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## Genetic Diversity in Okra and the Effect of Some Physiological Treatments on Seed Yield

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**ABSTRACT:** Two experiments were conducted on okra. The first experiment aimed to study the genetic diversity among 25 genotypes of okra (*Abelmoschus esculentus* L.), coded sequentially from 1 to 25, using 11 ISSR primers. Results showed that the maximum genetic distance between genotypes 2 and 13 was 0.5. The genotypes (16 and 17), (16 and 18), (17 and 18), and (17 and 19) recorded the lowest genetic distance of 0.03. The dendrogram of the genetic relationship among the genotypes also divided them into two main groups, A and B. Group B included most Iraqi cultivars, including the local cultivar Al-Bateerah (genotype 5), which was selected for the second experiment. During the spring of 2025, the second experiment was implemented in a field at the College of Agricultural Engineering Sciences, University of Baghdad, Al-Jadriyah. The objective was to examine the impact of certain physiological treatments on enhancing seed yield and its components. The experiment utilized a  $2 \times 2 \times 4$  factorial arrangement in a randomized complete block design (RCBD) with three replicates. It studied the effect of spraying amino acids, coded A1, a fruit-set regulator containing auxins, coded G1, and nutrient elements [Ca-Mg, coded N1; P-K, coded N2; and micronutrients + S, coded N3] on okra seed yield and its components. The results showed significant effects of the three-way interactions, two-way interactions, and individual factors on all studied traits. The treatment A1G1N3 achieved the highest values in seed yield (192.23 g plant<sup>-1</sup>), 1000-seed weight (75 g), seed bulk density, seed true density, germination percentage, and germination speed. The treatment A1G1N1 produced the highest number of dry pods.

## INTRODUCTION

Okra cultivars (*Abelmoschus esculentus* L.) vary in their productive performance because of differences in their genetic composition. This is reflected in their ability to grow, adapt, and produce. Therefore, genetic diversity is essential in crop breeding and genetic improvement programs (Mkhabela et al., 2022; Lata et al., 2025). Relying on morphological traits to evaluate genetic diversity among cultivars is not sufficient because these traits are affected by environmental factors and by the interaction between genetic and environmental effects. This may limit their accuracy in estimating genetic diversity among different genotypes (Napier et al., 2023; Zhang & Bell, 2024).

Therefore, there was a need to use more accurate techniques to detect genetic diversity. Molecular markers have become among the most important tools used to characterize and determine the degree of genetic relationship among different cultivars (Bunjkar et al., 2024; Nair & Pandey, 2024). The Inter Simple Sequence Repeat (ISSR) technique is considered one of the effective molecular techniques for studying genetic diversity. It is characterized by ease of application and does not require prior information about the plant genome sequence. In addition, it has a high ability to detect genetic polymorphism and high efficiency in distinguishing among different genotypes (Azizi et al., 2021; Murphy et al., 2024; Tran et al., 2025).

This technique contributes to identifying genetically related cultivars and directing breeding and genetic improvement programs towards developing their productivity and conserving their genetic resources, particularly desirable local cultivars

(Zheng et al., 2024; Altındal & Altındal, 2025). Analysis of genetic diversity using ISSR in a group of okra genotypes showed good polymorphism percentages, with the ability of these markers to distinguish among genotypes and form clear genetic patterns. This confirms their importance in preliminary studies of genetic diversity (Abd El-Fattah et al., 2020). The study of genetic diversity through whole-genome duplication revealed high genetic diversity. This helped to divide them into genetically distant groups, associate alleles with productive traits and fruit quality, and support improvement programs (Wang et al., 2023; Ma et al., 2024; Castellacci et al., 2026).

From the physiological aspect, okra suffers from the problem of flower abscission and a low fruit-set percentage. Its seed yield is negatively affected when it grows under stress environments. Therefore, researchers and producers need to address this problem by using some physiological treatments, such as spraying amino acids to overcome environmental stress, spraying nutrient elements, or spraying fruit-set regulators. These treatments help to increase fruit-set efficiency and reduce flower abscission, leading to an increase in okra seed yield (Deshmukh et al., 2024; Rajesaheb et al., 2025).

Foliar spraying with nutrients has also received wide attention in crop seed-production programs because it provides an effective means of supplying plants with nutrient elements during critical growth stages, particularly the flowering and fruit-set stages. Many studies have examined the effect of spraying nutrient elements, whether macronutrients or micronutrients, on improving the physiological performance of plants, increasing fruit-set percentage, reducing flower abscission, and consequently improving seed productivity and its traits (Ashraf et al., 2024; Alotaibi et al., 2024; Hossain et al., 2025; Rajesaheb et al., 2025).

Micronutrients play an essential role in activating enzymes and regulating the physiological processes associated with growth, flowering, and fruit set in okra. They also improve yield traits and pod quality in okra, particularly when used in integrated fertilization programs. This improves nutrient-use efficiency through enhancing plant activity, chlorophyll concentration in leaves, and metabolic processes in okra. This is positively reflected in growth and yield. It also enhances the balance of other nutrients, such as magnesium, potassium, and boron, particularly during the flowering and fruit-set stages (Romdhane et al., 2020; Jahan et al., 2020; Roslan et al., 2022; Singh et al., 2022; Jalal et al., 2024).

Several studies have demonstrated the importance of foliar spraying with growth regulators (fruit-set regulators) and their effects on plants. In okra, spraying with fruit-set regulators led to a significant increase in the number of flowers and fruit-set percentage, reduced flower abscission, and an increase in the number of pods, seed weight, and number of seeds per pod. It also increased fertilization efficiency, germination percentage, and sapling vigor. In addition, it improved seed viability, accelerated pod growth, enhanced stress tolerance, and improved nutrient distribution (Kumar et al., 2021; Hossain et al., 2022; Dhal et al., 2023; Hossain et al., 2023; Regar et al., 2024; Abdullah et al., 2024; Satpathy et al., 2025; Udpuay et al., 2025; Faldessai et al., 2025).

Amino acids are also vital compounds that play an essential role in building and regulating physiological processes within the plant. They enter into the composition of proteins and enzymes and contribute to improving the efficiency of many biological processes. They act as biostimulants when added to plants, particularly through foliar spraying. They also enhance vegetative growth, stimulate photosynthetic activity, and improve the efficiency of metabolic processes. This is positively reflected in the plant's ability to grow under different environmental conditions.

In okra, these compounds acquire special importance because of the sensitivity of the crop during the flowering and pod-formation stages. This is directly reflected in the physiological and nutritional status of the plant and affects the quantity and quality of the final yield. Several studies have indicated the importance and effects of using amino acids on okra plants. They contributed to increasing metabolic activity, improving nutrient-absorption efficiency, and increasing antioxidant activity. This was reflected in increased vegetative and floral growth, fruit set, pod formation, and seed yield (Sun et al., 2024; Henderson et al., 2025; Johari et al., 2020; Youssef et al., 2022; Benchasri, 2024; Ishfaq et al., 2025; Al Taie and Al Jubouri, 2025).

Based on the above, this research aims to determine the genetic distance among cultivars and to identify the best physiological combination of nutrients, amino acids, and fruit-set regulator for improving seed yield in okra.

## RESEARCH METHODOLOGY

Two experiments were conducted. The first experiment involved testing the genetic diversity of 25 genotypes of okra, coded from 1 to 25, using 11 ISSR primers. The primers were obtained from the Korean company Bioneer. DNA was extracted

according to the method of Sambrook and Russell (2001). Genetic distance was calculated according to the equation of Nei and Li (1979), and the dendrogram was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) according to the Statistical Package for the Social Sciences (SPSS) program.

The second experiment aimed to enhance the seed yield of the local Al-Bateerah cultivar. During the spring 2025 agricultural season, seeds were sown in a field at the College of Agricultural Engineering Sciences, University of Baghdad, Al-Jadriyah. The study utilized a  $2 \times 2 \times 4$  factorial arrangement based on a randomized complete block design (RCBD) with three replicates. Three factors of equal importance were studied.

The first factor was the effect of amino acids and included two treatments: without spraying, coded A0, and spraying with amino acids at a rate of 20 mL L<sup>-1</sup> of SCUDOR solution, which contains 6.6% free amino acids, weight volume<sup>-1</sup>, coded A1.

The second factor included the effect of the fruit-set regulator ALBENA and involved two treatments: without spraying, coded G0, and spraying with the fruit-set regulator at a rate of 1 g L<sup>-1</sup> of ALBENA fertilizer, which contains auxins:  $\beta$ -NOA (Beta-naphthoxyacetic Acid) at 1.8% weight volume<sup>-1</sup>, NAD (Amide Alpha-naphthalenacetic acid) at 1.5% weight volume<sup>-1</sup>, and NAA (Alpha-naphthalenacetic Acid) at 0.5% weight volume<sup>-1</sup>, coded G1.

The third factor included the effect of a nutrient group with four treatments. The first treatment was without spraying, coded N0. The second treatment was spraying with calcium and magnesium at a rate of 1 mL L<sup>-1</sup> of the Ca-Mg nutrient, which contains calcium oxide (CaO) at 8% by weight and magnesium oxide (MgO) at 6% by weight, coded N1. The third treatment was spraying with phosphorus and potassium at a concentration of 2.5 mL L<sup>-1</sup> of GRANFOL-K (P-K) nutrient, which contains 44.6% phosphorus (P<sub>2</sub>O<sub>5</sub>) and 31.7% potassium (K<sub>2</sub>O), coded N2. The fourth treatment was spraying with Micropol nutrient solution at a concentration of 0.5 g L<sup>-1</sup>, containing a group of chelated micronutrients and sulfur: boron (B) 6%, copper (Cu) 1%, manganese (Mn) 12%, molybdenum (Mo) 0.25%, zinc (Zn) 7.5%, and sulfur trioxide (SO<sub>3</sub>) 32%, coded N3.

The evaluated seed-yield parameters included the number of dry pods per plant, 1000-seed weight, seed count per 100 g, seed yield per plant (g), seed bulk density (g cm<sup>-3</sup>), seed true density (g cm<sup>-3</sup>), germination speed, and germination percentage. The data were analyzed in accordance with the previously described experimental design, and mean values were compared using the least significant difference (LSD) test at a 5% probability level.

## RESULTS AND DISCUSSION

### Genetic diversity among okra genotypes using the ISSR technique

It was observed from Table 1 that there was clear diversity among the genotypes at different levels, with the appearance of genetically close pairs and others that were somewhat distant. Low values of genetic distance indicate high similarity between two genotypes because they share a large number of molecular bands, whereas high values indicate greater differences in the band pattern and, consequently, clearer genetic divergence. This result agrees with An et al. (2022), who indicated that molecular markers in okra help analyze genetic diversity and genetic population structure, and with Nieuwenhuis et al. (2024), who stated that the complex genomic nature of okra makes the use of molecular markers important for detecting differences among genetic compositions.

The genetic-distance values in Table 1 showed that the highest genetic distance was between genotype2, which is a pure line derived from an Indian hybrid, and the local genotype13, reaching 0.58. Likewise, genotype4, which is a cultivar imported from Egypt, gave a high genetic-distance value of 0.54 with genotype13. These values indicate the possibility of using them as parents in hybridization programs. Some genotypes were also very closely related, particularly genotype16 with genotypes17 and 18, and genotype17 with genotypes18 and 19, as the genetic distance among them reached 0.03. These are local cultivars. There was also high similarity among genotypes21, 22, and 23, as the genetic distance between each pair was 0.05. This value reveals a substantial level of genetic similarity among them, which may be attributed to the closeness of their genetic origin, the similarity of their source, or their possession of a large number of shared ISSR loci amplified by the same primers.

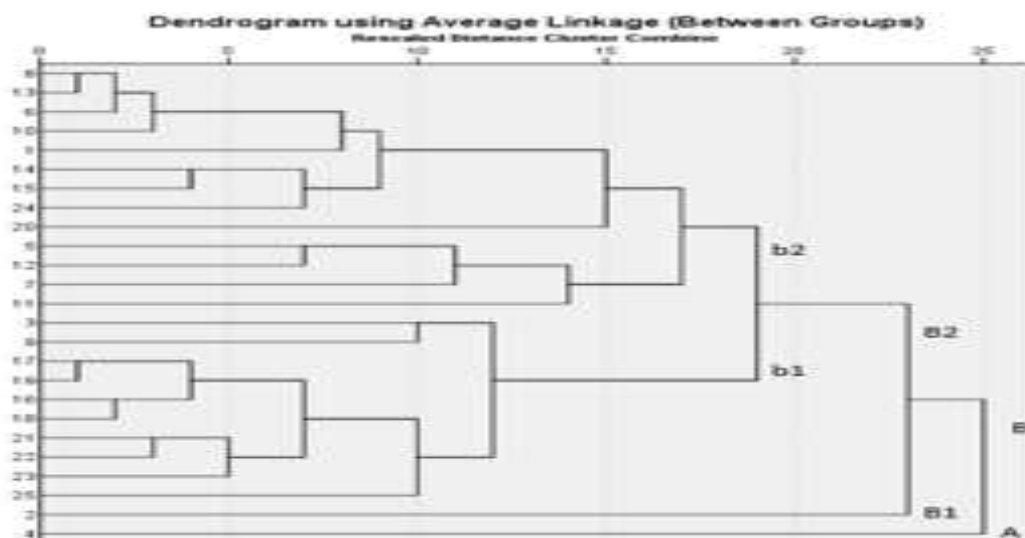
The genetic tree diagram in Figure 1 showed that the twenty-five genotypes were arranged into genetic groups that reflected the degrees of similarity and divergence among them. At the first level, the compositions were divided into two main groups, A and B. Group A included genotype4 alone, which is the Batra cultivar imported from Egypt. This indicates that it was the

most distinct and distant composition from the remaining genetic compositions. Its early separation in the tree indicates that it possesses a specific molecular pattern, possibly resulting from a difference in its genetic source or from possessing distinctive ISSR bands that were not common in the remaining compositions.

**Table 1:** Genetic distance values among 25 genetic compositions of okra based on ISSR molecular markers.

| Percent disagreement (Spreadsheet 1 (Recovered)) |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |
|--------------------------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Variable                                         | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14   | 15   | 16   | 17   | 18   | 19   | 20   | 21   | 22   | 23   | 24   | 25   |
| 1                                                | 0.00 | 0.45 | 0.38 | 0.41 | 0.39 | 0.42 | 0.43 | 0.34 | 0.42 | 0.36 | 0.55 | 0.45 | 0.34 | 0.42 | 0.36 | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 | 0.37 | 0.39 | 0.39 | 0.32 | 0.23 |
| 2                                                |      | 0.00 | 0.25 | 0.07 | 0.28 | 0.50 | 0.14 | 0.53 | 0.29 | 0.43 | 0.16 | 0.24 | 0.58 | 0.53 | 0.48 | 0.13 | 0.11 | 0.13 | 0.13 | 0.24 | 0.16 | 0.13 | 0.11 | 0.39 | 0.17 |
| 3                                                |      |      | 0.00 | 0.24 | 0.22 | 0.43 | 0.29 | 0.38 | 0.22 | 0.32 | 0.36 | 0.30 | 0.48 | 0.49 | 0.59 | 0.17 | 0.17 | 0.20 | 0.17 | 0.33 | 0.17 | 0.17 | 0.17 | 0.36 | 0.18 |
| 4                                                |      |      |      | 0.00 | 0.22 | 0.46 | 0.11 | 0.45 | 0.25 | 0.42 | 0.14 | 0.20 | 0.54 | 0.51 | 0.35 | 0.07 | 0.07 | 0.07 | 0.05 | 0.26 | 0.12 | 0.05 | 0.07 | 0.36 | 0.11 |
| 5                                                |      |      |      |      | 0.00 | 0.34 | 0.17 | 0.42 | 0.24 | 0.33 | 0.24 | 0.16 | 0.45 | 0.46 | 0.41 | 0.24 | 0.21 | 0.21 | 0.21 | 0.32 | 0.24 | 0.21 | 0.21 | 0.29 | 0.22 |
| 6                                                |      |      |      |      |      | 0.00 | 0.41 | 0.18 | 0.47 | 0.27 | 0.50 | 0.35 | 0.21 | 0.45 | 0.49 | 0.42 | 0.47 | 0.38 | 0.42 | 0.50 | 0.45 | 0.45 | 0.47 | 0.35 | 0.41 |
| 7                                                |      |      |      |      |      |      | 0.00 | 0.40 | 0.25 | 0.29 | 0.14 | 0.14 | 0.49 | 0.46 | 0.37 | 0.17 | 0.14 | 0.14 | 0.17 | 0.22 | 0.17 | 0.14 | 0.14 | 0.33 | 0.10 |
| 8                                                |      |      |      |      |      |      |      | 0.00 | 0.47 | 0.17 | 0.55 | 0.47 | 0.16 | 0.37 | 0.43 | 0.45 | 0.45 | 0.47 | 0.45 | 0.47 | 0.45 | 0.47 | 0.47 | 0.34 | 0.46 |
| 9                                                |      |      |      |      |      |      |      |      | 0.00 | 0.30 | 0.29 | 0.44 | 0.47 | 0.29 | 0.41 | 0.19 | 0.19 | 0.19 | 0.32 | 0.21 | 0.18 | 0.21 | 0.29 | 0.17 | 0.17 |
| 10                                               |      |      |      |      |      |      |      |      |      | 0.00 | 0.48 | 0.38 | 0.25 | 0.58 | 0.45 | 0.36 | 0.36 | 0.36 | 0.46 | 0.38 | 0.36 | 0.38 | 0.30 | 0.30 | 0.30 |
| 11                                               |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.21 | 0.63 | 0.41 | 0.42 | 0.23 | 0.18 | 0.18 | 0.21 | 0.32 | 0.24 | 0.21 | 0.18 | 0.42 | 0.24 |
| 12                                               |      |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.53 | 0.47 | 0.33 | 0.26 | 0.24 | 0.24 | 0.32 | 0.26 | 0.24 | 0.24 | 0.29 | 0.29 |      |
| 13                                               |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.25 | 0.42 | 0.30 | 0.58 | 0.47 | 0.53 | 0.50 | 0.50 | 0.55 | 0.37 | 0.51 |      |
| 14                                               |      |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.25 | 0.50 | 0.55 | 0.50 | 0.47 | 0.50 | 0.47 | 0.45 | 0.37 | 0.46 |      |
| 15                                               |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.46 | 0.46 | 0.42 | 0.42 | 0.28 | 0.42 | 0.42 | 0.42 | 0.29 |      |
| 16                                               |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.53 | 0.53 | 0.55 | 0.26 | 0.55 | 0.55 | 0.29 | 0.67 |      |
| 17                                               |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.53 | 0.25 | 0.55 | 0.05 | 0.05 | 0.05 | 0.29 |      |
| 18                                               |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.24 | 0.55 | 0.08 | 0.08 | 0.37 | 0.67 |      |
| 19                                               |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.29 | 0.05 | 0.05 | 0.08 | 0.25 |      |
| 20                                               |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.26 | 0.26 | 0.34 | 0.37 |      |
| 21                                               |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.05 | 0.26 | 0.12 |      |
| 22                                               |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.26 | 0.09 |      |
| 23                                               |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 | 0.32 |      |
| 24                                               |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 |      |
| 25                                               |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      |      | 0.00 |

The genotypes were: 1. Local Bateerah cultivar; 2. Pure line (7) derived from an Indian hybrid; 3. Mosuli Bateerah cultivar; 4. Imported Bateerah cultivar; 5. Local Bateerah cultivar; 6. Imported Clemson cultivar; 7. Imported cultivar (940); 8. Husainawiya cultivar; 9. Imported green okra cultivar; 10. Bateerah cultivar; 11. Pure line (2); 12. Pure line (8); 13. Pure line (30); 14. Khudairi cultivar; 15. Master cultivar; 16. Husainawiya cultivar; 17. Bateerah cultivar; 18. Long Bateerah cultivar; 19. Local Bateerah cultivar; 20. Mosuli okra cultivar; 21. Pure line (5); and 22. Pure line.



**Figure 1:** Dendrogram of the genetic relationship among 25 genetic compositions of okra based on the results of ISSR markers.

Group B included the remaining genetic compositions, indicating that they were more closely related to one another compared with genotype 4. This group was separated into two primary clusters, B1 and B2, with cluster B1 containing genotype 2, which is pure line 7 derived from an Indian hybrid, in a relatively separate position. This indicates that it was very distant from the remaining compositions of cluster B2, but at the same time it did not reach the degree of divergence shown

by genotype4. This makes it intermediate in terms of its genetic position, as it can be considered a good source of diversity within group B. Yuan et al. (2015) indicated that ISSR-based cluster analysis in okra is able to isolate divergent compositions and identify genetically related groups.

As for Cluster B2, it included the largest number of genotypes and divided into two subgroups. The first sub-group b1 included genotypes 3, 9, 17, 19, 16, 18, 21, 22, 23, and 25

It was observed within this group that there was very close similarity among genotypes16, 17, 18, and 19. This agrees with the low genetic-distance values recorded in Table 1. This means that these genotypes possess a closely related genetic background or a similar pattern of molecular bands.

The second secondary group, b2, included genotypes8, 13, 6, 10, 1, 14, 15, 24, 20, 5, 12, 7, and 11. Most of them were local cultivars, except genetic composition 6, which was the Clemson cultivar imported from the United States, and genotypes10 and 7, which were the Bateerah cultivar and cultivar 940, respectively, imported from Turkey.

It was observed that genotypes8 and 13 were the closest within group b2. Genotypes8 and 6, and 8 and 10, also showed clear similarity, as all of them were located within the same group. This was confirmed by the genetic-distance values in Table 1, which were 0.16 between genotypes8 and 13, and 0.18 and 0.17 between genotypes8 and 6 and between genotypes8 and 10, respectively. In group b1, genotypes16, 17, 18, and 19 were very closely related, as they were located within one group. This agrees with the genetic-distance values in Table 1, which recorded a distance of 0.03 between each pair. It was also found that three genetic compositions, 21, 22, and 23, were very closely related, as they were located within one group. This confirms the results of Table 1, as the genetic distance between each pair was 0.05, indicating a high degree of genetic similarity among them.

Group b2 was of special importance because it included most of the local genetic compositions. Among them was genetic composition 5, the local Al-Bateerah cultivar. Its presence within this group indicates that it is closely related to several other local cultivars. This indicates the possibility of adopting it as a representative model for this group when moving from the stage of genetic characterization to the stage of studying physiological response.

These results agree with the findings of Nieuwenhuis et al. (2024) regarding the efficiency of molecular techniques in detecting genetic diversity and determining evolutionary relationships among plant compositions. Abd El-Fattah et al. (2020) also confirmed the effectiveness of ISSR in analyzing genetic diversity in agricultural crops.

The results of Table 1 and the genetic tree showed that the local Al-Bateerah cultivar, which is genetic composition 5, was located within a genetic group that included a large number of relatively closely related local cultivars. This position gave it special importance because it made it a suitable representative of this local group, rather than selecting a genetically isolated composition that may not reflect the response of most local cultivars. Therefore, the selection of Al-Bateerah was not based only on its field observations, but also on molecular evidence showing its closeness to a wide group of local compositions.

The distinction of the Al-Bateerah cultivar under field conditions strengthened its nomination for the subsequent study. A cultivar that combines acceptable genetic representation and good field performance is more suitable for testing the effect of physiological treatments. Therefore, it was selected to study the effect of spraying amino acids, fruit-set regulator, macronutrients, and micronutrients, with the aim of improving flower set, seed yield, seed quality, and seed biological traits. This linkage between molecular characterization and field selection is an important approach in crop improvement because molecular markers provide an objective basis for selecting genetic materials included in subsequent experiments (An et al., 2022; Yuan et al., 2015).

From the research perspective, the selection of a representative local cultivar is an important step because the results of physiological treatments on it are closer to practical application under local environmental conditions. If the Al-Bateerah cultivar shows a positive response to amino acids, fruit-set regulator, and nutrient elements, this will provide an important indication of the possibility of improving the performance of local cultivars that are genetically close to it. The linkage between molecular characterization and field selection also strengthens the research design because it makes the transition from the genetic-diversity experiment to the physiological-treatment experiment a scientifically justified transition rather than a random selection. This approach agrees with what An et al. (2022) indicated regarding the importance of molecular markers in supporting the selection of appropriate genetic resources for breeding and improvement programs.

## Effect of amino acids, fruit-set regulator, and some nutrients on okra seed yield and its components

Seed-production traits are an important indicator of the efficiency of physiological processes, particularly during the seed-filling stage. They depend on the efficiency of nutrient translocation from source to sink, in addition to hormonal balance within the plant. These traits include seed weight, seed density, seed yield, germination rate, and germination percentage (Raza et al., 2024; Smeriglio et al., 2025).

Tables 2, 3, and 4 showed a significant effect of the three-way and two-way interaction treatments and the individual factors represented by amino acids (A), fruit-set regulator (G), and nutrient elements (N) on the traits of number of dry pods and seed-yield components of okra plants.

The treatments A1G1N3, A1N3, G1N3, A1G1, A1, G1, and N3 gave the highest values for seed-yield indicators, 1000-seed weight, seed bulk density, seed true density, germination percentage, and germination speed. The treatments A1G1N1, A1N1, G1N1, A1G1, A1, G1, and N1 yielded the highest number of dry pods.

All the above-mentioned three-way interaction, two-way interaction, and individual-factor treatments outperformed the control treatment.

In contrast, the control treatments for the three-way interactions, two-way interactions, and individual factors (A0G0N0, A0N0, G0N0, A0G0, A0, G0, and N0) yielded the highest values for the number of seeds per 100 g of seeds.

The results can be explained by the integrated effect of the studied factors on regulating the processes of seed formation, filling, and maturity, particularly when they were applied by foliar spraying, which increased absorption efficiency and the speed of compound translocation to the reproductive organs (Raza et al., 2024).

The superiority of the treatments A1G1N3, A1N3, G1N3, A1G1, A1, G1, and N3 in seed-yield indicators, 1000-seed weight, seed bulk density, seed true density, germination percentage, and germination speed was attributed to the vital role of micronutrients in combination with amino acids and auxins in improving the efficiency of physiological processes associated with seed formation and quality. Amino acids contributed to supporting metabolism by enhancing the synthesis of proteins and enzymes necessary for embryo formation and nutrient accumulation within seeds. They also reduced oxidative stress, which improved seed viability (Ishfaq et al., 2025; Raza et al., 2024). Auxins played an important role in enhancing the growth of reproductive tissues and regulating the translocation of photosynthetic products to the seeds, which increased their filling and improved their weight (Askr et al., 2018; Raza et al., 2024).

Micronutrients had an essential role in activating many enzymes associated with the formation of storage materials within seeds. Zinc contributed to regulating auxin and enzyme synthesis. Boron played an important role in improving pollen-grain formation and pollen-tube growth, which increased fertilization efficiency. Manganese contributed to supporting photosynthesis, while molybdenum participated in nitrogen metabolism, which was reflected in improving protein formation within seeds and increasing their quality. Copper also entered into many oxidation-reduction reactions important for seed maturity. This integration led to increased seed weight and density and improved seed viability, which was reflected in higher germination percentage and germination speed (Adhikari et al., 2024).

**Table 2:** Effect of the three-way interactions of amino acids, fruit-set regulator, and some nutrients on okra seed yield and its components.

| Treatments<br>A × G × N | Number of dry pods plant <sup>-1</sup> | Seed yield (g plant <sup>-1</sup> ) | Number of seeds per 100 g | 1000-seed weight (g) | Seed bulk density (g cm <sup>-3</sup> ) | Seed true density (g cm <sup>-3</sup> ) | Germination percentage (%) | Germination speed (seed day <sup>-1</sup> ) |
|-------------------------|----------------------------------------|-------------------------------------|---------------------------|----------------------|-----------------------------------------|-----------------------------------------|----------------------------|---------------------------------------------|
| A0G0N0                  | 27.64                                  | 55.98                               | 1806.67                   | 53                   | 0.60                                    | 1.06                                    | 78.06                      | 6.1                                         |
| A0G0N1                  | 29.05                                  | 67.43                               | 1756.67                   | 55                   | 0.61                                    | 1.08                                    | 80.80                      | 6.3                                         |
| A0G0N2                  | 28.82                                  | 77.21                               | 1700                      | 58                   | 0.63                                    | 1.10                                    | 82.10                      | 6.5                                         |
| A0G0N3                  | 28.18                                  | 90.06                               | 1653.33                   | 64                   | 0.65                                    | 1.12                                    | 84.12                      | 6.7                                         |
| A0G1N0                  | 32.55                                  | 81.57                               | 1726.67                   | 57                   | 0.62                                    | 1.09                                    | 81.87                      | 6.4                                         |
| A0G1N1                  | 34.52                                  | 99.34                               | 1620                      | 60                   | 0.66                                    | 1.13                                    | 83.13                      | 6.6                                         |

| Treatments<br>A × G × N | Number of dry pods plant <sup>-1</sup> | Seed yield (g plant <sup>-1</sup> ) | Number of seeds per 100 g | 1000-seed weight (g) | Seed bulk density (g cm <sup>-3</sup> ) | Seed true density (g cm <sup>-3</sup> ) | Germination percentage (%) | Germination speed (seed day <sup>-1</sup> ) |
|-------------------------|----------------------------------------|-------------------------------------|---------------------------|----------------------|-----------------------------------------|-----------------------------------------|----------------------------|---------------------------------------------|
| A0G1N2                  | 33.59                                  | 109.94                              | 1560                      | 63                   | 0.68                                    | 1.16                                    | 85.16                      | 6.8                                         |
| A0G1N3                  | 32.80                                  | 119.31                              | 1500                      | 65                   | 0.70                                    | 1.18                                    | 87.17                      | 7.0                                         |
| A1G0N0                  | 30.06                                  | 83.36                               | 1683.33                   | 59                   | 0.64                                    | 1.11                                    | 83.11                      | 6.6                                         |
| A1G0N1                  | 31.26                                  | 100.78                              | 1606.67                   | 62                   | 0.67                                    | 1.14                                    | 85.14                      | 6.8                                         |
| A1G0N2                  | 31.50                                  | 114.64                              | 1543.33                   | 65                   | 0.69                                    | 1.17                                    | 87.16                      | 7.0                                         |
| A1G0N3                  | 31.21                                  | 117.98                              | 1486.67                   | 63                   | 0.71                                    | 1.19                                    | 89.16                      | 7.2                                         |
| A1G1N0                  | 36.13                                  | 127.92                              | 1586.67                   | 68                   | 0.68                                    | 1.15                                    | 86.27                      | 6.9                                         |
| A1G1N1                  | 41.70                                  | 162.37                              | 1450                      | 67                   | 0.72                                    | 1.20                                    | 88.16                      | 7.1                                         |
| A1G1N2                  | 40.93                                  | 183.41                              | 1420                      | 71                   | 0.73                                    | 1.21                                    | 90.19                      | 7.3                                         |
| A1G1N3                  | 37.60                                  | 192.23                              | 1386.67                   | 75                   | 0.74                                    | 1.23                                    | 92.21                      | 7.5                                         |
| LSD 0.05                | 1.137                                  | 8.652                               | 23.468                    | 4.040                | 0.009                                   | 0.009                                   | 0.083                      | 0.119                                       |

Where: A1 represents spraying with amino acids, and A0 represents no spraying; G1 represents spraying with a fruit-set regulator, and G0 represents no spraying; N0 represents no nutrient spraying, N1 represents spraying with calcium and magnesium, N2 represents spraying with potassium and phosphorus, and N3 represents spraying with micronutrients and S.

**Table 3:** Effect of the two-way interactions of amino acids, fruit-set regulator, and some nutrients on okra seed yield and its components.

| Treatments   | Number of dry pods | Seed yield (g plant <sup>-1</sup> ) | Number of seeds | 1000-seed weight | Seed bulk density | Seed true density | Germination percentage | Germination speed |
|--------------|--------------------|-------------------------------------|-----------------|------------------|-------------------|-------------------|------------------------|-------------------|
| <b>A × N</b> |                    |                                     |                 |                  |                   |                   |                        |                   |
| A0N0         | 30.10              | 68.78                               | 1766.67         | 55.00            | 0.61              | 1.07              | 79.97                  | 6.25              |
| A0N1         | 31.79              | 83.38                               | 1688.33         | 57.50            | 0.63              | 1.10              | 81.97                  | 6.45              |
| A0N2         | 31.20              | 93.57                               | 1630.00         | 60.50            | 0.65              | 1.13              | 83.63                  | 6.65              |
| A0N3         | 30.49              | 104.69                              | 1576.67         | 64.33            | 0.67              | 1.15              | 85.65                  | 6.86              |
| A1N0         | 33.09              | 105.64                              | 1635.00         | 63.50            | 0.66              | 1.13              | 84.69                  | 6.74              |
| A1N1         | 36.48              | 131.58                              | 1528.33         | 64.50            | 0.69              | 1.17              | 86.65                  | 6.95              |
| A1N2         | 36.21              | 149.03                              | 1481.67         | 68.00            | 0.71              | 1.19              | 88.67                  | 7.13              |
| A1N3         | 34.41              | 155.10                              | 1436.67         | 69.00            | 0.72              | 1.21              | 90.69                  | 7.35              |
| LSD          | 0.804              | 6.118                               | 16.594          | 2.857            | 0.006             | 0.006             | 0.058                  | 0.084             |
| <b>G × N</b> |                    |                                     |                 |                  |                   |                   |                        |                   |
| G0N0         | 28.85              | 69.67                               | 1745.00         | 56.00            | 0.62              | 1.08              | 80.59                  | 6.35              |
| G0N1         | 30.16              | 84.11                               | 1681.67         | 58.50            | 0.46              | 1.11              | 82.97                  | 6.55              |
| G0N2         | 30.16              | 95.93                               | 1621.67         | 61.50            | 0.66              | 1.13              | 84.63                  | 6.74              |
| G0N3         | 29.69              | 104.02                              | 1570.00         | 63.33            | 0.68              | 1.15              | 86.64                  | 6.95              |
| G1N0         | 34.34              | 104.75                              | 1656.67         | 62.50            | 0.65              | 1.12              | 84.07                  | 6.63              |
| G1N1         | 38.11              | 130.86                              | 1535.00         | 63.50            | 0.69              | 1.16              | 85.65                  | 6.85              |
| G1N2         | 37.26              | 146.67                              | 1490.00         | 67.00            | 0.71              | 1.19              | 87.68                  | 7.05              |
| G1N3         | 35.20              | 155.77                              | 1443.33         | 70.00            | 0.72              | 1.20              | 89.69                  | 7.26              |
| LSD          | 0.804              | 6.118                               | 16.594          | 2.857            | 0.006             | 0.006             | 0.058                  | 0.084             |
| <b>A × G</b> |                    |                                     |                 |                  |                   |                   |                        |                   |
| A0G0         | 28.42              | 72.67                               | 1729.17         | 57.42            | 0.62              | 1.09              | 81.27                  | 6.40              |
| A0G1         | 33.37              | 102.54                              | 1601.67         | 61.25            | 0.66              | 1.14              | 84.33                  | 6.71              |
| A1G0         | 31.01              | 104.19                              | 1580.00         | 62.25            | 0.68              | 1.15              | 86.14                  | 6.89              |
| A1G1         | 39.09              | 166.48                              | 1460.83         | 70.25            | 0.72              | 1.20              | 89.21                  | 7.19              |
| LSD          | 0.569              | 4.326                               | 11.734          | 2.020            | 0.004             | 0.004             | 0.041                  | 0.060             |

**Table 4:** Effect of individual treatments of amino acids, fruit-set regulator, and some nutrients on okra seed yield and its components.

| Treatments      | Number of dry pods plant <sup>-1</sup> | Seed yield (g plant <sup>-1</sup> ) | Number of seeds per 100 g | 1000-seed weight (g) | Seed bulk density (g cm <sup>-3</sup> ) | Seed true density (g cm <sup>-3</sup> ) | Germination percentage (%) | Germination speed (seed day <sup>-1</sup> ) |
|-----------------|----------------------------------------|-------------------------------------|---------------------------|----------------------|-----------------------------------------|-----------------------------------------|----------------------------|---------------------------------------------|
| <b>A</b>        |                                        |                                     |                           |                      |                                         |                                         |                            |                                             |
| A0              | 30.89                                  | 87.61                               | 1665.42                   | 59.33                | 0.64                                    | 1.11                                    | 82.80                      | 6.55                                        |
| A1              | 35.05                                  | 135.34                              | 1520.42                   | 66.25                | 0.70                                    | 1.17                                    | 87.67                      | 7.04                                        |
| <b>LSD 0.05</b> | <b>0.402</b>                           | <b>3.059</b>                        | <b>8.297</b>              | <b>1.428</b>         | <b>0.003</b>                            | <b>0.003</b>                            | <b>0.029</b>               | <b>0.042</b>                                |
| <b>G</b>        |                                        |                                     |                           |                      |                                         |                                         |                            |                                             |
| G0              | 29.71                                  | 88.43                               | 1654.58                   | 59.83                | 0.65                                    | 1.12                                    | 83.71                      | 6.65                                        |
| G1              | 36.23                                  | 134.51                              | 1531.25                   | 65.75                | 0.69                                    | 1.17                                    | 86.77                      | 6.95                                        |
| <b>LSD 0.05</b> | <b>0.402</b>                           | <b>3.059</b>                        | <b>8.297</b>              | <b>1.428</b>         | <b>0.003</b>                            | <b>0.003</b>                            | <b>0.029</b>               | <b>0.042</b>                                |
| <b>N</b>        |                                        |                                     |                           |                      |                                         |                                         |                            |                                             |
| N0              | 31.60                                  | 87.21                               | 1700.83                   | 59.25                | 0.63                                    | 1.10                                    | 82.33                      | 6.49                                        |
| N1              | 34.13                                  | 107.48                              | 1608.33                   | 61.00                | 0.66                                    | 1.14                                    | 84.31                      | 6.70                                        |
| N2              | 33.71                                  | 121.30                              | 1555.83                   | 64.25                | 0.68                                    | 1.16                                    | 86.15                      | 6.89                                        |
| N3              | 32.45                                  | 129.90                              | 1506.67                   | 66.67                | 0.70                                    | 1.18                                    | 88.17                      | 7.10                                        |
| <b>LSD 0.05</b> | <b>0.569</b>                           | <b>4.326</b>                        | <b>11.734</b>             | <b>2.020</b>         | <b>0.004</b>                            | <b>0.004</b>                            | <b>0.041</b>               | <b>0.060</b>                                |

Where: A1 represents spraying with amino acids, whereas A0 represents no spraying; G1 represents spraying with a fruit-set regulator, whereas G0 represents no spraying; N0 represents no nutrient spraying, N1 represents spraying with calcium and magnesium, N2 represents spraying with potassium and phosphorus, and N3 represents spraying with micronutrients and sulfur.

The highest values for the number of dry pods were credited to the essential role that calcium and magnesium play in supporting physiological processes associated with pod formation and their continuation until the maturity stage. Calcium contributed to strengthening cell walls and reduced flower and fruit abscission, leading to an increased number of pods formed and retained until drying. Magnesium enhanced photosynthetic efficiency, which increased the production of carbohydrate materials required for pod growth and continuation (Kurul & Cevik, 2021). In addition, amino acids and auxins enhanced this effect by improving general growth and increasing fruit-set percentage, resulting in a higher number of dry pods.

The superiority of the control treatments A0G0N0, A0N0, G0N0, A0G0, A0, G0, and N0 in giving the highest values for the number of seeds per 100 g can be explained by the fact that the control treatments led to the formation of small-sized and lower-weight seeds, which increased their number at the expense of weight. The absence of amino acids, auxins, and nutrient elements led to reduced photosynthetic efficiency and metabolic processes, which resulted in lower dry-matter accumulation within seeds. Consequently, the seeds were less filled and more numerous per 100 g, which is an inverse relationship with seed weight (Raza et al., 2024).

The superiority of the above-mentioned three-way interaction, two-way interaction, and individual-factor treatments compared with the control treatment was attributed to the interrelated and integrated effects among amino acids, fruit-set regulator, and nutrient elements. This led to improved efficiency of the physiological processes associated with seed formation and maturity, represented by photosynthesis, metabolism, and nutrient translocation towards the seeds. This was reflected in improving seed yield and its qualitative traits (Raza et al., 2024; Ishfaq et al., 2025).

It is clear from the above that the improvement in dry pod and seed yield traits in okra plants is closely related to the efficiency of physiological processes during the seed-filling stage, particularly photosynthesis, metabolic processes, and nutrient translocation from source to sink. The best results were achieved through the integration of amino acids, fruit-set regulator, and nutrient elements. Micronutrients contributed to improving seed formation and increasing seed weight, density, and viability, whereas calcium and magnesium enhanced the increase in the number of dry pods and their continuation until maturity. This was confirmed by the superiority of the three-way interaction, two-way interaction, and individual-factor treatments, particularly A1G1N3, A1G1N1, and A1G1, in improving seed yield and its qualitative traits, compared with the reduced efficiency of physiological processes in the control treatment and its reflection in the production of smaller and more numerous seeds.

## CONCLUSION

It can be concluded that the ISSR technique was effective in estimating genetic diversity in okra and selecting suitable genotypes for breeding programs or for improving their physiological performance. Spraying with amino acids, fruit-set regulator, and micronutrients represents a promising treatment for improving seed yield and its traits in the local Al-Bateerah cultivar.

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