peas

(a)

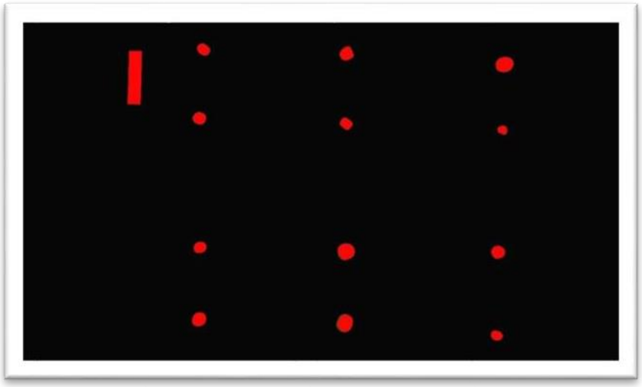


(b)

Threshold images

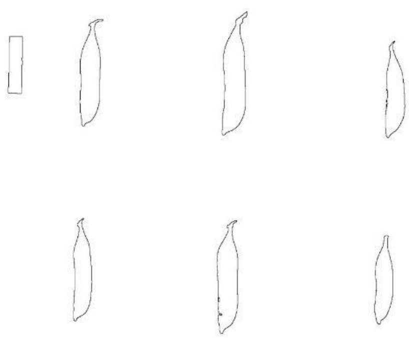


(c)

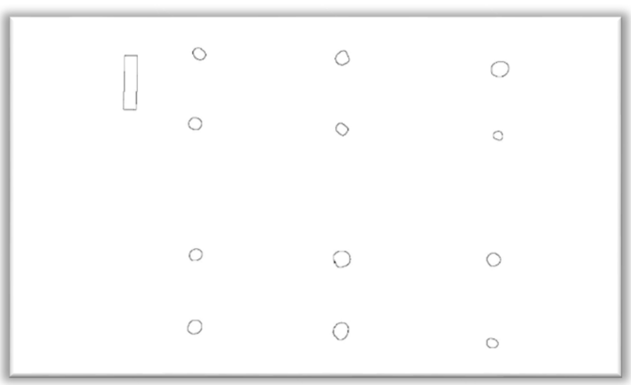


(d)

Outline Images



(e)



(f)

Statistical Analysis

Correlation analysis was performed to investigate the relationships between various traits using Kwon and Torrie's method (1964) approach by using R programming language. This analysis helped assess the direction and significance of the associations between the studied variables. Path coefficient analysis, as described by Wright (1921) and Dewey and Lu (1959), was used to explore the direct and indirect effects of traits on pod weight. Principal Component Analysis (PCA) was conducted using XLSTAT software. Genetic components, including genetic variance, phenotypic variance, environmental variance, and broad-sense heritability, were estimated using R Studio software.

Results

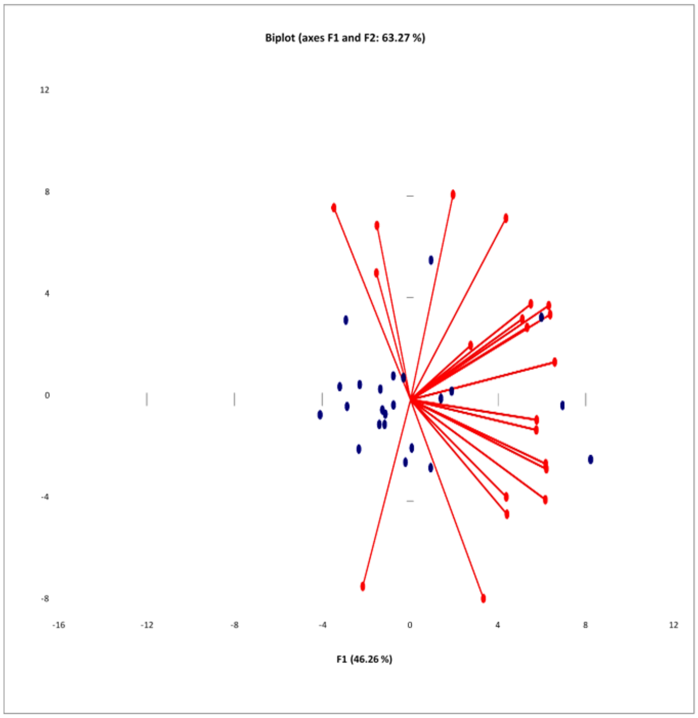
Principal Component Analysis (PCA)

Principal Component Analysis was performed to assess genetic diversity among the pea genotypes. The results of the PCA are presented in Table 1. Out of the 21 principal components (PCs) analyzed, only the first six had eigenvalues greater than one, and these were selected for further analysis. The eigenvalues for the first six PCs were as follows: the first PC had an eigenvalue of 9.715, the second PC had 3.573, the third PC had 1.945, the fourth PC had 1.581, the fifth PC had 1.269, and the sixth PC had 1.120. In 1st principle component the traits contributed more are pod area, pods per plant, pod length, pod weight per plant, pod weight, pod factor from density, seed area, seed perimeter, seed length and weight of seeds per pod. Four traits pod roundness, seed roundness, weight of pods per plant, number of pods per plant contributed negatively in 1st PC. The traits which contributed more in 2nd PC pod aspect ratio, seed roundness, number of seeds per pod, weight of pods per plant and number of pods per plant. While the traits pod weight per plant, pod roundness, seed area, seed perimeter, seed length and seed weight contributed negatively in 2nd PC.

The loading plot and biplot analysis are shown in Figure 2. The results indicated a positive correlation between traits such as pod area, pod perimeter, pod length, pod weight, pod aspect ratio, number of seeds per pod, seed weight per pod, and number of nodes, as evidenced by their angles of less than 90 degrees. In contrast, a negative correlation was observed among traits like seed area, seed perimeter, seed length, seed width, seed weight, seed aspect ratio, pod width, and seed factor from density, due to angles greater than 90 degrees between them. Genotypes 6, 5, 10, and 16, which are positioned farther from the center in the biplot, show greater genetic diversity and exhibit a positive nature in terms of their genetic traits.

Figure 2.

Biplot analysis of morphological traits



Cluster analysis of pea genotypes

Cluster analysis of the pea genotypes reveals substantial genetic diversity within the germplasm. The results, illustrated in Figure 3, show that the pea genotypes are generally categorized into two main clusters, further divided into four sub-clusters based on their morphological traits. The first cluster comprises approximately six varieties, the second cluster includes about ten varieties, the third cluster contains two varieties, and the fourth cluster consists of five varieties. Genotypes 2 and 3 are closely related to each other but exhibit greater diversity when compared to the other genotypes. The distance between the varieties in the cluster analysis reflects the degree of genetic diversity among them.

Table 1.

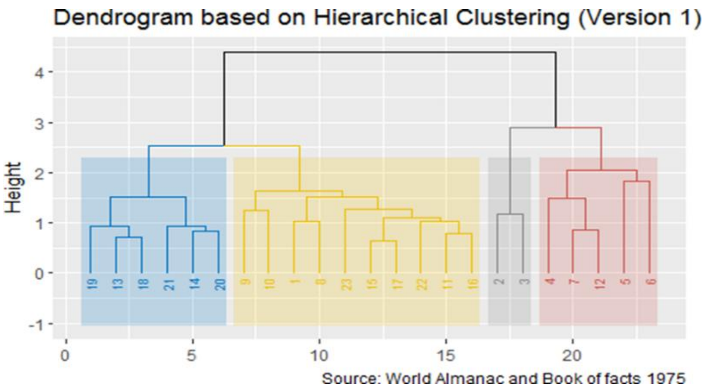
Principal component analysis of morphological traits

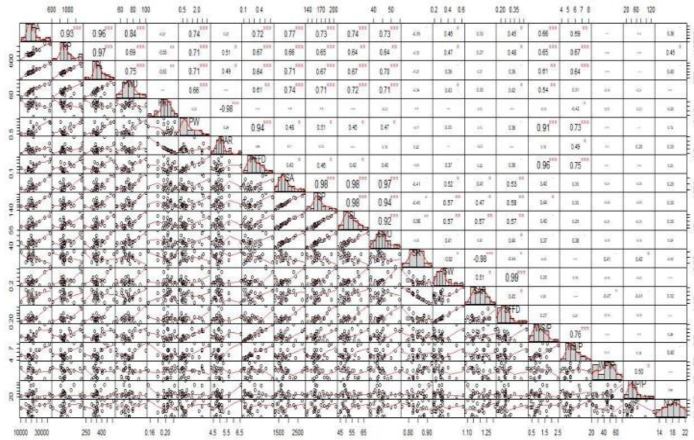
Traits	PC1	PC2	PC3
PA	0.2974	0.0667	0.0667
PP	0.2879	0.1515	-0.0765
PL	0.285	0.1675	-0.0749
PWD	0.2594	-0.0538	0.1184
PR	-0.0984	-0.3317	0.4459
PW	0.248	0.1701	0.3072
PAR	0.0876	0.3644	-0.4196
PFFD	0.2401	0.1287	0.3999
SA	0.2785	-0.1138	-0.1408
SP	0.28	-0.1231	-0.1222
SL	0.278	-0.1775	-0.1369
SWD	0.2601	-0.0357	-0.157
SR	-0.1576	0.341	0.0199
SW	0.1987	-0.2035	-0.0005
SAR	0.1504	-0.3532	-0.0421
SFFD	0.1974	-0.1728	-0.0241
WS/P	0.2304	0.1436	0.3461
NS/P	0.1966	0.3224	0.1497
WP/P	-0.07	0.225	0.2868
NP/P	-0.0689	0.3094	-0.0265
NON	0.1239	0.0965	-0.1864

Traits	PC4	PC5	PC6
PA	-0.0647	-0.0819	0.077
PP	0.08	0.037	-0.0002
PL	-0.0305	-0.0417	0.0336
PWD	-0.2063	-0.1329	-0.1559
PR	-0.1572	-0.113	0.1834
PW	0.0817	0.0056	-0.16
PAR	0.1532	0.1264	-0.1657
PFFD	0.1538	-0.0018	-0.0731
SA	-0.2692	-0.1312	0.05
SP	-0.2223	-0.0786	0.0871
SL	-0.1771	-0.1187	0.0862
SWD	-0.3727	-0.1645	0.0024
SR	-0.3747	0	-0.1966
SW	-0.0013	0.5951	-0.0015
SAR	0.383	-0.016	0.1963
SFFD	-0.0465	0.618	-0.0201
WS/P	0.2336	-0.0764	-0.1383
NS/P	0.1179	0.0396	0.0465
WP/P	-0.294	0.1732	0.4888
NP/P	-0.1336	0.2209	0.469
NON	0.3366	-0.2381	0.5489

Figure 3.

Cluster Analysis





Correlation analysis of different traits

Correlation analysis was conducted to examine the relationships between various seed, pod, and morphological traits. This analysis is crucial for identifying key yield components and understanding their interrelationships, which can guide the development of high-yielding pea varieties. The correlation coefficient values among the twenty-one traits of field pea, along with the correlation matrix, demonstrate the associations between different characteristics.

Figure 3 presents the correlation results for the 21 traits. Pod weight exhibited a strong and highly significant positive correlation with pod factor from density, seed weight per pod, and number of seeds per pod. A significant positive correlation was also observed between pod weight and seed area, seed perimeter, seed length, and seed width. Seed weight showed a highly significant and positive correlation with seed factor from density and a significant positive correlation with seed aspect ratio. Seed perimeter was positively and highly significantly correlated with seed length, seed weight, and seed factor from density, while also showing a significant positive correlation with seed area and weight per plant. Furthermore, seed weight per pod had a highly significant and positive correlation with the number of seeds per plant.

Path coefficient analysis of traits

Path coefficient analysis is used to examine the relationships between variables by considering both direct and indirect effects, allowing for the assessment of linearity and additivity. This analysis helps identify traits that contribute to indirect selection, which can be valuable for breeding programs. The results of the path analysis are presented in Table 2.

Pod area (PA) had a direct positive effect of 0.010 on pod weight (PW). It also showed a positive indirect effect on PW through various traits, including pod per plant (PP), pod weight, pod aspect ratio (PAR), pod factor from density (PFFD), seed perimeter (SP), seed weight (SW), seed aspect ratio (SAR), seed weight per pod (WS/P), and number of nodes (NON). However, it exhibited negative indirect effects via pod length (PL), pod roundness (PR), seed area (SA), seed length (SL), seed roundness (SR), seed factor from density (SFFD), and number of seeds per pod (NS/P).

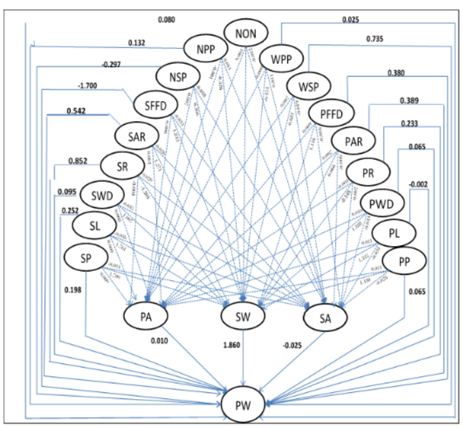
Pod per plant (PP) had a direct positive effect of 0.056 on PW. It showed a positive indirect effect on PW through pod area (PA), pod weight per plant (PWD), pod roundness (PR), pod aspect ratio (PAR), seed perimeter (SP), seed weight (SWD), seed weight per pod (WS/P), and number of nodes (NON). In contrast, PP had a negative indirect effect on PW through pod length (PL), pod roundness (PR), seed area (SA), seed length (SL), seed roundness (SR), seed factor from density (SFFD), number of seeds per pod (NS/P), weight per plant (WP/P), and number of pods per plant (NP/P).

Pod length (PL) had a direct negative effect of -0.002 on PW. It showed a positive indirect effect on PW via pod area (PA), pod per plant (PP), pod weight per plant (PWD), pod aspect ratio (PAR), seed factor from density (SFFD), seed perimeter

(SP), seed weight (SWD), seed weight per pod (WS/P), and number of nodes (NON). On the other hand, PL exhibited negative indirect effects via pod roundness (PR), seed area (SA), seed length (SL), seed roundness (SR), seed factor from density (SFFD), number of seeds per pod (NS/P), and weight per pod (WP/P). Figure 2 illustrates these relationships, providing a clearer understanding of the direct and indirect effects (Figure 4).

Figure 4

Path analysis for direct and indirect effects of pods, seeds & morphological descriptors to Pod Weight (PW). Dotted lines represent the indirect effects of the descriptors on Pod Weight (PW).



Genetic components

The results for the genetic components, including genotypic variance, phenotypic variance, environmental variance, and broad-sense heritability, are presented in Table 2. Genotypic variance estimates ranged from 41,108,350.00 for pod area to 0.00 for pod roundness. Similarly, phenotypic variance ranged from 50,332,040.00 for pod area to 0.00 for pod roundness. Environmental variance ranged from 9,223,681.00 for pod area to 0.00 for seed roundness.

The broad-sense heritability estimates were high for several traits, with the following results: pod area (0.82), pod roundness (0.75), pod weight (0.80), pod aspect ratio (0.79), seed width (0.42), seed weight per pod (0.81), pod weight per plant (0.85), number of pods per plant (0.81), and number of nodes (0.76). Other traits exhibited moderate heritability.

Table 2.

Genetic components of twenty-one pea traits

Traits	Genotypic Variance	Phenotypic Variance	Environmental Variance	Broad Sense
PA	41108350	50332040	9223681	0.82
PP	24178.77	49712.45	25533.68	0.49
PL	2684.07	5495.63	2811.56	0.49
PWD	88.57	191.3	102.73	0.46
PR	0	0	0	0.75
PW	0.5	0.63	0.12	0.8
PAR	0.2	0.25	0.05	0.79
PFFD	0.02	0.03	0.02	0.49
SA	55039.71	374412.85	319373.14	0.15
SP	194.73	479.19	284.46	0.41
SL	22.94	70.07	47.12	0.33
SWD	16.77	21.14	4.37	0.79
SR	0	0	0	0.68
SW	0.01	0.02	0.01	0.42
SAR	0	0.01	0.01	0.53
SFFD	0	0.01	0.02	0.45
WS/P	0.3	0.37	0.96	0.81
NS/P	1.29	1.82	4.41	0.71
WP/P	168.42	197.41	534.24	0.85
NP/P	792.14	981.08	2565.36	0.81
NON	6.79	8.92	22.5	0.76

Discussion

Principal component analysis (PCA) revealed that traits such as pod area, pod perimeter, pod length, pod weight, pod aspect ratio, number of seeds per pod, seed weight per pod, and number of nodes exhibited positive correlations, as indicated by angles of less than 90 degrees in the loading plot. These findings are consistent with previous studies by Abdullah et al. (2018), Dyulgerov (2018), Kaur et al. (2018), Bouziane et al. (2015), and Eticha et al. (2010), which also highlighted the genetic diversity within pea germplasm. Cluster analysis further supported the genetic diversity, showing a wide range of genetic origins. Ramzan et al. (2014) also reported high genetic diversity in pea genotypes based on Euclidean distance. The dendrogram generated in this study displayed four major clusters and six sub-clusters, indicating significant genetic variation among the genotypes. The distances between the varieties, particularly those of genotypes 2, 3, 19, and 13, suggest potential candidates for future crossing programs aimed at increasing genetic diversity. Correlation analysis showed a highly significant positive correlation between pod weight and traits like pod factor from density, seed weight per pod, and number of seeds per pod. A similar significant positive correlation was found between pod weight and seed area, perimeter, length, and width. These results align with studies by Singh et al. (2017) and Kumar and Sharma (2006), who emphasized the importance of pod weight in improving production.

The path coefficient analysis revealed that pod area had the highest positive and direct effect on pod weight, with a positive indirect effect through traits such as pod per plant, pod weight, pod aspect ratio, seed perimeter, seed weight, seed aspect ratio, weight of seed per pod, and number of nodes. These findings provide valuable insights for developing selection strategies for high-yielding genotypes. Similar observations were made by Rathi and Dhaka (2007), Tiwari et al. (2020), and Katoch et al. (2016), who found that pod length had an indirect but favorable effect on pod weight, number of pods per plant, and pod weight per plant.The phenotypic and genetic variance estimates for the traits under study were consistent with previous reports. Tiwari et al. (2012) observed similar trends, with genetic variance typically exceeding phenotypic variance. Our results showed the highest genotypic variance for pod area and the lowest for seed roundness, indicating that pod area is strongly influenced by genetic factors. The environmental variance for pod area and seed area was high, suggesting a considerable environmental influence on these traits, as reported by Gomez & Ligarreto (2012). High heritability estimates were observed for traits such as pod area, pod roundness, pod weight, pod aspect ratio, seed width, seed weight per pod, pod weight per plant, number of pods per plant, and number of nodes. These results are in line with the study by Kumar and Dubey (2001), who reported heritability estimates of 50% for seed number per plant and 45% for pod number. Miczak et al. (2001) also reported heritability estimates ranging from 0.72 to 0.88 for pod number and seed number per plant.

Conclusion and Recommendations

This study offers a comprehensive assessment of genetic diversity in field pea by integrating digital phenotyping data with traditional field observations. The application of diverse statistical approaches including PCA, cluster analysis, correlation and path analysis, and heritability estimates provided deeper insights into the population's genetic structure and the interrelationships among important agronomic traits. The results reveal substantial genetic variability, meaningful positive trait associations, and strong prospects for identifying and advancing superior genotypes. Collectively, these findings support informed selection strategies and highlight valuable opportunities for future field pea breeding programs.

Etik Komite Onayı: Bu çalışma için etik komite onayı gerekmiyor.
Hakem Değerlendirmesi: Dış bağımsız.
Yazar Katkıları: Fikir – M.S.; Tasarım – M.W.A., M.S.; Denetleme – M.S.*; Kaynaklar – M.W.A., M.S., M.Shn., S.Z., A.A.; Veri Toplanması ve/veya İşlemesi – M.W.A., M.Shn., S.Z., A.A., S.R., M.T.F.K.; Analiz ve/veya Yorum – M.W.A., M.S., T.N.K., M.A., M.M.; Literatür Taraması – M.W.A., S.Z., S.R., M.Y.M.; Yazıyı Yazan – M.W.A., M.S.; Eleştirel İnceleme – M.S.
Çıkar Çatışması: Yazarlar, çıkar çatışması olmadığını beyan etmiştir.
Finansal Destek: Yazarlar, bu çalışma için finansal destek almadığını beyan etmiştir.
Yapay Zeka Kullanımı: Yazar yapay zeka destekli uygulamaları kullanmadığını beyan etmiştir.

Ethics Committee Approval: This study does not require ethics committee approval.
Peer-review: Externally peer-reviewed.
Author Contributions: Concept – M.S.; Design – M.W.A., M.S.; Supervision – M.S.*; Resources – M.W.A., M.S., M.Shn., S.Z., A.A.; Data Collection and/or Processing – M.W.A., M.Shn., S.Z., A.A., S.R., M.T.F.K.; Analysis and/or Interpretation – M.W.A., M.S., T.N.K., M.A., M.M.; Literature Review – M.W.A., S.Z., S.R., M.Y.M.; Writing – M.W.A., M.S.; Critical Review – M.S.
Conflict of Interest: The authors have no conflicts of interest to declare.
Financial Disclosure: The authors declared that this study has received no financial support.
Use of Artificial Intelligence: The author has stated that they do not use AI-powered applications.

References

Abdullah, A.M., Subhani, MG., Ahmad, J., & Anwar, J. (2018). Multivariate analysis of some yield and yield related traits of barley (*Hordeum vulgare* L.) genotypes. *Academia Journal of Agricultural Research, 67*, 189- 197.

Assen, K. Y. (2020). Trait associations in prostrate and semi-leaf less type field pea (*Pisum sativum* L.) gene pools. *American Journal of Environmental Sciences, 4*, 54-60.

Bai, G., Ge, Y., Hussain, W., Baenziger, P.S., & Graef, G. (2016). A multi-sensor system for high throughput field phenotyping in soybean and wheat breeding. *Computures and Electronics in Agriculture, 128*, 181–192.

Bijalwan, P., Raturi, A., Mishra, A. C. (2018). Character Association and Path Analysis Studies in Garden Pea (*Pisum sativum* L.) for Yield and Yield Attributes. *International Journal of Current Microbiology and Applied Sciences, 7*(3), 3491-3495.

Bouziane, H. R., Berkani, S., Merdas, S., Merzoug, S. N., & Abdelguerfi, A. (2015). Genetic diversity of traditional genotypes of barley (*Hordeum vulgare* L.) in Algeria by pheno-morphological and agronomic traits. *African Journal of Agricultural Research, 10*(31), 3041-3048.

Cupic, T., Tucak, M., Popovic, S., Bolaric, S., Grljusic, S., &

Kosumplik, V. (2009). Genetic diversity of pea (*Pisum sativum* L.) genotypes assessed by pedigree, morphological and molecular data. *Journal of Food, Agriculture and Environment, 7*(3, 4), 343-348.

Daniel, I. O., Adeboye, K. A., Oduwaye, O. O., & Porbeni, J. (2012). Digital seed morpho-metric characterization of tropical maize inbred lines for cultivar discrimination. *International Journal of Plant Breeding and Genetics*, *6*(4), 245–251. Doi:10.3923/ijpbg.2012.245.251.

Dell'Aquila, A. (2006). Computerized seed imaging: a new tool to evaluate germination quality. *Commun Bio Crop Science*, *1*(1), 20–31.

Dewy, D. R., & Lu, K.H. (1959). A correlation and path coefficient analysis of components of crested wheat grass seed production. *Agronomy Journal, 51*, 515-518.

Dyulgerov, N., & Dyulgerova, B. (2018). Phenotypic diversity in six-rowed winter barley (*Hordeum sativum* L.) varieties. *Agricultural Science and Technology*, *10*(1), 16-20.

Eticha, F., Grausgruber, H., & Berghoffer, E. (2010). Multivariate analysis of agronomic and quality traits of hull-less spring barley (*Hordeum vulgare* L.). *Journal of Plant Breeding and Crop Science*, *2*(5), 81-95.

FAO. (2023). *FAOSTAT: FAO Statistical Databases*. Food and Agriculture Organization.

Fontes, M. M. P., Carvalho, C. R., Clarindo, W. R. (2014). Karyotype revised of Pisum sativum using chromosomal DNA amount. *Plant Systematics and Evolution, 300*(7), 1621–1626. 10.1007/s00606-014-0987-y.

Ghixari, B., Vrapi, H., & Hobdari, V. (2014). Morphological characterization of pea (*Pisum sativum* L.) genotypes stored in Albanian genebank. *Albanian Journal of Agricultural Sciences*. *Special Edition.* 169-173.

Gomez, G. E., & Ligarreto, G.A. (2012). Analysis of genetic effects of major genes on yield traits of a pea (*Pisum sativum* L.) cross between the Santa Isabel x WSU 31 varieties. *Agronomia Colombiana, 30*(3), 317-325.

Hornokova, O., Zavodna, M., Zakova, M., Kraic, J., & Debre, F. (2003). Diversity of common bean landraces collected in the western and eastern Carpatien. *Czech Journal of Genetics and Plant Breeding, 39*, 73-83.

Joshi, B. K., Mudwari, A., Bhatta, M. R., & Ferrara, G. O. (2004). Genetic diversity in Nepalese wheat cultivars based on agromorphological traits and coefficients of parentage. *Nepal Agriculture Research Journal, 5*, 7-17.

Katoch, V., Singh, P., Mayanglambam, B. D., Sharma, A., Sharma, G. D., & Sharma, J. K. (2016). Study of genetic variability, character association, path analysis and selection parameters for heterotic recombinant inbred lines of garden peas (*Pisum sativum* var. hortense L.) under mid- hill conditions of Himachal Pradesh, India. *Legume Research, 39*(2), 163-169.

Kaur, V., Kumari, J., Manju, M., Jacob, S. R., Panwar, B. S. (2018). Genetic diversity analysis of indigenous and exotic germplasm of barley (*Hordeum vulgare* L.) and identification of trait specific superior accessions. *Society for Advancement of Wheat and Barley Research, 10*, 190-197.

Keneni, G., Jarso, M., Wolabu, T., & Dino, G. (2005). Extent and pattern of genetic diversity for morpho-agronomic traits in Ethiopian highland pulse landraces: I. Field pea (*Pisum sativum* L.). *Genetic Resources and Crop Evolution,* 5, 539-549.

Kumar, S., & Dubey, D. K. (2001). Variability, heritability and correlation studies in grasspea (*Lathyrus sativus* L.). *Lathyrus Lathyrism Newsletter,* 2, 79-81.

Kumar, V. R., & Sharma, R. R. (2006). Character association studies in garden pea. *Indian Journal of Horticulture*, *63*, 185-187.

Kwon, S. H., & Torrie, J. H. (1964). Heritability and interrelationship among traits of two soybean populations. *Crop Science*, 4, 196–198.

Lewis, G., Schrirer, B., Mackinder, B., & Lock, M. (2005). *Legumes of the World; Royal Botanical Gardens: Kew*, UK, ISBN190 347806. Doi:10.1017/S0960428606190198.

Mahajan, R. C., Wadikar, P. B., Pole, S. P., & Dhuppe, M. V. (2011). Variability, correlation and path analysis studies in sorghum. *Research Journal of Agricultural Science*, *2*(1), 101-103.

Milczak, M., Pedzinski, M., Mnichowska, H., Szwed-Urbas, K., & Rybinski, W. (2001). Creative breeding of grasspea (*Lathyrus sativus* L.) in Poland. *Lathyrus Lathyrism Newsletter, 2*, 85-89.

Ramzan, A., Noor, T., Khan, T. N., & Hina, A. (2014). Correlation, cluster and regression analysis of seed yield and its contributing traits in pea (*Pisum sativum* L.). *Journal of Agricultural Research, 52*, 481-488.

Rathi, R., & Dhaka, R. (2007). Genetic variability, correlation and path analysis in pea (*Pisum sativum* L.). *Indian Journal of Plant Genetic Resources, 20*, 126-129.

Sarikamiş, G., Yanmaz, R., Ermis, S., Bakir, M., & Yüksel, C. (2010). Genetic characterization of pea (*Pisum sativum* L.) gemplasm from Turkey using morphological and SSR markers. *Genetics and Molecular Research, 9*(1), 591-600.

Sharma, V. K., & Bora, L. (2013). Studies on genetic variability and heterosis in vegetable pea (*Pisum sativum* L.) under high hills condition of Uttarakhand, India. *African Journal of Agricultural Research*, *8*(18), 1891-1895.

Singh, S., Ahmed, N., Singh, D., Srivastva, K., Singh, R., & Mir,

A. (2017). Genetic variability determination in garden pea (*Pisum sativum* L sub sp. hortense asch. and graebn.) by using the multivariate analysis. *Legume Research - An International Journal, 40*, 416-422.

Smykal, P., Corander, M. J., Jarkovsky, J., Flavell, A. J., & Griga, M. (2008). Genetic diversity and population structure of pea (*Pisum sativum* L.) varieties derived from combined retro-transposon, microsatellite and morphological marker analysis. *Theoretical and Applied Genetics, 117*, 413-424.

Tanabata, T., Shibaya, T., Hori, K., Ebana, K., & Yano, M. (2012). Smartgrain: High-throughput phenotyping software for measuring seed shape through image analysis. *Plant Physiology*, *160*, 1871–1880.

Tiwari, G., & Lavanya, G.R. (2012). Genetic variability, character association and component analysis in F4 generation of field pea (*Pisum sativum* var. arvense L.). *Karnataka Journal of Agricultural Sciences, 25*, (2),173-175.

Tiwari, S., Sharma, R., Kushwah, S., & Pandey, B. (2020). Correlation analysis on different characters in garden pea (*Pisum sativum* var hortense L.). *International Journal of Chemical Studies, 8*, 1180-1183.

Williams, K., Munkvold, J., & Sorrells, M. (2013). Comparison of digital image analysis using elliptic Fourier descriptors and major dimensions to phenotype seed shape in hexaploid wheat (*Triticum aestivum* L.). *Euphytica, 190*(1), 99–116.

Wright, S. (1921). Correlation and causation. *Journal of Agricultural Research*, *20*, 557-585.

Yang, W., Duan, L., Chen, G., Xiong, L., & Liu, Q. (2019). Plant phenomics and high-throughput phenotyping: accelerating rice functional genomics using multidisciplinary technologies. *Current Opinion in Plant Biology*, *54*, 31-39.