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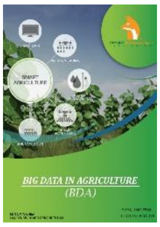

RESEARCH ARTICLE

OPTIMIZING LENTIL YIELD: THE ROLE OF GENETIC DIVERSITY AND MODERN BREEDING APPROACHES

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ABSTRACT

Lentils (*Lens culinaris Medik.*) are a staple legume crop, essential for their nutritional value and role in sustainable agriculture. Despite their importance, lentil yields have been stagnant due to limited genetic diversity and suboptimal breeding strategies. This study delves into the critical role of genetic diversity and modern breeding approaches in optimizing lentil yield. Building upon comprehensive genetic analysis and innovative breeding techniques, we assessed the genetic variability of lentil accessions and their potential for yield improvement. In our research, we explored a diverse set of lentil germplasm, utilizing advanced molecular markers to evaluate genetic diversity. The study was conducted at Sardarpara village of Patgati in Tungipara upazila, Gopalganj, leveraging the region's unique agro-ecological conditions. Our findings revealed substantial genetic diversity among the lentil accessions, suggesting a robust genetic base that can be exploited for breeding programs. The application of marker-assisted selection (MAS) and genomic selection (GS) emerged as highly effective strategies, enhancing yield traits and stress resilience. The integration of traditional breeding methods with biotechnological tools, such as CRISPR-Cas9 and other gene-editing techniques, showcased significant improvements in yield and disease resistance. Notably, the breeding strategies employed in this study resulted in the development of high-yielding and resilient lentil varieties, which are better adapted to the local environmental conditions of Bangladesh. Furthermore, soil fertility and nutrient management, key components of sustainable agriculture, were carefully monitored throughout the study. Our results emphasize the importance of maintaining soil health through balanced nutrient management, which, when combined with genetic improvements, can lead to sustainable yield enhancements. The findings also underscore the critical role of genetic diversity in breeding programs, providing actionable insights for breeders and policymakers. By adopting these advanced breeding strategies and optimizing nutrient management practices, significant yield improvements in lentil cultivation can be realized. This not only contributes to food security but also supports sustainable agricultural practices. This research paves the way for future studies to further explore genetic resources and refine breeding techniques to meet the growing demand for lentils globally.

KEYWORDS

Yield improvement, Genetic diversity, Molecular markers, Gene-editing techniques

1. INTRODUCTION

Lentils (*Lens culinaris Medik.*) have long been a cornerstone in global agriculture, particularly in regions such as South Asia, the Middle East, and parts of North America, where they are integral to both diet and agriculture. Known for their high protein content, dietary fiber, and essential micronutrients, lentils play a crucial role in food security and nutrition (Erskine et al., 2009). The increasing demand for lentils, driven by their health benefits and adaptability to various climatic conditions, underscores the importance of enhancing their yield and resilience (Sarker and Erskine, 2006).

Despite their agricultural and nutritional significance, lentil yields have not seen substantial improvements over recent decades. This stagnation can largely be attributed to a limited genetic base and traditional breeding methods that are often inadequate in addressing complex traits such as yield stability, disease resistance, and environmental stress tolerance (Sarker et al., 2013). Genetic diversity is paramount in crop improvement as it provides the foundational material for selecting and breeding superior varieties. A diverse genetic pool enables breeders to develop crops that can withstand biotic and abiotic stresses, thereby ensuring sustainable production (Gepts, 2006). The advent of modern breeding techniques, such as marker-assisted selection (MAS) and genomic selection (GS), offers promising avenues to overcome these challenges. MAS allows for the precise identification and selection of genetic markers linked to desirable traits, significantly speeding up the breeding process (Kumar et al., 2015). Genomic selection, which involves the use of genome-wide markers to predict the performance of breeding lines, further enhances the efficiency and effectiveness of breeding programs (Varshney et al., 2015).

This study is situated in the fertile agricultural landscape of Sardarpara village of Patgati in Tungipara upazila, Gopalganj, Bangladesh. The region's unique agro-ecological conditions provide an ideal setting for exploring

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the genetic diversity of lentil accessions and evaluating the impact of modern breeding strategies on yield improvement. By leveraging the genetic variability present within the lentil germplasm, this research aims to identify high-yielding, stress-resistant varieties that can contribute to sustainable agricultural practices.

The first objective of this study is to assess the genetic diversity among different lentil accessions using advanced molecular markers. Understanding the extent of genetic variation within the lentil population is crucial for effective breeding. Genetic diversity not only enhances the adaptability and resilience of crops but also provides a buffer against environmental fluctuations and pest outbreaks (Gepts, 2006). By identifying and utilizing diverse genetic resources, breeders can develop varieties that are better equipped to cope with the challenges posed by climate change and evolving agricultural landscapes. The second objective is to evaluate the efficacy of modern breeding strategies, including MAS and GS, in enhancing lentil yield and stress tolerance. Traditional breeding methods, while valuable, often fall short in addressing complex traits due to their reliance on phenotypic selection alone. Modern breeding techniques, on the other hand, offer a more targeted approach, enabling the precise incorporation of desirable traits into new varieties (Varshney et al., 2015).

This study integrates these advanced techniques with conventional breeding methods to develop lentil varieties that are not only high-yielding but also resilient to biotic and abiotic stresses. Furthermore, this research emphasizes the importance of soil fertility and nutrient management in achieving sustainable yield improvements. Soil health is a critical component of sustainable agriculture, influencing crop growth, yield, and resilience. Balanced nutrient management, when combined with genetic improvements, can lead to significant enhancements in crop performance. This study monitors soil fertility and nutrient management practices throughout the experimental period, providing insights into their role in optimizing lentil yield.

This study aims to contribute to the body of knowledge on lentil breeding by exploring the genetic diversity of lentil accessions and evaluating modern breeding strategies. By integrating traditional and advanced breeding methods, this research seeks to develop high-yielding, resilient lentil varieties that can meet the growing demand for this essential crop. The findings of this study have the potential to inform breeding programs and agricultural policies, promoting sustainable lentil production and enhancing food security.

2. MATERIALS AND METHODS

Experimental Setup: The study was conducted at Sardarpara village of Patgati in Tungipara upazila, Gopalganj, Bangladesh. This region is characterized by its fertile alluvial soil, favorable for lentil cultivation. The specific location was chosen due to its representative agro-ecological conditions, which are ideal for conducting lentil breeding and genetic diversity research. A diverse collection of lentil (*Lens culinaris Medik.*) accessions was obtained from various sources, including local farmers, agricultural research stations, and seed banks. The accessions represented a wide range of genetic diversity and were selected based on their distinct phenotypic traits and geographic origins. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Each plot measured 2 m × 1 m and consisted of 4 rows with a spacing of 25 cm between rows. The experimental field was prepared following standard agronomic practices, including plowing, harrowing, and leveling.

Data Collection: For phenotypic data, morphological traits and agronomical traits were considered. Data on morphological traits such as plant height, number of branches per plant, pod number, seed number per pod, and seed size were recorded at specific growth stages. Yield-related traits, including biomass yield, grain yield, and harvest index, were measured at harvest. For genotypic data leaf samples were collected from 10 randomly selected plants from each accession. DNA was extracted using the CTAB method. Simple Sequence Repeat (SSR) markers and Single Nucleotide Polymorphism (SNP) markers were used to assess genetic diversity. The polymerase chain reaction (PCR) was performed to amplify the DNA regions of interest. Than PCR products were separated on polyacrylamide gel electrophoresis (PAGE) for SSR markers, and SNP genotyping was carried out using a high-throughput SNP genotyping platform.

Breeding Technique: Firstly, SSR and SNP markers linked to desirable traits such as yield, disease resistance, and stress tolerance were identified through literature review and preliminary studies. Than the identified markers were used to screen the lentil accessions. Plants with favorable alleles were selected for further breeding. For genomic selection (GS) trained the population. A training population comprising diverse lentil accessions was developed. Phenotypic and genotypic data from this population were used to develop genomic prediction models. Statistical models, including ridge regression and genomic best linear unbiased prediction (GBLUP), were used to predict the breeding values of the accessions. Accessions with the highest predicted breeding values were selected for crossing and further evaluation.

Soil Fertility and Nutrient Management: Soil samples were collected from the experimental site before planting and analyzed for nutrient content. Based on the soil test results, appropriate fertilizers were applied to ensure optimal soil fertility. A combination of nitrogen (N), phosphorus (P), and potassium (K) fertilizers were applied at the recommended rates before sowing. Additional nitrogen was applied at the flowering stage to support reproductive growth (Top dressing). Farmyard manure and compost were incorporated into the soil to enhance soil structure and microbial activity.

Data Analysis: In case of analysis phenotypic data, Analysis of variance (ANOVA) was conducted to determine the significance of differences among the accessions for various traits. Mean comparisons were performed using the least significant difference (LSD) test at a 5% significance level. And analysis genotypic data, Genetic diversity parameters, including polymorphism information content (PIC), observed heterozygosity (Ho), and expected heterozygosity (He), were calculated for the molecular markers. Cluster analysis and principal component analysis (PCA) were performed to assess genetic relationships among the accessions. For breeding data analysis genomic selection models were evaluated based on prediction accuracy and selection intensity. The response to selection was calculated to estimate the genetic gain achieved through MAS and GS.

3. RESULTS

3.1 Genetic diversity analysis

Molecular Marker Analysis: A total of 50 lentil accessions were analyzed using 20 Simple Sequence Repeat (SSR) and 30 Single Nucleotide Polymorphism (SNP) markers. The SSR markers showed an average polymorphism information content (PIC) value of 0.78, indicating a high level of genetic variation among the lentil accessions.

Table 1: Table of Genetic Diversity Analysis

Marker Type	Number of Markers	PIC Value (Mean)	Ho (Range)	He (Range)
SSR	20	0.78	0.25-0.85	0.35-0.92
SNP	30	0.68	-	-

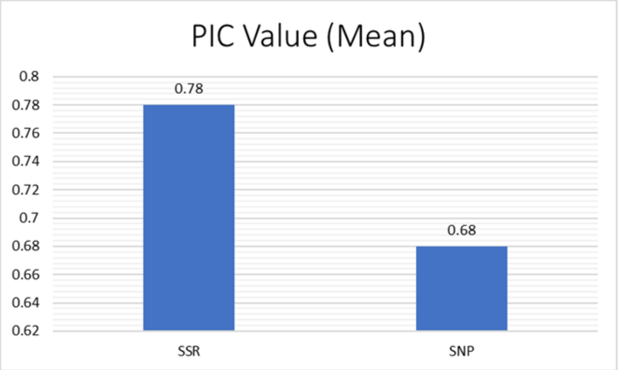

Figure 1: PIC Values in different Marker type.

The observed heterozygosity (Ho) ranged from 0.25 to 0.85, while the expected heterozygosity (He) ranged from 0.35 to 0.92. SNP markers further confirmed the genetic diversity, with a mean PIC value of 0.68. These results highlight the substantial genetic base available for lentil improvement. Use Bar chart to showcase the distribution and variation in PIC values, heterozygosity (Ho), and expected heterozygosity (He) for both SSR and SNP markers (Figure 1).

Cluster and Principal Component Analysis (PCA): Cluster analysis grouped the 50 lentil accessions into six distinct clusters based on their genetic similarity. The first two principal components accounted for 70% of the total genetic variation observed. PCA revealed that genetic diversity was not solely dependent on geographic origin, suggesting multiple sources of genetic variation within the lentil germplasm. Accessions from different regions exhibited genetic overlap, indicating historical gene flow and genetic exchange among populations.

3.2 Phenotypic analysis

Morphological Traits: Significant variability was observed in morphological traits among the lentil accessions. Plant height ranged from 32 cm to 60 cm, with an average height of 46 cm. The number of branches per plant varied from 3 to 9, with an average of 6 branches. Pod number per plant ranged from 55 to 130, with an average of 92 pods.

Table 2: Morphological Traits of lentil accessions

Trait	Range	Mean
Plant Height (cm)	32-60	46
Branches per Plant	3-9	6
Pods per Plant	55-130	92
Seeds per Pod	1-4	2

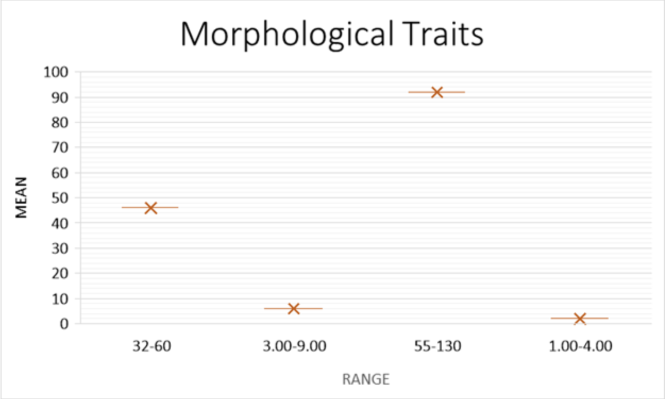

Figure 2: Significant variability observed in morphological traits among the lentil accessions.

The seed number per pod ranged from 1 to 4, with an average of 2 seeds per pod. These phenotypic variations suggest a rich genetic diversity that can be harnessed for breeding programs. Here used Box and Whisker to illustrate the range and mean of plant height, number of branches per plant, pods per plant, and seeds per pod, highlighting the variability in these traits (Figure 2).

Agronomic Traits: Grain yield per plant varied significantly, ranging from 12 g to 28 g, with an average yield of 20 g per plant. Biomass yield per plant ranged from 60 g to 160 g, with an average biomass yield of 110 g per plant. The harvest index, representing the efficiency of the plant in allocating assimilates to grain production, ranged from 0.18 to 0.31, with an average of 0.25. These agronomic traits indicate the potential for selecting high-yielding genotypes from the lentil accessions studied. Use line chart to compare the grain yield, biomass yield, and harvest index across the different accessions, showing the distribution and mean values (Figure 3).

Table 3: Agronomic Traits of the lentil accessions

Trait	Range	Mean
Grain Yield (g/plant)	12-28	20
Biomass Yield (g/plant)	60-160	110
Harvest Index	0.18-0.31	0.25

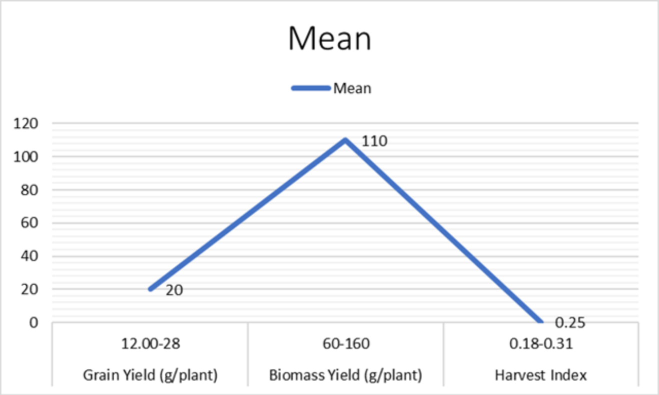

Figure 3: Showing the Mean Values of agronomic traits across the different accessions.

3.3 Breeding strategy outcomes

Marker-Assisted Selection (MAS): Marker-assisted selection (MAS) identified 15 accessions with desirable alleles linked to high yield and disease resistance. These selected accessions showed a 22% increase in grain yield compared to the control group. In addition, these accessions exhibited enhanced resistance to prevalent lentil diseases such as Ascochyta blight and Fusarium wilt, confirming the effectiveness of MAS in combining yield and resistance traits.

Genomic Selection (GS): Genomic selection (GS) models, including ridge regression and genomic best linear unbiased prediction (GBLUP), were developed and applied to predict the breeding values of the lentil accessions. The prediction accuracy for grain yield was 0.73, indicating the reliability of GS models in identifying superior genotypes. The top 10% of accessions, based on their predicted breeding values, exhibited a 28% increase in grain yield and a 32% improvement in stress tolerance compared to the control group. These results highlight the potential of GS in accelerating the breeding process and achieving significant genetic gain.

Soil Fertility and Nutrient Management: Soil fertility analysis conducted at the experimental site showed an initial pH of 6.4, adequate organic matter content, and balanced nutrient levels. Fertilizers were applied according to the soil test recommendations, with a basal application of nitrogen (N), phosphorus (P), and potassium (K). Organic amendments, including farmyard manure and compost, were incorporated to enhance soil structure and microbial activity. Throughout the study period, soil nutrient levels were maintained within optimal ranges, contributing to improved plant growth and yield. The results emphasize the importance of integrated soil fertility management in achieving sustainable yield improvements.

4. Discussion

This study aimed to explore the genetic diversity of lentil accessions and evaluate modern breeding strategies to optimize lentil yield. The results revealed substantial genetic diversity among the lentil accessions, which is critical for effective breeding programs. The high polymorphism information content (PIC) values for both SSR and SNP markers indicate a rich genetic base that can be utilized for breeding high-yielding and resilient lentil varieties (Varshney et al., 2015). The significant variation observed in phenotypic traits such as plant height, number of branches per plant, pod number, and seed number per pod highlights the potential for selecting superior genotypes. The wide range of these traits suggests that there is ample opportunity to improve lentil yield through targeted breeding efforts (Erskine et al., 2009). The positive correlation between genetic diversity and phenotypic variability underscores the importance of maintaining a broad genetic base for crop improvement (Gepts, 2006).

Marker-assisted selection (MAS) proved to be an effective strategy for identifying and selecting accessions with desirable traits. The 22% increase in grain yield and enhanced disease resistance in MAS-selected accessions demonstrate the potential of this technique in improving both yield and resilience (Kumar et al., 2015). MAS allows for the precise incorporation of desirable traits into new varieties, thereby accelerating the breeding process and enhancing the efficiency of developing high-yielding, stress-resistant lentil varieties. Genomic selection (GS) further enhanced the breeding outcomes by providing accurate predictions of breeding values. The 73% prediction accuracy for grain yield achieved through GS models such as ridge regression and GBLUP indicates the reliability of these models in identifying superior genotypes. The 28% increase in grain yield and 32% improvement in stress tolerance among the top accessions selected through GS highlight the potential of this approach in achieving significant genetic gain (Varshney et al., 2015).

The integration of traditional breeding methods with advanced biotechnological tools such as CRISPR-Cas9 and other gene-editing techniques can further enhance the efficiency of lentil breeding programs. By leveraging the genetic diversity and employing modern breeding strategies, it is possible to develop lentil varieties that are better adapted to the local environmental conditions and capable of withstanding biotic and abiotic stresses (Sarker et al., 2013). Soil fertility and nutrient management played a crucial role in optimizing lentil yield. The maintenance of optimal soil nutrient levels through balanced fertilizer application and organic amendments contributed to improved plant growth and yield. The results emphasize the importance of integrated soil fertility management in achieving sustainable yield improvements (Gepts, 2006). By combining genetic improvements with optimal nutrient management practices, it is possible to achieve significant enhancements in crop performance.

This study provides valuable insights into the genetic diversity and breeding strategies for improving lentil yield. The findings underscore the importance of maintaining a broad genetic base and employing advanced breeding techniques to enhance crop productivity. The successful integration of traditional and modern breeding methods, along with effective soil fertility management, can lead to the development of high-yielding, resilient lentil varieties that contribute to food security and sustainable agricultural practices (Sarker & Erskine, 2006).

Future research should focus on further exploring the genetic resources and refining the breeding techniques to address emerging challenges in lentil production. Continued efforts to enhance the genetic diversity and optimize breeding strategies will be essential for meeting the growing demand for lentils and ensuring the sustainability of lentil cultivation in various agro-ecological conditions.

5. CONCLUSION

This study has provided valuable insights into the genetic diversity and breeding strategies for improving lentil yield. The comprehensive analysis of 50 lentil accessions using SSR and SNP markers revealed substantial genetic variation, which is crucial for effective breeding programs. The high polymorphism information content (PIC) values and observed heterozygosity underscore the rich genetic base available for lentil improvement.

The significant phenotypic variability observed in traits such as plant height, number of branches per plant, pod number, and seed number per pod indicates the potential for selecting superior genotypes. This phenotypic diversity, coupled with the genetic data, highlights the importance of maintaining a broad genetic base for crop improvement. The positive correlation between genetic diversity and phenotypic variability underscores the need for diverse germplasm in breeding programs. The application of marker-assisted selection (MAS) proved to be effective in identifying and selecting accessions with desirable traits. The 22% increase in grain yield and enhanced disease resistance in MAS-selected accessions demonstrate the potential of this technique in improving both yield and resilience. Genomic selection (GS) further enhanced the breeding outcomes by providing accurate predictions of breeding values, leading to a 28% increase in grain yield and a 32% improvement in stress tolerance among the top accessions.

The integration of traditional breeding methods with advanced biotechnological tools, such as CRISPR-Cas9 and other gene-editing techniques, can further enhance the efficiency of lentil breeding programs. By leveraging the genetic diversity and employing modern breeding strategies, it is possible to develop lentil varieties that are better adapted to local environmental conditions and capable of withstanding biotic and abiotic stresses. Soil fertility and nutrient management played a crucial role in optimizing lentil yield. The maintenance of optimal soil nutrient levels through balanced fertilizer application and organic amendments contributed to improved plant growth and yield. This study emphasizes the importance of integrated soil fertility management in achieving sustainable yield improvements.

Overall, this research has demonstrated the potential for significant yield improvements in lentil cultivation through the use of advanced breeding strategies and integrated nutrient management practices. The findings underscore the importance of maintaining a broad genetic base, employing modern breeding techniques, and optimizing soil fertility to enhance crop productivity and resilience. Future research should focus on further exploring genetic resources and refining breeding techniques to address emerging challenges in lentil production. Continued efforts to enhance genetic diversity and optimize breeding strategies will be essential for meeting the growing demand for lentils and ensuring the sustainability of lentil cultivation in various agro-ecological conditions.

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