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The influence of foliar application of calcium-containing preparations on apple orchard productivity and fruit quality

Abstract. Effective production of high-quality and high-yielding fruit and berries is essential for addressing shortages. However, producers often encounter challenges related to calcium deficiency in apples. This study aimed to evaluate the productivity of late-ripening apple cultivars – Ligol, Jonagold Early Queen, and Fuji – following foliar application of calcium-containing preparations, as well as to assess the quality of their fruit. Field and laboratory research methods were employed to achieve this objective, and the results were subjected to statistical analysis. The study revealed that the Ligol cultivar exhibited the highest yield (19.3 t/ha) when treated with Brexil Ca at a rate of 1.8 L/ha. This represented an 8.3% increase compared to the control and a 12.9% increase compared to the variant treated with HelpRost at a rate of 1.0 L/ha. The highest fruit weight (296.5 g) was recorded for the Ligol cultivar when treated with Brexil Ca at a rate of 1.8 L/ha. Foliar application of Brexil Ca at the same rate was most effective for the Fuji cultivar, resulting in a yield of 24.8 t/ha, which was 9.8% higher than the control and 16.1% higher than the variant treated with Biocalcium (2.0 mL/ha) and HelpRost (1.0 L/ha). For the Jonagold Early Queen cultivar, the highest yield (16.2 t/ha, 8.6% above the control) and fruit weight (249.8 g, 5.4% above the control) were achieved with HelpRost at 1.0 L/ha. The highest flesh firmness in the Ligol cultivar was observed with Biocalcium treatment (2.0 mL/ha), while in the Jonagold Early Queen and Fuji cultivars, it was achieved with HelpRost (1.0 L/ha). The findings provide practical insights for enhancing apple production efficiency, improving fruit quality, and extending storage life

Keywords: Ligol; Jonagold Early Queen; Fuji, calcium-containing preparations; yield; fruit weight; flesh firmness

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INTRODUCTION

According to Law of Ukraine No. 8370-1 “On Food Security in Ukraine” (2011), fruits and berries must be included in the food basket as they are vital to the food balance. Fruit products serve as valuable raw materials for processing (freezing, juice production, jams, baby food, etc.) and provide the human body with easily digestible carbohydrates, organic acids, and vitamins.

In apple cultivation, calcium deficiency has become increasingly evident. According to Z. Wu & P. Chen (2021), extensive orchard management, excessive crop loads, and early harvesting exacerbate the issue of functional disorders in apples. Such disorders significantly affect the economic value of the produce by reducing its marketable quality, diminishing its visual appeal and taste, and, consequently, lowering consumer interest. Under these conditions, the economic efficiency of apple production inevitably declines, reducing growers’ motivation to remain engaged in the apple industry.

F. Carrasco-Cuello *et al.* (2024) identified the application of calcium-containing fertilisers as an effective measure to address these challenges. The foliar application method, as examined by J. Morales *et al.* (2023), compared to soil fertilisation, facilitates better absorption of micro- and macronutrients, including calcium, by the fruit.

The use of organo-mineral nutrient complexes significantly enhances the adaptive properties of plants, increases productivity, and improves crop quality. Calcium is among the least efficiently absorbed nutrients, and its deficiency leads to various physiological disorders. Effective and rapid mitigation of calcium deficiency can be achieved through foliar feeding. Calcium fertilisers not only enhance photosynthesis and the growth rate of vegetative plant organs (Liu *et al.*, 2022) but also significantly improve fruit quality (Lin *et al.*, 2020) and increase disease resistance (Yang *et al.*, 2020).

Research by G. Wang *et al.* (2022) demonstrated that spraying apple trees with calcium-containing fertilisers increases chloroplast content and enhances the photosynthetic capacity of apple leaves. Compared to the control, the photosynthetic rate of apple leaves sprayed with Niucui increased by 4.3-34.6%, while those

treated with Naipu 9 exhibited a 15.0-57.4% increase. In addition, spraying with calcium fertilisers improved fruit quality and facilitated the accumulation of essential mineral elements (Ca, Mg, and B) in both fruit and leaves while suppressing copper (Cu) accumulation. The weight and firmness of apples harvested from orchards treated with Niucui and Naipu 9 were higher than those from the control group. Apples from trees treated with Naipu 9 contained higher levels of soluble solids, sugars, and vitamin C, whereas those from orchards treated with Niucui showed a reduction in titratable acids. This effect is attributed to Naipu 9 enhancing the activity of sugar metabolism enzymes, while Niucui increased the activity of enzymes associated with acid synthesis. The researchers concluded that the consumer quality of Fuji apples was optimal, with the most balanced taste when the orchards were treated with Naipu 9.

Y. Xu *et al.* (2022) investigated the mechanism by which calcium regulates fruit ripening. To examine changes in proteins and enriched phosphopeptides, labelling was performed on calcium-treated Golden Delicious apples. The resulting dataset provided a comprehensive overview of critical pathways associated with fruit ripening. A total of 47 proteins and 124 phosphoproteins were significantly altered in calcium-treated apples, playing key roles in regulating cell walls, ethylene production, protein modification (especially degradation), as well as primary and secondary metabolism.

P. Kowalik *et al.* (2020) conducted comparative studies on the ultrastructure of epidermal and hypodermal cells, assessing the content and distribution of calcium within the cell walls, cytoplasmic membranes, and cytoplasm of Champion apples. Their findings demonstrated a positive impact of calcium treatments on the ultrastructure of epidermal and hypodermal cells, as evidenced by the preservation of continuous cytoplasmic membranes and stabilised cell structures.

P. Wójcik *et al.* (2019) investigated the yield and fruit quality of Elstar apple trees. Their findings revealed that pre-harvest spraying with CaCl₂ increased calcium concentrations in leaves and fruit by an average of 22% and 39%,

respectively. Additionally, an improvement in apple flesh firmness was observed.

The study aimed to examine the effects of foliar fertilisation of apple orchards with calcium-containing preparations on yield and the physical quality parameters of the fruit.

The aim was achieved through the following objectives:

- n analysing the yield of apple orchards of various cultivars treated with foliar applications of calcium-containing preparations;
- n determining the firmness of apple flesh as an indicator of quality and market value;
- n identifying optimal application rates of calcium-containing preparations for foliar treatment in apple orchards.

MATERIALS AND METHODS

Recognising the critical importance of biological diversity and the fact that its preservation is a shared responsibility of humanity, particularly the scientific community, the research was conducted in alignment with the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973) to support environmental conservation and the sustainable use of its components.

The study was carried out at the educational laboratory "Fruit and Vegetable Garden" of the National University of Life and Environmental Sciences of Ukraine, located in the natural and climatic zone of Ukrainian Polissia. The area is characterised by sod-medium podzolic soils formed on fluvio-glacial deposits, suitable for horticulture, and a moderately continental climate with mild winters and warm summers.

The research was conducted in 2023 in apple orchards established in 2019, with a planting scheme of 4×2.5 m, M.106 rootstock, rainfed conditions, and a protection and fertilisation system consistent with practices typical of this natural and climatic zone. The study examined the effects of foliar treatments on apple orchard yield, fruit weight, and flesh firmness. The analysis focused on late-ripening apple cultivars, including Fuji, Ligol, and Jonagold Early Queen.

The research was conducted as a three-factor experiment, with the factors being cultivar, type of preparation, and application rate. Each treatment was replicated three times. The

following calcium-containing preparations were used for foliar application in the orchards: Biocalcium (Biotech LTD), HelpRost (BTU-Center), Brexil Ca (Valagro), and Avangard (AgroAntal).

The experimental design included: control – water treatment (H₂O); variant 1 – Biocalcium (2 mL/ha); variant 2 – Biocalcium (4 mL/ha); variant 3 – HelpRost (1.0 L/ha); variant 4 – HelpRost (3 L/ha); variant 5 – Brexil Ca (1.8 kg/ha); variant 6 – Brexil Ca (2.0 kg/ha); variant 7 – Avangard (3 L/ha); and variant 8 – Avangard (6 L/ha).

Foliar treatments were performed following the manufacturer's instructions for each preparation. One set of treatments was applied at the dosage recommended by the manufacturer, while another set used a dosage reduced by half. Applications were carried out at four intervals: the first occurred after the June ovary drop (late June), with subsequent treatments at 20-day intervals, and the final application conducted two weeks before harvest. Spraying took place before 10:00, with the volume adjusted to ensure the formation of water droplets on the surface of the leaves or fruit.

Yield was determined by weighing the harvested fruit from each replication of a variant, subsequently recalculated into tonnes per hectare for each treatment. The weight of apples was measured using the gravimetric method with KERN EHA 500-1 scales (Germany). For this purpose, 10 apples were randomly selected from each replication of a treatment, weighed individually, and the average weight per replication and variant was calculated. Apple flesh firmness was assessed using a portable penetrometer, "Wagner FRUIT TEST" (USA), with an FT 30FT716 (USA) probe, 11 mm in diameter. At harvest maturity, three apples were sampled from each replication of a variant. The skin was removed on two opposite sides of each apple using a sharp tool provided by the device, and the probe was slowly inserted into the fruit's flesh. The average value for each fruit was recorded, followed by the calculation of mean values for each replication and treatment.

RESULTS AND DISCUSSION

The yield of the Ligol apple cultivar ranged from 16.8 t/ha in the variant treated with HelpRost at a rate of 1 L/ha to 19.3 t/ha with foliar application

of Brexil Ca at 1.8 L/ha. In the variant treated with Avangard at 6 L/ha, the yield exceeded 18.0 t/ha. The fruit weight was also highest in the Brexil Ca treatment at 296.5 g and in the Avangard treatment (3 L/ha) at 294.7 g. The lowest fruit weight for Ligol (252.0 g) was recorded in the variant treated with HelpRost at 1 L/ha (Table 1).

The yield of the Jonagold Early Queen cultivar ranged from 14.7 to 16.2 t/ha, depending on

the treatment. The lowest yields were recorded in variants with foliar applications of HelpRost (3 L/ha) and Brexil Ca (2.0 kg/ha), while the highest yield was observed in the variant treated with HelpRost (1.0 L/ha). Similarly, the largest fruit weight for this cultivar (249.8 g) was recorded in the same treatment, while the lowest weight (228.3 g) was observed in apples harvested from trees treated with Biocalcium at 2 mL/ha (Table 1).

Table 1. Yield and average fruit weight of apples, harvest 2023

Cultivar	Ligol		Jonagold Early Queen		Fuji	
	Fruit weight, g	Yield, t/ha	Fruit weight, g	Yield, t/ha	Fruit weight, g	Yield, t/ha
Control	271.7±10.2	17.7±0.7	236.2±21.4	14.8±0.5	162.1±10.1	22.0±1.1
Biocalcium (2.0 mL/ha)	277.6±11.0	17.3±0.4	228.3±10.9	15.0±0.6	155.1±14.4	21.1±1.4
Biocalcium (4.0 mL/ha)	285.6±10.9	18.6±0.6	231.2±11.2	15.6±0.8	167.5±11.5	22.8±1.1
HelpRost (1.0 L/ha)	252.0±10.9	16.8±0.6	249.8±29.9	16.2±0.9	155.2±15.6	21.1±1.4
HelpRost (3.0 L/ha)	268.3±14.8	17.4±0.5	234.5±13.4	14.7±0.6	180.3±10.7	24.5±1.0
Brexil Ca (1.8 kg/ha)	296.5±17.1	19.3±2.1	240.0±16.1	15.0±1.0	182.3±14.8	24.8±0.6
Brexil Ca (2.0 kg/ha)	275.7±12.2	17.9±0.8	235.3±18.2	14.7±1.1	179.1±11.6	24.3±1.4
Avangard (3.0 L/ha)	294.7±15.3	19.2±2.5	237.3±12.4	14.8±0.8	172.6±10.7	23.5±0.7
Avangard (6.0 L/ha)	277.5±16.9	18.0±1.1	238.8±21.0	14.9±1.5	177.2±12.6	24.1±1.1
<i>Mean ± SE</i>	<i>278.3 ± 13.2</i>	<i>18.0 ± 1.0</i>	<i>236.8 ± 17.2</i>	<i>15.1 ± 0.8</i>	<i>170.1 ± 12.4</i>	<i>23.1 ± 1.1</i>
<i>Min</i>	<i>252.0 ± 10.9</i>	<i>16.8 ± 0.6</i>	<i>228.3 ± 10.9</i>	<i>14.7 ± 0.6</i>	<i>155.1 ± 14.4</i>	<i>21.1 ± 1.4</i>
<i>Max</i>	<i>285.6 ± 10.8</i>	<i>19.3 ± 2.1</i>	<i>249.8 ± 29.9</i>	<i>16.2 ± 0.9</i>	<i>182.3 ± 14.8</i>	<i>24.8 ± 0.6</i>

Source: developed by the authors

The yield of the Fuji apple cultivar varied between 21.1 and 24.8 t/ha, depending on the method of foliar application. The lowest yield was recorded in variants treated with Biocalcium at a rate of 2 mL/ha and HelpRost at 1 L/ha. The highest yield was achieved in the variant treated with Brexil Ca (1.8 kg/ha). The heaviest fruit weight was also observed in the Brexil Ca treatment (1.8 kg/ha) at 182.3 g, while the lightest fruits were recorded following treatments with Biocalcium (2 mL/ha) and HelpRost (1.0 L/ha), at 155.1 g and 155.2 g, respectively.

When comparing foliar treatments for the Ligol cultivar, Brexil Ca applied at 1.8 kg/ha proved to be the most effective. The least

favourable result was obtained in the variant treated with HelpRost at 1 L/ha (Fig. 1). The fruit weight of apples harvested from trees treated with Brexil Ca (1.8 kg/ha) was 24.8 g (9.1%) greater than in the control and 44.5 g (17.6%) higher than the weight of apples from trees treated with HelpRost (1.0 L/ha). Similarly, the yield of apple trees treated with Brexil Ca at 1.8 kg/ha exceeded the control by 9.0% or 1.6 t/ha. Compared to HelpRost (1.0 L/ha), Brexil Ca (1.8 kg/ha) resulted in a 14.9% or 2.5 t/ha higher yield. When comparing treatment methods for the Jonagold Early Queen apple cultivar, the most effective was the application of HelpRost at a rate of 1 L/ha, while the least effective was HelpRost at 3 L/ha (Fig. 2).



Figure 1. Yield and average fruit weight of Ligol apple cultivar, 2023

Source: developed by the authors

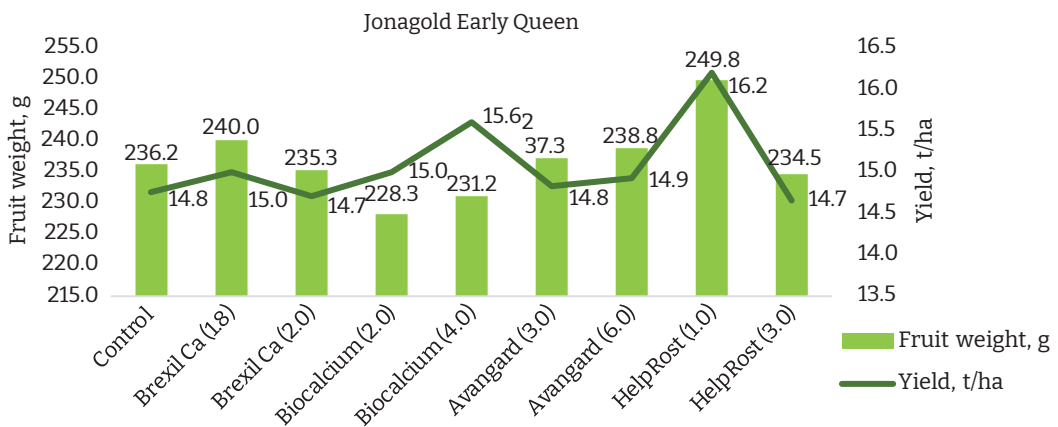


Figure 2. Yield and average fruit weight of Jonagold Early Queen apple cultivar, 2023

Source: developed by the authors

The fruit weight of Jonagold Early Queen apples grown in orchards treated with HelpRost (1 L/ha) was 13.6 g (5.7%) greater than in the water-treated control and 15.3 g (6.5%) higher than in the variant treated with HelpRost at 3 L/ha. Similarly, the yield of apple trees treated with HelpRost (1 L/ha) exceeded the control by 9.4% or 1.4 t/ha. The yield in this variant was also 10.2% or 1.5 t/ha higher than that obtained from trees treated with HelpRost (3 L/ha) (Fig. 2).

The fruit weight of Jonagold Early Queen apples treated with calcium-based foliar applications was generally higher compared to the control. Specifically, treatments with Avangard at 3.0 L/ha increased fruit weight by 1.1 g (0.5%), Avangard at 6.0 L/ha by 2.6 g (1.1%), Brexil Ca at

1.8 kg/ha by 3.8 g (1.6%), and HelpRost at 1.0 L/ha by 13.6 g (5.7%). The yield of Jonagold Early Queen orchards treated with foliar applications was also higher compared to the control. For example, Avangard (6.0 L/ha) increased yield by 0.1 t/ha (0.7%), Biocalcium (2.0 mL/ha) by 0.2 t/ha (1.3%), Brexil Ca (1.8 kg/ha) by 0.2 t/ha (1.3%), Biocalcium (4.0 mL/ha) by 0.8 t/ha (5.4%), and HelpRost (1.0 L/ha) by 1.4 t/ha (9.4%) (Fig. 2).

The yield of apple orchards treated with HelpRost at 3.0 L/ha and Brexil Ca at 2.0 kg/ha was 0.7% lower than the control. Similarly, the average fruit weight was reduced in variants treated with Biocalcium (2.0 mL/ha), Biocalcium (4.0 mL/ha), HelpRost (3.0 L/ha), and Brexil Ca (2.0 kg/ha) by 3.3%, 2.1%, 0.7%, and 1.6%, respectively (Fig. 2).

For foliar treatments in Fuji apple orchards, Brexil Ca at 1.8 kg/ha proved the most effective.

The least effective treatment was Biocalcium at 2.0 mL/ha (Fig. 3).

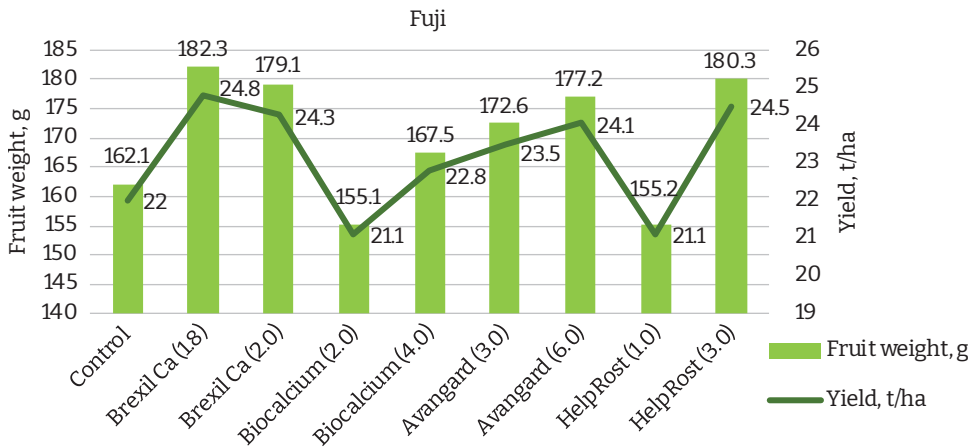


Figure 3. Yield and average fruit weight of Fuji apple cultivar, 2023

Source: developed by the authors

The fruit weight of Fuji apples harvested from trees treated with Brexil Ca (1.8 kg/ha) was 20.0 g (12.4%) higher than the control and 27.2 g (17.5%) greater than apples grown on trees treated with Biocalcium (2.0 mL/ha). Correspondingly, the yield of orchards treated with Brexil Ca (1.8 kg/ha) exceeded the control by 12.7% or 2.8 t/ha and surpassed the yield of Biocalcium-treated trees (2.0 mL/ha) by 17.5% or 3.7 t/ha (Fig. 3).

Variants treated with Brexil Ca and Avangard, in both application rates, as well as HelpRost (3.0 L/ha) and Biocalcium (4.0 mL/ha), demonstrated higher yields and fruit weights for the Fuji cultivar compared to the control (Fig. 3). In contrast, lower yields were observed

in orchards treated with Biocalcium (2.0 mL/ha) and HelpRost (1.0 L/ha), which were 4.1% lower than the control. Fruit weights in these variants were also lower by 4.3% and 4.2%, respectively, compared to the control (Fig. 3). The firmness of fruit flesh is a critical parameter influencing consumer and market value. It serves as an indicator of fruit quality, ripeness, and suitability for long-term storage and transportation.

The firmness of the flesh in the Jonagold Early Queen cultivar at harvest maturity was highest in the variant treated with HelpRost (1.0 L/ha). In other variants, the flesh firmness ranged from 9.3 kg/cm² (control) to 10.7 kg/cm² (variant treated with HelpRost (3.0 L/ha)) (Table 2).

Table 2. Flesh firmness of apple cultivars under study, kg/cm², 2023

Experimental variants	Cultivars		
	Jonagold Early Queen	Ligol	Fuji
Control	9.3±0.2	9.6±0.2	10.4±0.3
Biocalcium (2.0 mL/ha)	10.1±0.3	10.1±0.3	10.1±0.3
Biocalcium (4.0 mL/ha)	9.8±0.2	9.5±0.3	9.5±0.3
HelpRost (1.0 L/ha)	10.8±0.2	9.7±0.3	10.8±0.5
HelpRost (3.0 L/ha)	10.7±0.4	9.7±0.3	10.7±0.3
Brexil Ca (1.8 kg/ha)	10.1±0.4	9.0±0.3	10.1±0.3
Brexil Ca (2.0 kg/ha)	9.8±0.4	9.3±0.3	9.8±0.3
Avangard (3.0 L/ha)	9.3±0.4	9.3±0.3	9.3±0.2

Table 2, Continued

Experimental variants	Cultivars		
	Jonagold Early Queen	Ligol	Fuji
Avangard (6.0 L/ha)	9.8±0.1	9.8±0.3	9.8±0.4
Mean±SE	10.1±0.3	9.6±0.3	10.2±0.3
Min	9.3±0.2	9.0±0.3	9.3±0.2
Max	10.8±0.2	10.1±0.3	10.8±0.5

Source: developed by the authors

For the Ligol cultivar, the highest flesh firmness at harvest maturity was recorded in the variant treated with Biocalcium at 2.0 mL/ha, reaching 10.1 kg/cm². In other variants, flesh firmness varied from 9.8 kg/cm² (in the variant treated with Avangard at 6.0 L/ha) to 9.3 kg/cm² (in variants treated with Brexil Ca at 2.0 kg/ha and Avangard at 3.0 L/ha).

In the Fuji cultivar, the firmest flesh (10.8 kg/cm²) was observed in apples treated with HelpRost at 1.0 L/ha. In other variants, firmness ranged from 9.3 kg/cm² (variant treated with Avangard at 3.0 L/ha) to 10.7 kg/cm² (variant treated with HelpRost at 3.0 L/ha).

The role of calcium and calcium-sensitive receptors in plant physiology has been extensively studied. Q. Gao *et al.* (2019) investigated their involvement in regulating fruit development and ripening, as well as the molecular mechanisms underlying these physiological processes. The findings of A.G. Levin *et al.* (2019) confirm a direct or indirect correlation between calcium content in fruits and the health and resistance of apple trees to various diseases, including bitter pit, core rot, and cracking at different stages of fruit maturity. L.L. Yang *et al.* (2020) proposed solutions to these issues through the application of calcium-based fertilisers. In experimental conditions, the most satisfactory results were achieved with three applications of calcium nitrate, amounting to a total of 0.72 kg per tree.

In examining the key aspects of calcium's influence on the quality and yield of fruits and berries through an analysis of existing research in the field, Y. Huang *et al.* (2020) identified its positive effects on yield, product quality, and post-harvest storage duration. Following foliar application of amino acid-chelated calcium, grape berry mass increased by 22.5%, vertical

diameter by 8.8%, and transverse diameter by 10.8%. Additionally, fruit firmness improved by 35.7%, titratable acidity decreased by 23.2%, and soluble sugar content increased by 38.7%. L. Xinming *et al.* (2021) corroborated these findings, demonstrating that calcium fertilisation enhanced grape quality, with calcium content in the skin and pulp exceeding the control by 1.36 and 1.73 times, respectively. Pulp firmness increased by 19.28%, and soluble solids content rose by 5.71% compared to the control. Calcium treatment also influenced the qualitative and physiological characteristics of Japanese pears. The results indicated preserved fruit firmness, significant reductions in ethylene production and malondialdehyde accumulation, increased protopectin content, and reduced soluble pectin levels. Furthermore, there was a decrease in polygalacturonase and cellulase activity. J. Zhou *et al.* (2018) concluded that foliar calcium application significantly improved pear fruit storage quality. Similarly, M. Matteo *et al.* (2024) confirmed the effectiveness of foliar calcium fertilisation in sweet cherries, which reduced fruit cracking during rainy periods, enhanced firmness, and prolonged shelf life during storage. These researchers noted that calcium uptake by fruits was most effective in the early stages of development. The mechanism of calcium penetration through the skin and pedicel of cherries was further validated by A. Winkler & M. Knoche (2021). They demonstrated that spraying calcium salt solutions on berries increased calcium content within the fruit. Collectively, these findings underline the scientific interest in studying calcium's effects on fruit and berry quality. The observed mechanisms generally confirm their beneficial impact, regardless of the research subject, suggesting a consistent trend. However, unresolved issues related specifically to apple

production remain, motivating the authors to continue their investigations.

Y.X. Xu *et al.* (2023) elucidated a potential mechanism for delaying post-harvest ripening in apples through the application of calcium ions. Their research confirmed the suppression of ethylene biosynthesis in fruits, which extends storage life – a subject of significant interest to the authors. Y.X. Xu *et al.* (2023) identified a transcription factor in apples that functions as an activator of a gene involved in ethylene biosynthesis. Upon calcium treatment, a phosphorylation-mediated process was observed, leading to the degradation of this transcription factor. These findings demonstrated the efficacy of calcium ions in extending storage duration and improving product quality. However, the authors' hypothesis regarding significant variations in results among different apple cultivars was not substantiated. Furthermore, alternative biochemical pathways, beyond ethylene production, may contribute to post-harvest ripening. Various researchers have periodically investigated the inhibition of ethylene synthesis (Soto *et al.*, 2012) and chlorophyll degradation (Wei *et al.*, 2021) in apples to mitigate spoilage during storage. The findings of P.T. Yue *et al.* (2020) indicate that auxin, a plant hormone, induces ethylene biosynthesis in apple fruits by activating the expression of auxin response transcription factors via naphthaleneacetic acid. Such mechanisms encourage the exploration of counteracting factors, as identified by A. Soto *et al.* (2012). Their study demonstrated that methyl jasmonate, a source of the phytohormone jasmonate, inhibits the expression of ethylene-related genes, thereby delaying fruit ripening. Y. Wei *et al.* (2021) investigated the regulation of chlorophyll degradation through post-translational modifications. The overexpression of the ethylene-related module Md-PUB24 in apple fruits was found to accelerate chlorophyll degradation by increasing the expression of chlorophyll catabolic genes (CCG). These studies revealed potential mechanisms for mitigating undesirable processes, among which exogenous calcium may prove effective. However, the primary focus of the research was the impact of calcium on apple yield and physical quality attributes. G. Wang *et al.* (2022)

demonstrated that calcium-based fertiliser sprays improved the quality of apple fruits. Compared to the control, the mass of individual fruits, firmness, soluble solid content, and vitamin C concentration increased by 12.1%, 16.1%, 12.0%, and 29.4%, respectively. Additionally, the application of calcium fertilisers enhanced sugar content and reduced acidity, improving the flavour profile of the apples. In a subsequent study, G. Wang *et al.* (2023) developed calcium-encapsulated carbon dots as a nanofertiliser for apple production. The results indicated that this approach facilitated efficient nutrient uptake and increased calcium concentration in the fruits. Post-harvest spraying with nano-calcium carbonate yielded the lowest levels of total fruit damage (3.569%), respiratory intensity (2.636 mg CO₂/kg/hour), and total acidity (0.214%), alongside the highest fruit firmness (1.007 kg/cm²), sugar content (12.06%), ascorbic acid concentration (15.93 mg/100 mL), and calcium content in the fruits (895.9 mg/L).

Studies presented in the scientific literature confirm the effectiveness of calcium-containing fertilisers in horticultural production. However, each study has its specific context, limiting the generalisation of results across all cultivars, application methods, and other conditions. This consideration was acknowledged by the authors, who identified this as a critical objective at the outset of their research.

CONCLUSIONS

Foliar application of calcium-based fertilisers to apple orchards significantly affects productivity and fruit quality, with a specific impact depending on the application rate and the source of the active substance. The highest yield for Ligol apples was observed with the application of Brexil Ca at a rate of 1.8 L/ha, reaching 19.3 t/ha, which was 8.3% higher than the control and 12.9% greater than the lowest yield in the study. This treatment also produced the highest fruit mass – 296.5 g – exceeding the control by 8.4% and the lowest recorded value by 15.0%. For Jonagold Early Queen, the highest yield was achieved with the application of HelpRost at a rate of 1.0 L/ha, exceeding the control by 8.6% and the lowest value in the study by 9.2%. The same treatment also resulted in the highest fruit

mass, which was 5.4% greater than the control and 8.6% higher than the lowest recorded value. The highest yield for Fuji apples was achieved with Brexil Ca applied at 1.8 L/ha, producing 24.8 t/ha – 9.8% more than the control. This treatment also resulted in the largest fruit mass of 182.3 g, 11.1% higher than the control.

The firmness of Ligol apple flesh was greatest with the application of Biocalcium at 2.0 mL/ha, showing a 4.9% increase compared to the control. For Jonagold Early Queen and Fuji apples, the greatest flesh firmness was observed with

the application of HelpRost at 1.0 L/ha, showing increases of 13.9% and 3.7% over the control, respectively. Future research will focus on evaluating the effects of calcium-containing nanofertilisers on the organic matter content and mineral composition of apples.

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None.

CONFLICT OF INTEREST

None.

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Вплив позакореневого обробітку насаджень яблуні кальцієвмісними препаратами на урожайність та якість плодів

Анотація. Ефективне виробництво плодів та ягід з високою якістю та урожайністю є важливим для уникнення дефіциту, але виробники часто стикаються з проблемами, пов'язаними з нестачею кальцію в яблуках. Метою проведення досліджень стало вивчення продуктивності насаджень яблуні, сортів пізніх термінів достигання – Лігол, Джонаголд Ерлі Квін, Фуджі, позакоренево оброблених кальцієвмісними препаратами, а також дослідження якості їх плодів. Для реалізації поставленої мети застосовували польові та лабораторні методи досліджень, отримані результати були статистично опрацьовані. У результаті досліджень встановлено, що урожайність сорту Лігол була найвищою (19,3 т/га) у варіанті із застосуванням препарату Vrexit Ca у нормі 1,8 л/га, що більше ніж у контролі на 8,3 % та на 12,9 % ніж у варіанті з обробкою Helprost у нормі 1,0 л/га. Маса плоду була найвищою у сорту Лігол (296,5 г) у варіанті із застосуванням Vrexit Ca (1,8 л/га). Позакоренева обробка насаджень сорту Фуджі препаратом Vrexit Ca у нормі 1,8 л/га виявилася найбільш ефективною, зокрема урожайність становила 24,8 т/га, що на 9,8 % вище ніж у контролі та на 16,1 % ніж у варіанті із Біокальцієм (2,0 мл/га) і препаратом Helprost (1,0 л/га). Найкращу урожайність та найвищу масу плоду сорт Джонаголд Ерлі Квін мав у варіанті із використанням препарату Helprost у нормі 1 л/га – відповідно 16,2 т/га, що на 8,6 % більше ніж у контролі та 249,8 г на 5,4 % вище за контроль. Твердість м'якоті плодів сорту Лігол була найбільша у варіанті з обробкою препаратом Біокальцій (2,0 мл/га), а сортів Джонаголд Ерлі Квін і Фуджі у варіанті, де застосовували Helprost (1,0 л/га). Отримані результати практично дають можливість підвищити ефективність виробництва плодів яблуні, покращити їх якість та подовжити тривалість зберігання

Ключові слова: Лігол; Джонаголд Ерлі Квін; Фуджі; кальцієвмісні препарати; урожайність; маса плоду; твердість м'якоті

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**Genetic variability, combining ability,
and stability analysis studies on traditional okra genotypes
and their hybrids (*Abelmoschus esculentus* (L.) Moench)
using line × tester design**

Abstract. Improving fruit yield in traditional okra varieties is vital for food security, sustainable agriculture, and the conservation of landrace diversity. Evaluating their genetic potential and stability with modern testers is key to achieving higher yields and advancing crop improvement.

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The study aimed to assess the genetic variability, combining ability, gene action, and stability of traditional okra genotypes and their hybrids using a line × tester design to identify high-yielding, stable hybrids with specific environmental adaptability and enhanced fruit yield. A line × tester analysis was conducted during the period 2022–2024 at Annamalai University, Chidambaram (India), using seven lines and three testers to investigate combining ability, gene action, and heterosis in traditional okra genotypes (*Abelmoschus esculentus* (L.) Moench). The parents were selected from a diverse group of 32 traditional landraces. The study evaluated ten economically important traits, including days to 50% flowering, plant architecture, and yield components. Combining ability analysis indicated the predominance of nonadditive gene action for all traits studied. Among the parents, Heirloom White, Anchita Local (lines), and Arka Anamika (tester) exhibited superior general combining ability for multiple traits, including fruit yield. The crosses Green Long Okra × Arka Anamika and Anchita Local × Arka Anamika emerged as superior hybrids, demonstrating significant specific combining ability effects and desirable standard heterosis for fruit yield and its component traits. AMMI analysis confirmed these hybrids' environmental adaptability, with Anchita Local × Arka Anamika showing particular stability across environments. Strong correlations were observed between yield components, notably between fruit dimensions and yield per plant. The high heritability combined with the prevalence of nonadditive gene action suggests that hybrid breeding would be more effective than pure line selection for crop improvement. These findings provide valuable insights for okra breeding programmes aimed at developing high-yielding, stable hybrids with specific adaptation.

Keywords: okra breeding; environmental adaptability; hybrid performance; gene action; AMMI analysis; heterosis

INTRODUCTION

Okra (*Abelmoschus esculentus*), commonly known as lady's fingers, is an annual plant of significant agronomic and nutritional importance. As a mallow family (*Malvaceae*) member, okra is cultivated globally, particularly in tropical and subtropical regions, for its edible pods, which are rich in dietary fibre, vitamins, and antioxidants. It is an allopolyploid species with a chromosome number ranging from $2n=72$ to $2n=130$, a characteristic that underpins its genetic complexity and potential for breeding. Despite its wide cultivation, enhancing okra's productivity, disease resistance, and adaptability to diverse growing conditions remains a key challenge for breeders. The relevance of this research lies in addressing the increasing demand for high-yielding and disease-resistant okra varieties to ensure food security and sustainable agriculture.

Okra production substantially contributes to the income of smallholder farmers and helps ensure nutritional security in these regions (Kshash & Oda, 2022). Developing high-yielding and stable okra varieties faces several challenges, including environmental stressors and

disease pressure, particularly from the yellow vein mosaic virus (Rahman, 2020). A comprehensive study by A. Das *et al.* (2022b) examined okra's population structure and genetic diversity, revealing significant variation between cultivated and wild germplasm. This variation indicates potential for genetic improvement through systematic breeding strategies. Their research, utilising microsatellite markers, identified distinct genetic clusters that could be utilised in heterotic breeding programmes. The value of traditional germplasm in okra improvement has garnered renewed attention. Genetic variability studies by M. Reddy *et al.* (2016) and C. Alake (2020) on okra landraces showed significant diversity in agro-morphological traits and mineral content, highlighting their potential utility in breeding programmes. This research underscored the importance of characterising and preserving traditional varieties for future breeding efforts.

Recent advancements in molecular breeding have enhanced the understanding of disease resistance mechanisms. According to G. Singh *et*

al. (2023), significant progress in identifying SSR markers associated with YVMV resistance using bulk segregant analysis illustrates how resistance genes are inherited from parents to offspring. This breakthrough provides valuable resources for marker-assisted selection in breeding initiatives. Environmental stability is crucial for developing new varieties. S. Sanwal *et al.* (2021) evaluated genotype \times environment interactions for fruit yield across various alkaline environments, emphasising the significance of stability analysis in variety development. Their use of AMMI analysis (Additive Main Effects and Multiplicative Interaction) established a framework for identifying stable, high-performing genotypes across diverse conditions.

Exploiting heterosis in okra has shown promising outcomes for enhancing both yield and quality traits. Research by A. Das *et al.* (2022a) on heterotic potential for antioxidant and nutritional traits revealed significant positive heterosis for quality parameters. These findings suggest the potential to develop superior hybrids with enhanced nutritional value. Recent metabolic profiling research by A. Elshafy *et al.* (2024) further demonstrated the potential for enhancing phytochemical content through hybrid breeding. A comprehensive review by B. Singh *et al.* (2023) stressed the need to integrate traditional breeding methods with modern genomic tools to improve okra. They highlighted the importance of systematically evaluating combining ability and stability in developing superior varieties, especially in the context of changing climatic conditions.

Given these recent advances and ongoing challenges, systematic evaluation of genetic variability, combining ability, and stability in okra improvement programmes remains essential. This line \times tester mating design, combined with stability analysis, offers a robust framework for identifying superior hybrid combinations while accounting for genotype \times environment interactions. This research aimed to assess the genetic variability, combining ability, heterotic potential, and stability of traditional okra genotypes and their hybrids, providing valuable insights for future breeding programmes.

MATERIALS AND METHODS

The present line \times tester study was carried out at the Plant Breeding Farm, Department of Genetics and Plant Breeding, Faculty of Agriculture, Annamalai University, Chidambaram, India, during the period from December 2022 to August 2024. A total of 21 hybrids were developed using a line \times tester mating design, with lines serving as females and testers as males (pollen source). Ten parents were selected from a pool of 32 traditional genotypes. The seeds of mostly selected traditional okra genotypes were obtained from the Rangamalai Seed Bank Community, Karur, India. The list of the 10 selected parents used in the hybridisation programme is provided in Table 1. These parents were evaluated for yield and yield-related component traits alongside the 32 traditional genotypes. The evaluation of F_1 hybrids was conducted using a Randomised Complete Block Design (RCBD) with three replications per location.

Table 1. List of hybrids used in the programme

No.	Lines	Sl. No	Testers
1	L ₁ - Salkeerthi	1	T ₁ - Arka Anamika
2	L ₂ - Red Long Okra	2	T ₂ - Pusa A4
3	L ₃ - Green Round Okra	3	T ₃ - Kashi Chaman
4	L ₄ - Anchita Local	Arka Anamika – standard check	
5	L ₅ - Sri Lankan Candle Okra		
6	L ₆ - Heirloom White		
7	L ₇ - Green Long Okra		

Source: compiled by the authors

A multi-location trial was conducted to evaluate the stability and performance of F_1 hybrids using AMMI stability analysis during 2023 and

2024. Fifty per cent of the F_1 hybrid seeds from 21 crosses, developed through a line \times tester mating design described by O. Kempthorne (1957), were

sown in the multi-location fields along with their parental lines. The trial was conducted across three environments to assess the performance of the 21 hybrids and 10 parents included in this

line \times tester study. The environments were ENV-1 (Malliakarai, Attur, India), ENV2 (Salem, India), and ENV-3 (Chidambaram, India), with further details provided in Table 2.

Table 2. Environment details of experimental sites

Code	Location	Sowing date	Season	Latitude	Longitude	Year
ENV-1	Malliakarai, Attur, India	24.04.2023	Summer	11°34'35.3"N	78°29'29.4"E	2023
ENV-2	Kannankurichi, Salem, India	18.07.2023	Kharif	11.697412°N	78.176074°E	2023
ENV-3	Chidambaram, India	07.03.2024	Summer	11.385494°N	79.722557°E	2024

Source: compiled by the authors

All the parents and hybrids were evaluated, and the observed data were analysed to assess combining ability, heterosis, and stability parameters. Ten quantitative traits were examined from five randomly selected plants of each parent and F_1 generation viz. DFF – days to 50% flowering; NPB – number of primary branches per plant; PHT – plant height at maturity (cm); IL – internode length (cm), FG – fruit girth (cm); FL – fruit length (cm); SFW – single fruit weight (g); NFP – number of fruits per plant; FYP – fruit yield per plant; NSPP – number of seeds per pod. One of the parents, Arka Anamika, was chosen as the standard check variety. The performance of the F_1 hybrids was evaluated for heterosis compared to both the superior parent and the standard check, using the method described by S. Fonseca & F. Patterson (1968). To calculate heterosis, the percentage increase or decrease in the F_1 hybrid's performance was determined relative to the superior parent and the standard check.

AMMI analysis was performed for 31 okra genotypes, comprising G_{1-21} hybrids and G_{21-31} parents. The study of okra yield across different genotypes and environments was conducted using AMMI biplots. Two types of biplots, AMMI1 and AMMI2, provided insights into genotype performance, stability, and adaptability across varying environmental conditions. The AMMI model fit was achieved using two components: the main effect, representing the additive part of the model, was analysed using ordinary analysis of variance (ANOVA), while the non-additive residual (the genotype \times environment interaction, the multiplicative part of the model) was analysed using principal component analysis (PCA). The main effect of a genotype \times environment

combination was calculated by adding the genotype mean to the environment mean and subtracting the grand mean. The interaction effect was determined by multiplying the genotype's PCA scores by the environment's PCA scores.

The experimental research conducted in this study adhered to institutional, national, and international guidelines for plant research. The collection of traditional okra genotypes, including seeds obtained from the Rangamalai Seed Bank Community, Karur, India, was carried out in compliance with the principles outlined in the Convention on Biological Diversity (CBD) (1992) and the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) (1973). No endangered or protected plant species were involved in this study.

RESULTS AND DISCUSSION

Diversity analysis for traditional genotypes – dendrogram

The diverse selection from various clusters indicates that these lines likely exhibit distinct morphological and yield-related traits among the 32 traditional landraces, focusing on selecting those with high fruit yield potential. Such genetic divergence among parents is crucial for a line \times tester breeding programme as it can lead to higher heterosis (hybrid vigour) in the resulting crosses. Based on the phylogenetic tree diagram, the selection of okra lines for the line \times tester breeding programme appears to be strategically chosen to maximise genetic diversity and capture a broad range of desirable yield characteristics. Based on Ward's cluster analysis method, six clusters have been formed using the Euclidean distance (Caliński

8 Harabasz, 1974). The selected lines represent distinct genetic clusters from different branches of the dendrogram, which suggests good genetic divergence among the chosen parents. Specifically, Salkeerthi, Green Long Okra, Kashi Chaman, and Arka Anamika are from the bottom cluster (high-yield clusters), while Red Long Okra comes from a separate middle cluster (long fruit length). Green Round Okra

(short internode and plant height) and Anchita Local represent different intermediate branches. Sri Lankan Candle Okra and Heirloom White are selected from the upper portion of the tree (moderate fruit yield), while Pusa A-4 is distributed across a different cluster. All lines possessed a significant degree of phenotypic variation, with most of them being traditional okra genotypes (Fig. 1).

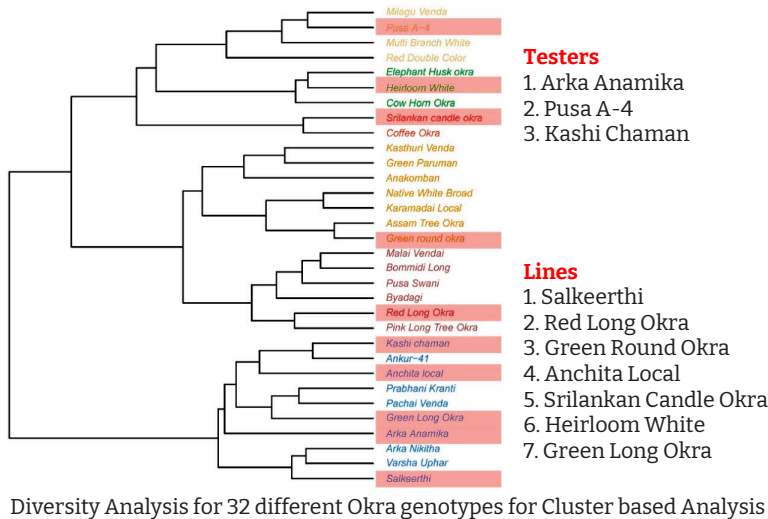


Figure 1. Diversity analysis for 32 traditional genotypes

Source: compiled by the authors

The wide genetic base also increases the probability of obtaining superior recombinants in segregating generations. Additionally, selecting representatives from different clusters helps maintain genetic diversity while pursuing yield improvement objectives in the breeding programme.

Correlation studies on traditional genotypes

The correlation analysis of various traits in okra genotypes reveals significant genotypic and phenotypic relationships that are valuable for breeding. Days to 50% flowering (DFF) has a strong positive genotypic correlation with plant height (PHT), fruit length (FL), and number of fruits per plant (NFPP), suggesting that selecting for early flowering could be associated with increased plant height and fruit size genetically. Phenotypically, DFF exhibits negative correlations with the number of seeds per pod

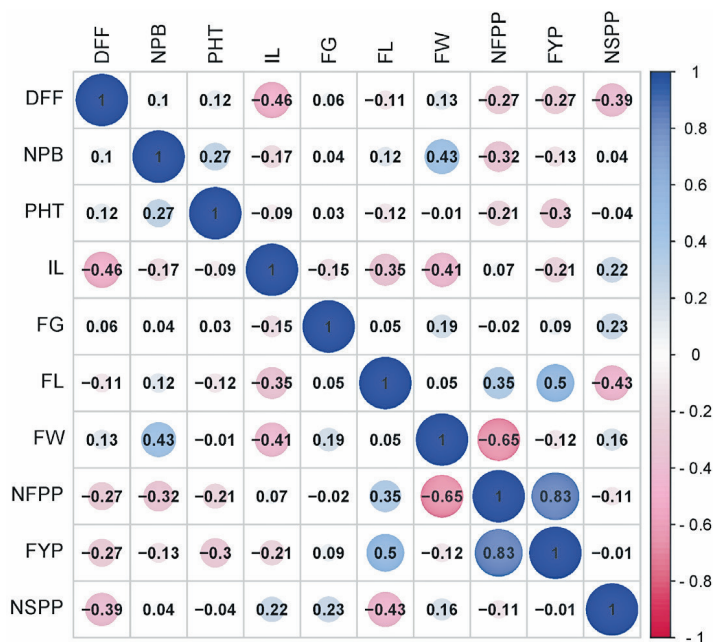
(NSPP), indicating a possible trade-off between early flowering and seed number (Table 3). Similarly, the number of primary branches (NPB) is positively correlated with plant height and fruit length at the genotypic level, indicating that plants with more branches could also grow taller and produce longer fruits. Traits such as plant height (PHT) and internode length (IL) show strong genotypic correlations with fruit length, suggesting that taller plants with longer internodes may have larger fruits. However, phenotypic correlations are weaker, hinting at environmental influences. Positive genotypic correlations between fruit girth (FG), fruit length (FL), and the number of fruits per plant (NFPP) suggest that selecting for larger fruits could also increase fruit yield. Furthermore, fruit width (FW) shows a positive genotypic correlation with the number of fruits per plant, suggesting that wider fruits may accompany higher yields (Fig. 2).

Table 3. Correlation results: Genotypic correlations are presented in the upper diagonal, while phenotypic correlations are shown in the lower diagonal for the 32 traditional genotypes

	DFF	NPB	PHT	IL	FG	FL	FW	NFPP	FYP	NSPP
DFF		0.00074	0.018	-0.43*	0.15	-0.03	0.2	-0.23	-0.16	-0.46*
NPB	0.004		0.331	-0.16	-0.04	0.07	0.35	-0.33	-0.18	0.09
PHT	0.02	0.32*		-0.11	0.032	-0.18	-0.08	-0.22	-0.34	0.09
IL	-0.4**	-0.16	-0.11		-0.13	-0.35	-0.4	0.074	-0.2	0.23
FG	0.13	-0.03	0.031	-0.13		0.12	0.25	-0.01	0.14	0.09
FL	-0.03	0.06	-0.17	-0.34**	0.11		0.09	0.371	0.54*	-0.46*
FW	0.16	0.34**	-0.06	-0.38**	0.23	0.08		-0.61**	-0.05	0.12
NFPP	-0.2	-0.32**	-0.22	0.07	-0.01	0.35**	-0.61**		0.82**	-0.14
FYP	-0.15	-0.17	-0.33**	-0.19	0.14	0.518**	-0.05	0.81**		-0.07
NSPP	-0.43**	0.08	0.09	0.23	0.09	-0.45**	0.11	-0.14	-0.07	

Note: DFF – days to 50% flowering; NPB – number of primary branches per plant; PHT – plant height at maturity (cm); IL – internode length (cm); FG: fruit girth (cm); FL – fruit length (cm); SFW – single fruit weight (g); NFP – number of fruits per plant; FYP – fruit yield per plant; NSPP – number of seeds per pod; * – significant at the 5% level; ** – significant at the 1% level

Source: compiled by the authors

**Figure 2.** Correlation results

Source: compiled by the authors

However, the number of seeds per pod (NSPP) tends to have negative correlations with several traits, indicating a trade-off between seed quantity and fruit size or number.

Variability parameters for 32 traditional genotypes

The analysis of variability parameters for various traits in okra demonstrated substantial

genetic diversity among the 32 traditional genotypes studied (Table 4). The phenotypic expression showed considerable variation, with plant height ranging from 92.60 cm to 144.60 cm, fruit yield per plant varying from 331.90 g to 484.80 g, and the number of seeds per pod ranging from 42.67 to 59.33. High broad-sense heritability estimates (>0.90) were observed for most traits, with values ranging from 0.88 to 0.99, indicating strong genetic control over trait expression and minimal environmental influence on phenotypic manifestation. Days to 50% flowering (DFF) exhibited a relatively high genotypic variance (1.12) and phenotypic variance (1.28), indicating a significant influence of genetic factors on this trait. High heritability (0.88) and moderate genetic advance as a percentage of the mean (4.97%) suggest that selection could effectively improve early flowering in okra. Similarly, the number of primary branches (NPB) exhibited high heritability (0.98) and genetic advance (25.61%), implying strong genetic

control and potential for improvement through selection. Plant height (PHT) displayed high genotypic (142.47) and phenotypic variance (145.36), with very high heritability (0.98), indicating that height is largely genetically determined and can be effectively selected for breeding programmes. Traits such as fruit girth (FG) and fruit length (FL) also demonstrated high heritability (0.98 and 0.96, respectively) with considerable genetic advance as a percentage of the mean (27.31% for FG and 17.28% for FL), suggesting that selection for larger fruit size could yield significant improvements in yield. The yield-related trait, fruit yield per plant (FYP), showed extremely high genotypic and phenotypic variance coupled with high heritability (0.98) and genetic advance (21.90%), highlighting its genetic control and responsiveness to selection. The number of fruits per plant (NFPP) also displayed high genetic variance and heritability, supporting the potential for yield improvement through selection for fruit number.

Table 4. Variability parameters for 32 traditional genotypes

Sl. No.	Variability parameters	DFF	NPB	PHT	IL	FG	FL	FW	NFPP	FYP	NSPP
1	Maximum mean	51.45	3.45	194.60	7.49	6.41	24.74	26.53	27.25	539.80	59.33
2	Minimum mean	42.16	2.17	92.60	4.76	3.56	14.87	15.29	16.52	211.90	42.67
3	Grand mean	41.16	2.82	123.89	6.27	4.75	17.80	18.28	21.97	399.36	49.03
4	Standard error of the mean (SEm)	0.23	0.03	0.98	0.04	0.05	0.17	0.22	0.33	3.81	0.02
5	Critical difference (CD) at 5%	0.65	0.09	2.81	0.10	0.14	0.48	0.63	0.96	10.88	0.01
6	Critical difference (CD) at 1%	0.87	0.12	3.76	0.14	0.19	0.65	0.85	1.28	14.56	0.01
7	Environmental variance	0.16	0.00	2.89	0.01	0.01	0.09	0.15	0.34	43.46	0.01
8	Genotypic variance	1.12	0.13	142.47	0.54	0.40	2.31	1.61	8.58	1,844.54	22.43
9	Phenotypic variance	1.28	0.13	145.36	0.54	0.41	2.40	1.75	8.92	1,888.0	22.43
10	Environmental coefficient of variance	0.96	2.00	1.37	1.01	1.80	1.65	2.10	2.64	1.65	0.01
11	Genotypic coefficient of variance (GCV)	2.57	12.59	9.63	11.68	13.38	8.54	6.93	13.33	10.75	9.66
12	Phenotypic coefficient of variance (PCV)	2.75	12.75	9.73	11.73	13.50	8.70	7.24	13.59	10.88	9.66
13	Heritability (Broad sense)	0.88	0.98	0.98	0.99	0.98	0.96	0.92	0.96	0.98	0.99
14	Genetic advance (GA)	2.04	0.72	24.34	1.50	1.30	3.07	2.50	5.92	87.45	9.76
15	Genetic advance as a percentage of the mean	4.97	25.61	19.65	23.98	27.31	17.28	13.67	26.94	21.90	19.90

Note: DFF – days to 50% flowering; NPB – number of primary branches per plant; PHT – plant height at maturity (cm); IL – internode length (cm); FG: fruit girth (cm); FL – fruit length (cm); SFW – single fruit weight (g); NFP – number of fruits per plant; FYP – fruit yield per plant; NSPP – number of seeds per pod

Source: compiled by the authors

The genotypic coefficient of variation (GCV) demonstrated substantial genetic variability, with the highest magnitude recorded for the number of fruits per plant (13.33%) and fruit girth (13.38%). The relatively small difference between the phenotypic coefficient of variation (PCV) and GCV for most traits further confirmed the limited environmental influence on trait expression. This conclusion is substantiated by the low environmental coefficient of variance and the considerably smaller environmental variance compared to genotypic variance, suggesting that phenotypic expression is predominantly under genetic control. Similar results were

reported by O. AdeOluwa & O. Kehinde (2011). The genetic advance (GA) as a percentage of the mean showed promising values for several traits, notably the number of primary branches (25.61%), fruit girth (27.31%), and the number of fruits per plant (26.94%).

ANOVA for L × T, combining ability analysis, *per se* performance of L × T parents and hybrids

The ANOVA results for parents and hybrids across ten traits, as shown in Table 5, indicated significant differences among lines, testers, and hybrids for all traits. Both lines and testers exhibited significant variance for each of the ten traits.

Table 5. ANOVA for L × T and combining ability analysis in okra

Source of variation	Df	MSS									
		DFF	NPB	PHT	IL	FG	FL	SFW	NFF	NSPP	FYP
Replication	2	0.154	0.055	2.796	0.003	0.013	0.067	0.197	0.459	7.311	9.991
Hybrid	20	3.7**	0.4**	430.3**	1.6**	1.2**	7.4**	5.0**	26.1**	232.3**	5577.1**
Lines	6	6.3**	0.6**	650.8**	2.0**	1.9**	8.1**	6.2**	41.7**	615.4**	6099.7**
Tester	2	9.2**	0.1**	342.1**	0.5**	5.6**	40.5**	2.4**	31.3**	297.6**	12642.1**
L × T	12	1.4**	0.3**	334.7**	1.6**	0.1**	1.6**	4.8**	17.4**	29.8**	4138.3**
Error	60	0.137	0.018	3.009	0.007	0.008	0.061	0.109	0.246	2.956	30.199

Note: DFF – days to 50% flowering; NPB – number of primary branches per plant; PHT – plant height at maturity (cm); IL – internode length (cm); FG – fruit girth (cm); FL – fruit length (cm); SFW – single fruit weight (g); NFP – number of fruits per plant; FYP – fruit yield per plant; NSPP – number of seeds per pod; * – significant at the 5% level; ** – significant at the 1% level

Source: compiled by the authors

The importance of high *per se* performance, according to V. Singh *et al.* (1983), in breeding programmes was emphasised. In this study, parental lines Anchita, Red Long Okra, Salkeerthi, and Green Long Okra, along with testers Kashi Chaman and Arka Anamika, exhibited maximum fruit yield per plant. Anchita showed remarkable performance in traits such as primary branches, plant height, internode length, fruit length, girth, single fruit weight, and fruit count. Green Long Okra also excelled in fruit yield, with notable performance in days to 50% flowering, fruit girth, length, fruit count, and seeds per pod. Salkeerthi and Red Long Okra performed well in both fruit yield and fruit number. Among the testers, Arka Anamika displayed excellent performance in fruit yield and traits like days to 50%

flowering, plant height, fruit length, single fruit weight, and fruit count, while Kashi Chaman demonstrated favourable traits including days to 50% flowering, primary branches, internode length, fruit count, and seeds per pod. These results show the genetic potential of the parents used for hybridisation programmes.

The hybrids like Green Long Okra × Arka Anamika, Anchita Local × Arka Anamika, Heirloom White × Arka Anamika, Heirloom White × Pusa A-4, Heirloom White × Kashi Chaman, and Salkeerthi × Pusa A-4 demonstrated higher fruit yield per plant. Anchita Local × Arka Anamika showed high yield along with favourable traits such as days to 50% flowering, primary branches, internode length, fruit length, girth, single fruit weight, and fruit count. Green Long Okra ×

Arka Anamika also excelled in fruit yield, days to 50% flowering, internode length, fruit length, seeds per pod, and fruit count. Salkeerthi × Pusa A-4 exhibited desirable traits like primary branches, internode length, fruit girth, and single fruit weight. Overall, Anchita Local × Arka Anamika and Green Long Okra × Arka Anamika hybrids demonstrated superior performance in fruit yield and most traits under study, while Heirloom White × Arka Anamika and Salkeerthi

× Pusa A-4 also performed favourably across several traits.

General combining ability effect of parents

The parent lines Heirloom White, Salkeerthi, and the tester Arka Anamika showed strong positive effects for general combining ability (GCA) regarding fruit yield per plant (Table 6). This suggests that they could be valuable in breeding new varieties aimed at increasing yield.

Table 6. GCA effect of parents

L/T	Parental Genotypes	DFF	NYB	PHT	IL	FG	FL	FW	NFF	NSPP	FYP
L1	Salkeerthi	0.94**	0.06**	3.60**	-0.22**	0.29**	0.14	-0.41**	1.65**	7.64**	19.52**
L2	Red Long Okra	-0.67**	0.01	3.27**	0.77**	-0.29**	-0.50**	-0.89**	1.72**	5.9**	9.41**
L3	Green Round Okra	0.40**	-0.31**	-14.28**	0.59**	-0.23**	-1.15**	0.24	-0.57**	8.95**	-3.61
L4	Anchita Local	0.08	0.29**	-3.43**	-0.20**	-0.20**	1.61**	1.38**	-2.15**	-11.73**	-8.74**
L5	Srilankan Candle Okra	0.95**	0.02	7.82**	-0.30**	0.83**	-0.92**	0.73**	-3.44**	1.13*	-47.73**
L6	Heirloom White	-0.45**	-0.38**	-6.51**	-0.45**	0.17**	0.40**	-0.30*	2.23**	-2.25**	34.87**
L7	Green Long Okra	-1.26**	0.32**	9.53**	-0.19**	-0.56**	0.41**	-0.76**	0.56**	-9.64**	-3.71
T1	Arka Anamika	-0.33**	0.001	-4.15**	0.11**	0.53**	1.35**	0.13	0.95**	1.17**	28.17**
T2	Pusa A-4	0.76**	-0.08**	0.25	-0.19**	-0.50**	0.08	-0.38**	-0.74**	3.04**	-16.71**
T3	Kashi Chaman	-0.43**	0.08**	3.91**	0.07**	-0.03	-1.43**	0.26**	-0.21	-4.21**	-11.46**

Note: DFF – days to 50% flowering; NPB – number of primary branches per plant; PHT – plant height at maturity (cm); IL – internode length (cm); FG – fruit girth (cm); FL – fruit length (cm); SFW – single fruit weight (g); NFP – number of fruits per plant; FYP – fruit yield per plant; NSPP – number of seeds per pod; * – significant at the 5% level; ** – significant at the 1% level

Source: compiled by the authors

Heirloom White also showed positive GCA for multiple traits, including days to 50% flowering, plant height, internode length, fruit length, number of fruits, and seeds per pod. Green Long Okra demonstrated desirable GCA effects for six traits, including days to 50% flowering, primary branches, fruit girth, and fruit length. Anchita Local ranked second in positive GCA for traits like primary branches, plant height, and fruit length but had negative GCA for fruit yield per plant. Among testers, Arka Anamika was portrayed as a good general combiner for most traits, while Kashi Chaman showed desirable GCA effects for flowering, primary branches, and single fruit weight but was negative for fruit

yield. Based on the GCA scores, Arka Anamika and Pusa A-4 were average combiners, while Kashi Chaman was a poor combiner.

Specific combining ability effect of hybrids

Specific combining ability (SCA) represents the deviation from expected performance based on GCA and is influenced by non-additive gene action (Sprague & Tatum, 1942). Significant positive SCA effects for fruit yield per plant were perceived in cross combinations such as Anchita Local × Arka Anamika, Green Long Okra × Arka Anamika, Heirloom White × Pusa A-4, and others, making them ideal candidates for heterosis breeding to enhance fruit yield. Each of these

crosses included at least one strong parent contributing desirable traits.

The hybrid Anchita Local × Arka Anamika ranked as the top performer, showing strong SCA effects for traits such as fruit length, primary branches, and single fruit weight, despite negative SCA effects for some traits such as days to 50% flowering and plant height. Heirloom White × Pusa A-4 also performed well, with superior SCA effects for traits such as days to 50% flowering, internode length, fruit length, and fruit yield. Green Round Okra × Kashi Chaman and other hybrids, including Sri Lankan Candle Okra × Kashi Chaman, exhibited desirable

SCA effects for traits such as fruit girth, fruit number, and yield. Based on SCA scores, hybrids such as Heirloom White × Pusa A-4, Green Round Okra × Kashi Chaman, Salkeerthi × Kashi Chaman, and Anchita Local × Arka Anamika showed the highest SCA effects, indicating their strong potential as specific combiners. Similar results were reported by V. Bendalel *et al.* (2004) and A. Shwetha *et al.* (2018). Overall, the results highlight the superior performance of Anchita Local and Arka Anamika in various hybrid combinations, contributing to enhanced fruit yield and trait improvement across multiple hybrids (Table 7).

Table 7. SCA effect of hybrids

Hybrids	DFF	NYB	PHT	IL	FG	FL	FW	NFF	NSPP	FYP
L ₁ ×T ₁	0.13	0.10**	-1.24	1.00**	0.01	-0.57**	-2.23**	1.73**	2.60**	-25.77**
L ₁ ×T ₂	0.35*	0.28**	5.40**	-0.32**	-0.04	-0.01	1.96**	-1.55**	2.39*	20.36**
L ₁ ×T ₃	-0.48**	-0.38**	-4.16**	-0.67**	0.03	0.58**	0.27	-0.18	-4.99**	5.4
L ₂ ×T ₁	-0.08	0.27**	-7.77**	-0.24**	0.10*	-0.51**	1.05**	-2.59**	3.40**	-19.78**
L ₂ ×T ₂	0.75**	-0.22**	-3.57**	0.53**	-0.20**	-0.08	-1.66**	3.67**	-1.9	22.57**
L ₂ ×T ₃	-0.67**	-0.04	11.34**	-0.29**	0.1	0.59**	0.61**	-1.08**	-1.5	-2.79
L ₃ ×T ₁	-0.01	-0.17**	5.43**	-0.04	-0.02	0.3	-0.08	-1.18**	0.48	-25.33**
L ₃ ×T ₂	-0.64**	-0.20**	13.92**	0.16**	0.13*	-0.1	-0.50*	-0.04	2.01*	-8.60*
L ₃ ×T ₃	0.65**	0.37**	-19.35**	-0.12**	-0.10*	-0.2	0.57*	1.22**	-2.49*	33.93**
L ₄ ×T ₁	-0.97**	0.12**	-3.24**	-1.33**	-0.07	1.36**	0.62**	2.27**	-2.27*	59.65**
L ₄ ×T ₂	0.42*	-0.35**	-12.23**	0.58**	-0.10*	-0.32*	-0.57*	-0.54	-1.63	-19.80**
L ₄ ×T ₃	0.55**	0.23**	15.47**	0.75**	0.17**	-1.04**	0.06	-1.73**	3.90**	-39.86**
L ₅ ×T ₁	0.2	-0.08*	0.08	0.24**	0.22**	-0.56**	-0.58*	-0.90**	-3.12**	-27.27**
L ₅ ×T ₂	0.29	0.40**	3.34**	-0.77**	0.03	-0.22	1.25**	1.48**	1.09	-3.02
L ₅ ×T ₃	-0.48**	-0.32**	-3.42**	0.52**	-0.25**	0.78**	-0.67**	2.39**	2.03*	30.29**
L ₆ ×T ₁	0.86**	-0.04	1.44	0.63**	-0.40**	-0.31	0.92**	-2.24**	0.47	-18.49**
L ₆ ×T ₂	-0.79**	-0.12**	-0.72	-0.40**	0.23**	0.39*	-0.91**	2.07**	-2.69**	15.19**
L ₆ ×T ₃	-0.07	0.17**	-0.72	-0.23**	0.17**	-0.08	-0.01	0.17	2.22*	3.3
L ₇ ×T ₁	-0.13	-0.20**	5.30**	-0.26**	0.16**	0.29	0.29	2.91**	-1.57	56.99**
L ₇ ×T ₂	-0.38*	0.21**	-6.13**	0.22**	-0.04	0.33*	0.42	-2.12**	0.74	-26.71**
L ₇ ×T ₃	0.50**	-0.01	0.84	0.03	-0.12*	-0.62**	-0.71	-0.79*	0.82	-30.28**

Note: DFF – days to 50% flowering; NPB – number of primary branches per plant; PHT – plant height at maturity (cm); IL – internode length (cm); FG – fruit girth (cm); FL – fruit length (cm); SFW – single fruit weight (g); NFP – number of fruits per plant; FYP – fruit yield per plant; NSPP – number of seeds per pod; * – significant at the 5% level; ** – significant at the 1% level

Source: compiled by the authors

The analysis of variance components for combining ability demonstrated that SCA variance exceeded GCA variance across all evaluated traits (Table 8).

Table 8. Estimation of combining ability variance

VARIANCE	DFP	NYB	PHT	IL	FG	FL	FW	NFF	NSPP	FYP
GCA variance	0.06	0.002	2.48	0.05	0.03	0.15	0.00	0.22	37.46	5.27
SCA variance	0.45	0.09	110.6	0.53	0.04	0.49	1.55	5.69	1,364.93	9.03
GCA/SCA variance	0.13	0.02	0.02	0.094	0.67	0.31	0.00	0.04	0.03	0.58

Note: DFP – days to 50% flowering; NPB – number of primary branches per plant; PHT – plant height at maturity (cm); IL – internode length (cm); FG – fruit girth (cm); FL – fruit length (cm); SFW – single fruit weight (g); NFP – number of fruits per plant; FYP – fruit yield per plant; NSPP – number of seeds per pod

Source: compiled by the authors

This observation suggests that non-additive gene action plays a significant role in the inheritance of these traits, highlighting the complexity and adaptive potential of the genetic mechanisms involved.

Estimation of heterosis concerning fruit yield

The top five hybrids based on heterobeltiosis for fruit yield per plant were Heirloom White × Pusa A4 (48.31%), Green Long Okra × Arka Anamika (22.32%), Heirloom White × Arka Anamika (19.11%), Sri Lankan Candle Okra × Pusa A-4 (9.57%), and Green Round Okra × Arka Anamika (6.94%) (Fig. 3). These hybrids also exhibited desirable heterobeltiosis for traits such as days to 50% flowering, plant height, internode length, fruit length, and fruit number. For standard heterosis, the top five hybrids were Green Long Okra × Arka Anamika (29.00%), Anchita Local × Arka Anamika (28.37%), Heirloom White × Arka Anamika (19.11%), Heirloom White × Pusa A-4 (16.1%), and Heirloom White × Kashi Chaman (14.32%). These crosses also recorded significant standard heterosis for traits such as fruit number, primary branches, internode length, and seed number per pod. Hybrids like Green Long Okra × Arka Anamika, Heirloom White × Pusa A-4, and Heirloom White × Arka Anamika showed high heterosis for both heterobeltiosis and standard heterosis, making them promising candidates for fruit yield improvement. According to S. Rynjah *et al.* (2020), the F_1 hybrids with high heterosis for fruit yield also exhibited favourable heterosis in other traits, supporting their use for okra yield enhancement.

The findings have significant implications for okra breeding programmes. The combination of high heritability and substantial genetic advance indicates the effectiveness of selection breeding, while the predominance of non-additive gene action supports the adoption of hybrid breeding approaches. The minimal environmental effects suggest stable expression of traits across environments, which is favourable for breeding programmes. The SCA variances were higher than the GCA variance for all ten characters, *viz.* days to 50% flowering, plant height at maturity, number of primary branches per plant, internode length, fruit length, fruit girth, single fruit weight, number of fruits per plant and fruit yield per plant. The ratio of GCA/SCA was less than unity, indicating the pre-dominance of nonadditive gene action. For traits such as plant height at maturity and fruit yield per plant, high SCA variance suggests the influence of non-additive gene effects or dominant gene action, indicating that hybridisation could be effective in improving these traits. Selecting hybrids for heterosis breeding based on *per se* performance, specific combining ability (SCA) effects and standard heterosis is highly effective. Among the 21 hybrids evaluated, Anchita Local × Arka Anamika and Heirloom White × Pusa A-4 emerged as superior hybrids based on all three criteria. Anchita Local × Arka Anamika excelled across nine out of ten traits, making it the top-performing hybrid. Heirloom White × Pusa A4 also demonstrated desirable performance for most traits, making it a strong candidate for hybrid breeding.



Figure 3. Relative, heterobeltiosis, and standard heterosis in per cent (%)

Source: compiled by the authors

Additionally, the hybrids Green Long Okra × Arka Anamika and Salkeerthi × Pusa A-4 showed notable fruit yield per plant. The strong correlation observed between *per se* performance, SCA effects, and standard heterosis suggests that SCA effects can serve as a useful biometrical marker for hybrid breeding in okra. This study highlights the significance of non-additive gene action for fruit yield and its related traits, indicating that developing hybrid varieties is the best approach to capitalise on the genetic gain in F_1 generations. While recurrent selection, as proposed by C. Andrus (1963), can produce high-performing pure lines and hybrids, it is time-consuming. Combining pedigree breeding with recurrent selection may offer a more efficient strategy for developing high-yielding segregants and maximising hybrid potential.

Diallel selective mating design, according to N. Jensen (1970), can also be adopted to encourage recombination. The hybrid Heirloom White × Arka Anamika showed high combining ability with positive, non-significant SCA effects for plant height and negative, non-significant values for fruit length. Similarly, the hybrid Green Long Okra × Kashi Chaman had non-significant SCA effects for seeds per pod and negative values for primary branches. These crosses suggest additive genes contributing to traits such as plant height and earliness in later generations. Green Long Okra × Arka Anamika and Heirloom White × Arka Anamika were selected for recombination breeding due to their superior fruit yield and component traits. Therefore, crosses involving Arka Anamika, Heirloom white, and Green long okra are expected to yield superior segregants for fruit yield improvement.

The results of AMMI analysis of hybrids

The AMMI1 biplot shows yield (YLD) on the x-axis and the first principal component (PC1) on the y-axis. This visualisation facilitates the assessment of the yield performance and stability of 31 different okra genotypes across multiple environments (ENV-1, ENV-2, and ENV-3). Genotypes positioned further along the x-axis towards higher yield values indicate superior performance in terms of yield (Patel *et al.*, 2023). For example, hybrids Red Long Okra × Arka Anamika, Anchita Local × Arka Anamika, and the tester Arka

Anamika exhibit relatively high yields, making them potential candidates for yield-focused breeding programmes. Stability, in this context, refers to a genotype's consistency across different environmental conditions. Genotypes located near the origin, such as Heirloom White × Pusa A-4, display low interaction effects, indicating stable performance across environments. In contrast, genotypes farther from the origin, such as Red Long Okra and Heirloom White × Kashi Chaman, exhibit higher interaction effects, suggesting variability in yield depending on the environment. Thus, while Heirloom White × Pusa A-4 is stable across conditions, Red Long Okra and Heirloom White × Kashi Chaman may be better suited to specific environments rather than being broadly adapted. The AMMI2 biplot displays genotype × environment interactions by plotting the first principal component (PC1) on the x-axis and the second principal component (PC2) on the y-axis. In this biplot, the proximity of genotypes to specific environments reveals patterns of adaptability. For instance, Red Long Okra aligned closely with ENV-2, indicating a particular suitability for that environment, while the hybrid Heirloom White × Kashi Chaman showed a similar alignment with ENV-3. Stability and specific adaptation were also apparent in this biplot. Genotypes positioned near the origin, such as Anchita Local × Arka Anamika and Heirloom White × Pusa A-4, showed low interaction effects, indicating stable performance across environments. Similar genotype × environment interaction techniques were reported by C. Alake & O. Ariyo (2012) (Fig. 4). In contrast, genotypes positioned farther from the origin, such as Heirloom White × Kashi Chaman and Red Long Okra, demonstrated specific adaptability to certain environments (ENV-3 and ENV-2, respectively).

Environmental influence is represented by the direction and length of the arrows from the origin toward each environment (ENV-1, ENV-2, and ENV-3). Longer arrows, such as the one for ENV-2, indicate a stronger interaction effect on the genotypes aligned along that vector, emphasising the environment's impact on yield performance in that direction. ENV-1 exhibits greater stability as it is positioned closer to the origin in both the AMMI biplot for yield and the AMMI1 biplot. This proximity indicates minimal

interaction effects with genotypes, making ENV-1 a reliable environment for consistent performance across a wide range of genotypes. In contrast, ENV-2 and ENV-3 are located farther from the origin, reflecting stronger interaction effects. These environments are more suitable for specific adaptation, as they may favour certain genotypes and potentially lead to higher yield performance. In terms of variance explained, the contributions of PC1 (73.6%) and PC2 (26.4%) account for the full variance of the genotype ×

environment interactions in the AMMI2 biplot (16). Genotypes near the origin, such as Heirloom White × Pusa A-4 and Anchita Local × Arka Anamika, emerge as ideal candidates due to their stability across environments. Meanwhile, high-yielding genotypes like Red Long Okra × Arka Anamika and Arka Anamika show promise for breeding programmes aimed at yield improvement and multi-location trials to confirm broad adaptability and yield consistency across environments.

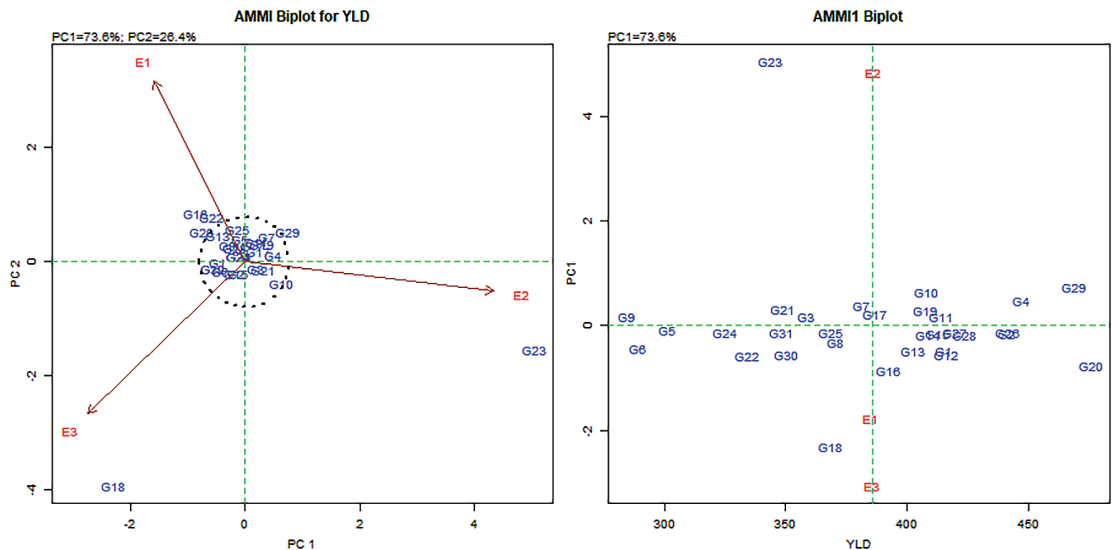


Figure 4. AMMI analysis biplot

Source: compiled by the authors

The study demonstrated that improving traits and fruit yield per plant through standard pedigree selection may be challenging. Correlation analysis revealed that days to 50% flowering (DFF) is negatively correlated with internode length (IL) and number of seeds per pod (NSPP), suggesting that early-flowering plants may have shorter internodes and fewer seeds. The number of primary branches (NPB) is positively correlated with plant height (PHT) and fruit width (FW), while fruit girth (FG) and fruit length (FL) are positively correlated with the number of fruits per plant (NFPP) and fruit yield per plant (FYP). However, wider fruits were negatively correlated with NFPP, indicating potential tradeoffs. The genetic parameters indicated that most traits are under genetic control, with high

heritability (0.88-0.99) and narrow gaps between genotypic and phenotypic variances. Days to 50% flowering exhibited high heritability (0.88) and moderate genetic advance (4.97%), making it a promising trait for developing early-maturing varieties (Reddy *et al.*, 2012; Patel *et al.*, 2023). Traits such as plant height and primary branches demonstrated high heritability (0.98) and genetic advance (19.65% and 25.61%, respectively), highlighting additive gene action and their amenability to selection. Fruit-related traits, including fruit girth and the number of fruits per plant, displayed high genetic variability and heritability, supporting their effectiveness for selection, consistent with findings from P. Sharma *et al.* (2016). Fruit yield per plant showed high heritability (0.98) and genetic

advance (21.90%), confirming the potential for yield improvement via selection (Basir, 2020). Stable genetic expression across traits supports reliable phenotypic selection, although multi-location trials remain necessary to confirm superior genotypes (Hill & Mulder, 2010). The predominance of specific combining ability (SCA) over general combining ability (GCA) for most traits underscores the significance of non-additive gene action, suggesting hybrid breeding is more effective than pure-line selection for crop improvement, as mentioned by V. Verma *et al.* (2018) and M. Srivastava *et al.* (2023).

Green Long Okra demonstrated desirable GCA effects for traits like primary branches and fruit girth, while Anchita Local showed positive GCA effects for plant height and primary branches but was less favourable for yield. Among testers, Arka Anamika emerged as the best general combiner for multiple traits, while Kashi Chaman showed favourable GCA effects for flowering and fruit weight but not for yield. For SCA, high-performing hybrids such as Anchita Local \times Arka Anamika and Heirloom White \times Pusa A-4 displayed strong potential, particularly for fruit yield and key traits like fruit length and primary branches. These crosses benefited from at least one high-GCA parent, underscoring their suitability for yield-oriented breeding. Top hybrids for heterosis included Heirloom White \times Pusa A-4 (48.31%) and Green Long Okra \times Arka Anamika (22.32%), which showed desirable performance in days to 50% flowering, plant height, and fruit number. Overall, hybrids like Green Long Okra \times Arka Anamika and Anchita Local \times Arka Anamika displayed promising heterosis. The hybrid Heirloom White \times Arka Anamika exhibited high combining ability with positive, non-significant SCA effects for plant height at maturity and negative, non-significant values for fruit length. The hybrid Green Long Okra \times Kashi Chaman showed high combining ability with non-significant SCA effects for the number of seeds per pod and negative, non-significant values for the number of primary branches. This indicated that these crosses possessed additive genes from the parents which are likely to produce desirable recombinants for traits like plant height, internode length, and earliness in later segregating generations. The hybrids selected

for recombination breeding were Green Long Okra \times Arka Anamika and Heirloom White \times Arka Anamika, as they excelled in fruit yield per plant and most of their component traits. Therefore, the crosses involving the parents *viz.* Arka Anamika, Heirloom White, and Green Long Okra were also expected to throw superior segregants for pedigree breeding with favourable genes for improving fruit yield and its component traits.

The AMMI analysis identified hybrids such as Anchita Local \times Arka Anamika, Green Long Okra \times Arka Anamika, and Heirloom White \times Pusa A-4 as high-yielding genotypes with strong adaptability to specific environments. This aligns with the combining ability analysis, which highlighted these hybrids for their superior specific combining ability (SCA) and high heterosis for fruit yield per plant and related traits. The dominance of SCA variance over general combining ability (GCA) variance, as observed in traits like fruit yield, days to flowering, and fruit size, supports the AMMI results. This indicates that non-additive gene action plays a major role in determining these traits. This non-additive genetic control aligns with the variability observed across environments. This captured by AMMI biplots, where specific genotypes excelled in particular environments, suggests strong genotype \times environment interactions and highlights the need for multi-location trials to confirm broad adaptability and yield consistency across environments.

In both analyses, the hybrids Anchita Local \times Arka Anamika and Green Long Okra \times Arka Anamika emerged as high performers for key traits, including fruit yield per plant, fruit length, and reduced days to flowering. In the AMMI biplots, these hybrids displayed high yield and specific adaptability to certain environments, while in the combining ability analysis, they demonstrated significant SCA effects and heterosis. These findings indicate that these hybrids are not only highyielding but also capable of thriving under specific environmental conditions, which is beneficial for region-specific cultivation. The AMMI analysis highlighted Anchita Local \times Arka Anamika as stable across different environments, which correlates well with its high SCA effects and superior performance across multiple traits in the combining ability study,

positioning it as an ideal candidate for stable yield improvement (Komolafe *et al.*, 2022). Instead, fruit yield improvement may be achieved by delaying selection to later generations and using intermating of segregants followed by recurrent selection. Okra breeders have the choice between lines and hybrids. Single crosses can be justified at the beginning, while lines can be prioritised later if all heterosis is fixable.

CONCLUSIONS

The authors concluded that the high heritability and genetic variance observed in these traits indicated the potential for effective yield improvement through selective breeding. The parental genotypes, such as Anchita Local, Green Long Okra, and Arka Anamika, were identified as superior combiners for key traits like fruit yield and plant height in the combining ability analysis. The AMMI biplots further highlighted the adaptability and stability of these genotypes, particularly in specific hybrid combinations, across different environments. Heirloom White stood out for its high general combining ability (GCA) effects on yield-related traits, signifying its potential to enhance overall yield in hybrids. This high GCA effect aligned with its performance in the AMMI analysis, where hybrids like Heirloom White × Pusa A-4 demonstrated strong adaptability and high yield potential. The integration of AMMI results with combining ability analysis confirmed that most hybrids were stable across all three environments, exhibited high specific combining ability (SCA), and adapted well to diverse climatic conditions, minimising environmental influences on quality. Hybrids such as Anchita Local × Arka Anamika and Green Long Okra × Arka Anamika displayed both strong

genetic potential and environmental adaptability, making them promising candidates for breeding programmes aimed at improving yield stability and performance.

In the near future, okra breeding should focus on integrating molecular techniques with traditional breeding approaches to enhance selection accuracy and efficiency. Using genomic selection, marker-assisted breeding, and transcriptomics can help understand the genetic factors behind important traits such as fruit yield, earliness, and adaptability to different environments. Multi-location trials are also important to study how different genotypes interact with their environments, helping identify hybrids that can adapt broadly as well as specifically. Exploring underutilised genetic resources and the wild relatives of okra can provide new genes for trait improvement. Breeding programmes should aim to develop hybrids and lines that can withstand climate challenges and perform well under stress, ensuring sustainable production and resilience against pests and environmental stressors. Integrating biochemical trait analysis into breeding programmes can reveal new genetic variations and improve the selection process for superior genotypes, aligning future breeding efforts with market demands.

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

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Генетична варіативність, комбінаційна здатність та аналіз стабільності традиційних генотипів бамії та їх гібридів (*Abelmoschus esculentus* (L.) Moench) із використанням схеми «лінія × тестер»

Анотація. Покращення врожайності традиційних сортів бамії має важливе значення для забезпечення продовольчої безпеки, сталого сільського господарства та збереження генетичного різноманіття сортів. Оцінка їх генетичного потенціалу та стабільності за допомогою сучасних тестерів є ключовим фактором для досягнення вищої врожайності та вдосконалення культури. Дослідження мало на меті оцінити генетичну варіативність, комбінаційну здатність, дію генів та стабільність традиційних генотипів бамії та їх гібридів за допомогою схеми «лінія × тестер» для визначення високоврожайних, стабільних гібридів зі специфічною адаптацією до умов середовища. Аналіз за схемою «лінія × тестер» було проведено у 2022-2024 роках в Університеті Аннамалай, Чідамбарам (Індія), із використанням семи ліній і трьох тестерів для вивчення комбінаційної здатності, генетичних дій та гетерозису у традиційних генотипах бамії (*Abelmoschus esculentus* (L.) Moench). Батьківські лінії були обрані з групи 32 місцевих сортів. У дослідженні оцінювали 10 економічно важливих ознак, включаючи кількість днів до 50 % цвітіння, архітектуру рослини та компоненти врожайності. Аналіз комбінаційної здатності показав перевагу неадитивної дії генів для всіх досліджуваних

ознак. Серед батьківських рослин Heirloom White, Anchita Local (лінії) та Arka Anamika (тестер) продемонстрували вищу загальну комбінаційну здатність за кількома ознаками, включаючи врожайність плодів. Гібриди Green Long Okra × Arka Anamika та Anchita Local × Arka Anamika виявилися кращими, демонструючи значні ефекти специфічної комбінаційної здатності та бажаний стандартний гетерозис за врожайністю та її складовими ознаками. Аналіз АММІ підтвердив екологічну адаптивність цих гібридів, при цьому Anchita Local × Arka Anamika відзначився особливою стабільністю в різних умовах. Висока спадковість у поєднанні з перевагою неадитивної дії генів свідчить про те, що гібридизація є ефективнішим підходом для вдосконалення культури, ніж селекція чистих ліній. Отримані результати дають цінну інформацію для реалізації програм із селекціонування бамії, спрямованих на створення високоврожайних, стабільних гібридів зі специфічною адаптацією

Ключові слова: селекція бамії; адаптивність до навколишнього середовища; продуктивність гібридів; дія генів; аналіз АММІ; гетерозис

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Effect of a growth regulator on the salt resistance of soybean Zheng 196 at the seeding stage

Abstract. Soil salinity is an increasingly pressing issue for global agriculture. Of the 230 million hectares of irrigated farmland worldwide, 20% is affected by salinity, and this percentage continues to rise annually due to improper irrigation practices. Consequently, advancing research into salt tolerance presents an appealing and cost-effective solution for addressing this challenge. The main goal of this study was to examine the effectiveness of a growth regulator in increasing salt

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tolerance in soybeans at the seedling stage. A hydroponic experiment on soybeans was conducted at the Henan Institute of Science and Technology, Xinxiang, China. The seedlings were grown in an artificial climate chamber. Superoxide dismutase activity was measured using a reference method involving nitrotetrazolium blue chloride, while ascorbate peroxidase activity was measured using a standard assay. The use of growth regulators has been shown to mitigate the damage caused by salt stress in plants. In this study, the ability of the growth regulator to alleviate salt stress during the seedling stage of the soybean variety Zheng 196 was evaluated through analysis and measurement of antioxidant enzymes and malondialdehyde. This process provided indirect verification of its role in enhancing the salt resistance of soybean seedlings. The activities of ascorbate peroxidase and catalase were found to increase significantly at a salt concentration of 100 mmol/L, reaching 30% and 35.96%, respectively, while malondialdehyde levels were significantly reduced by 33% under the same conditions. These results offer valuable insights for the development of new growth regulators and scientifically support their feasibility in addressing the current trend of increasing soil salinity

Keywords: *Glycine max* (L.); amino regulator; salt stress; enzyme activity; seedling leaves

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is the world's most important oil crop and a high-protein grainfeeding crop. The soybean industry has economic, and political attributes, making it and is of great importance to a country's food security, economic growth, and social stability. This strategic significance is highlighted in the study of V. Borovyk *et al.* (2024). Soybean is a leguminous plant native to East Asia, specifically China and is now widely cultivated across the globe, as indicated by S. Kalenska *et al.* (2022).

P. Jia *et al.* (2021) stated that soil salinisation and drought stress are significant abiotic factors affecting crop yield. According to T. Eryiğit *et al.* (2022) and K. Saravanan *et al.* (2023), environmental degradation and unsustainable cultivation practices are exacerbating soil salinisation, which in turn affects the quality and yield of soybeans. There is a dynamic equilibrium between the production and elimination of reactive oxygen species generated by plants during life processes. Salt stress disrupts this equilibrium, as noted by M. Osman *et al.* (2021). The accumulation of reactive oxygen species, such as superoxide anions and hydrogen peroxide, induces membrane lipid peroxidation, increasing membrane permeability, damaging the membrane system, and causing severe harm to plants. The use of growth regulators and hormones can improve plant salt resistance, mitigate salt stress, and promote plant growth, as highlighted by S. Butenko *et al.* (2022).

According to I. Didur (2023), one of the most effective measures to increase soybean grain production while reducing anthropogenic pressure on ecosystems and conserving energy resources – especially given the exceptionally high cost of mineral fertilisers – is the optimal use of biological intensification factors. These include exploiting symbiotic potential as a cost-effective natural nitrogen source. In recent years, there has been growing scientific and practical interest in the use of biological fertilisers and foliar treatments with various mechanisms of action. These treatments enhance plants' ability to utilise available resources, thereby improving productivity. Thus, it is highly relevant to study not only the efficiency of seed inoculation but also the creation of optimal growth and development conditions for plants by combining inoculation with foliar feeding. This combined approach amplifies the effects of bioactive substances, enhances photosynthetic activity and symbiotic productivity, and ultimately contributes to higher yields.

“Amino VG-Antistress” is a liquid fertiliser containing a high concentration of free amino acids (of vegetable origin) that are quickly absorbed by plant tissues. Amino acids play an important role in vegetative growth and the accumulation of nutrients for protein synthesis. The special composition of this growth regulator promotes balanced growth and accelerates plant recovery from chemical and mechanical damage.

The presence of the anti-stress agent glycine betaine helps plants overcome abiotic stresses and improves metabolic processes within the plant. Amino acids, as the fundamental components of biological functional macromolecular proteins and important plant metabolites, play a key role in nitrogen metabolism and stress resistance (Didora *et al.*, 2019). Glycine betaine can mitigate the inhibitory effects of stress on plants and enhance their stress tolerance. Studies conducted by H. Deng *et al.* (2024) have shown that betaine can regulate the osmotic pressure of plant cells, remove reactive oxygen species, and maintain the stability of biological membranes. Research has also demonstrated that betaine significantly enhances the drought and salt tolerance of plants by stabilising the structure and activity of key enzymes under stress conditions. Furthermore, it improves photosynthetic efficiency and growth by protecting the photosystem and ensuring optimal water balance within plant tissues.

The free amino acids and betaine contained in the investigated regulator are known to have a significant impact on the salt resistance of soybeans. However, there are limited reports on the effects of this regulator on the salt resistance of soybean seedlings. In this study, the growth regulator was applied to soybean seedlings exposed to different levels of salinity stress. The experimental investigation focused on the effects of the regulator on antioxidant enzyme activity in soybean seedlings, revealing its impact on salt resistance. The study aimed to identify changes in the enzymatic activity of soybean seedlings treated with the growth regulator under varying salinity conditions. Demonstrating the influence of the regulator on enzymatic activity provides a scientific basis for increasing soybean resistance to salinity.

MATERIALS AND METHODS

The objective of the research was to evaluate changes in the enzymatic activity of soybean seedlings treated with a growth regulator under varying levels of environmental salinity. The focus of the study included *Glycine max* L. (variety Zheng 196), a plant growth regulator ("Amino VG-Antistress"), abiotic stress (salinity), and enzyme activity (superoxide dismutase, peroxidase, catalase, ascorbate peroxidase). The experiment was conducted in Xinxiang, China,

from March to May 2023. "Amino VG-Antistress" was provided by the Greenhas Group, Italy, while the soybean variety Zheng 196 was obtained from the Henan Academy of Agricultural Sciences, China. The research was conducted in an artificial climate chamber at the Henan Institute of Science and Technology, Xinxiang, China.

Soybean seeds were grown in 7×7 cm nutrient pots filled with vermiculite. The growth conditions were set as follows: 16 hours of light with an intensity of 8,000 lumens, and day/night temperatures of 25°C/18°C. Once the seedlings developed two leaves and one cotyledon, they were subjected to stress treatment by irrigating them with sodium chloride (NaCl) solutions at concentrations of 50 mmol/L, 75 mmol/L, and 100 mmol·L⁻¹. The control group (CK) was irrigated with distilled water, while the experimental group (VG) received the growth regulator.

To minimise variability in antioxidant enzyme content across different plant parts, all samples were collected from the third or fourth fully expanded leaves at the base of the plant, while root samples were taken from the tips of the taproots. Superoxide dismutase (SOD) activity was measured using the method of C. Beauchamp & I. Fridovich (1971), with absorbance recorded at 560 nm. Peroxidase (POD) activity was analysed using guaiacol as a substrate, following the established protocol, with absorbance measurements taken at 470 nm within 3 minutes. Catalase (CAT) activity was determined using a modified version of Neto's method, which calculates based on the rate of H₂O₂ decomposition, measured at 240 nm. Ascorbate peroxidase (APX) activity was evaluated using the method of Y. Nakano & K. Asada (1981), with absorbance recorded at 290 nm. Lipid peroxidation, indicated by malondialdehyde (MDA) content, was measured using the thiobarbituric acid (TBA) method. The reaction mixture was incubated at 95°C for 30 minutes, then rapidly cooled in an ice bath. Following centrifugation at 10,000 g for 20 minutes, the absorbance of the supernatant was recorded at 450 nm, 532 nm, and 600 nm.

Statistical analysis was conducted using SPSS 22 (IBM, Armonk, New York, USA). Duncan's multiple range test was used to identify significant differences, with a significance level of $P < 0.05$. Multivariate analysis of variance (ANOVA) was also applied.

RESULTS AND DISCUSSION

As demonstrated by the findings of P. Dang et al. (2024), the active oxygen scavenging system is coordinated by superoxide dismutase, catalase, ascorbate peroxidase, and other substances. The results of this study indicated that the use of the “VG-Antistress” regulator significantly improved the content of superoxide dismutase in soybean seedlings (Fig. 1). With increasing in salt concentrations, the SOD content in soybean seedling leaves gradually increased, and the VG group exhibited higher

levels than the CK group. A two-factor analysis of variance was conducted (F_{CK} is the F value for salt concentration, and F_{VG} is the F value for the growth regulator), yielding $F_{CK} = 30.84$, $p = 0 < 0.01$. The main effect of salt concentration was significant, indicating that increasing salt concentration had a pronounced impact on the SOD content of soybean seedling leaves. Similarly, $F_{VG} = 18.40$, $p = 0 < 0.01$, revealed that the growth regulator also had a significant effect, highlighting its substantial influence on SOD content in soybean seedling leaves.

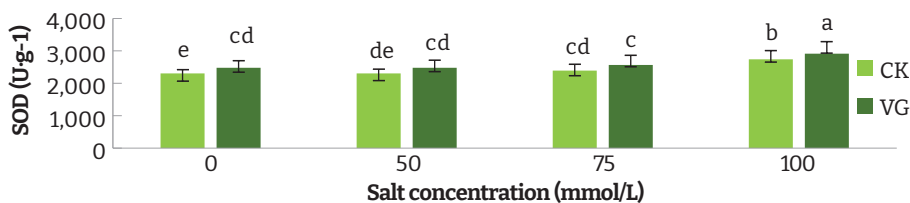


Figure 1. Effect of the growth regulator on superoxide dismutase (SOD) under different salt stress conditions

Note: the experiment was conducted in an artificial climate chamber at the Henan Institute of Science and Technology, Xinxiang, China

Source: authors' development

Different salt concentrations and the application of the regulator significantly influenced changes in SOD content, with levels in the VG group consistently higher than those in the CK group. Under treatments with 0 mmol/L, 50 mmol/L, 75 mmol/L, and 100 mmol/L salt

concentrations, the SOD content in soybean seedlings increased by 6.86%, 6.83%, 6.59%, and 5.78%, respectively, following the application of the regulator. The regulator also substantially increased the content of ascorbate peroxidase in soybean seedlings (Fig. 2).

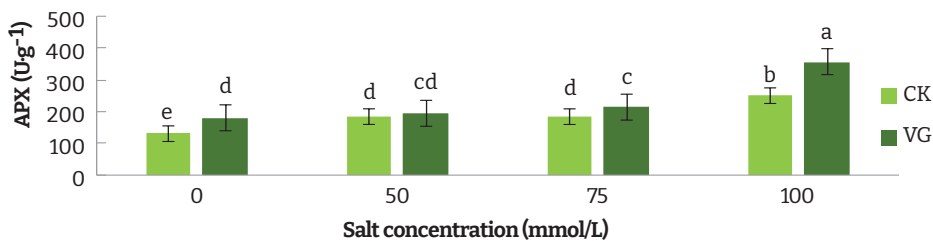


Figure 2. Effect of the growth regulator on ascorbate peroxidase (APX) under different salt stress conditions

Note: the experiment was conducted in an artificial climate chamber at the Henan Institute of Science and Technology, Xinxiang, China

Source: authors' development

As the concentration of salt increased, ascorbate peroxidase activity gradually increased, and with the application of the regulator, the

APX activity was higher than that of the control group. After conducting a two-factor analysis of variance, $F_{CK} = 45.29$, $p = 0 < 0.01$, indicating that

the main effect of salt concentration was significant and that increasing salt concentration had a substantial impact on the content of ascorbate peroxidase in soybean seedling leaves. $F_{VG} = 27.08$, $p = 0 < 0.01$, further demonstrated that the main effect of the growth regulator was significant, confirming that the use of the regulator VG significantly influenced the content of

ascorbate peroxidase in soybean seedling leaves. Under salt concentrations of 0 mmol/L, 50 mmol/L, 75 mmol/L, and 100 mmol/L, ascorbate peroxidase activity increased by 27.72%, 5.41%, 14.08%, and 30.00% following the use of the regulator, respectively. The application of the regulator notably increased the content of catalase in soybean seedlings (Fig. 3).

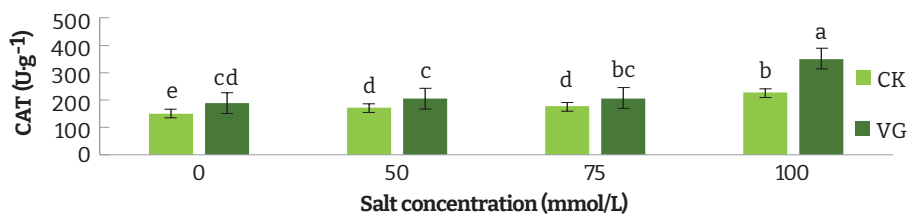


Figure 3. Effect of the growth regulator on catalase (CAT) under different salt stress conditions

Note: the experiment was conducted in an artificial climate chamber at the Henan Institute of Science and Technology, Xinxiang, China

Source: authors' development

As the salt concentration increased, the activity of catalase also increased. While the growth regulator was applied, CAT activity was higher than that observed in the control group. Following a two-factor analysis of variance, $F_{CK} = 29.93$, $p = 0 < 0.01$, indicated that the main effect of salt concentration was substantial, showing that increasing salt concentration significantly influenced the content of catalase in soybean seedling leaves. $F_{VG} = 35.02$, $p = 0 < 0.01$, showed that the main effect of the regulator had a significant effect, demonstrating its notable influence on catalase content. Under salt concentrations of 0 mmol/L,

50 mmol/L, 75 mmol/L, and 100 mmol/L, the regulator increased catalase activity by 20.14%, 16.89%, 15.62%, and 35.96%, respectively.

As noted by O. Laslo & A. Melnychuk (2021), malondialdehyde is a commonly used indicator to measure the degree of oxidative stress, reflecting the extent of membrane lipid peroxidation in plants. Under salt stress, antioxidant enzymes play a crucial role in scavenging plant reactive oxygen species, as observed in the research of C. Accoroni *et al.* (2020). The application of the VG regulator reduced the content of malondialdehyde in soybean seedlings (Fig. 4).

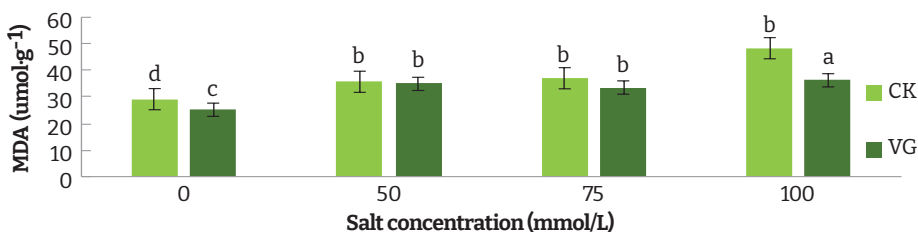


Figure 4. Effect of the growth regulator on malondialdehyde (MDA) under different salt stress conditions

Note: the experiment was conducted in an artificial climate chamber at the Henan Institute of Science and Technology, Xinxiang, China

Source: authors' development

As the salt concentration increased, the malondialdehyde content gradually decreased, and with the application of the regulator, the MDA content was lower than that of the control group. After a two-factor analysis of variance, $F_{CK} = 45.29$, $p = 0 < 0.01$, the main effect of salt concentration was significant, indicating that increasing salt concentration significantly influenced the content of MDA in soybean seedling leaves. $F_{VG} = 27.08$, $p = 0 < 0.01$, therefore, the main effect of the growth regulator was notable, indicating that its use had a significant effect on the content of MDA in soybean seedling leaves. At salt concentrations of 0 mmol/L, 50 mmol/L, 75 mmol/L, and 100 mmol/L, the use of the regulator decreased MDA levels by 15.71%, 2.31%, 10.14%, and 33.33%, respectively.

The research showed that under salt stress, the antioxidant enzyme activity of soybeans increased, as noted by A. Melnyk *et al.* (2022). The plant's antioxidant enzyme system can effectively remove reactive oxygen species generated by metabolic activities in plants (Dudkina & Bondareva, 2019; Hasanuzzaman *et al.*, 2022). Reactive oxygen species and free radicals can cause varying degrees of oxidative damage to plants. According to A. Eliçin *et al.* (2021), the antioxidant enzymes can convert excess reactive oxygen species and free radicals in plants into less toxic or harmless substances, maintaining the balance of reactive oxygen species. Furthermore, increased antioxidant enzyme activity can also indicate that plants have experienced environmental stress. As is known from the results of M. Iqbal *et al.* (2021), membrane lipid peroxidation may occur when plant organs age or are damaged under adverse circumstances. MDA is the final decomposition product of membrane lipid peroxidation, and its content reflects the degree of environmental damage to plants (Hadzovskiy *et al.*, 2020; Zhou *et al.*, 2024).

Under salt stress, soybean superoxide dismutase, ascorbate peroxidase, and catalase activities showed an increasing trend, while MDA content also gradually accumulated (Osman *et al.*, 2021; Saravanan *et al.*, 2023). In this study, the activity of antioxidant enzymes and MDA content gradually increased with higher salt concentrations. This observation aligns with findings from previous studies.

When using the VG growth regulator, the activities of CAT, SOD and APX increased more than in the control group, indicating that the regulator enhanced antioxidant enzyme activity under salt stress and mitigated the damage caused by oxygen-free radicals in soybeans. At a salt concentration of 100 mmol/L, SOD activity increased by 5.78%, with no significant increase at lower concentrations. The maximum increase in APX activity was 30%, while CAT activity increased by up to 35.96%. The content of MDA decreased under different salt treatment conditions following the use of the regulator, indicating that the regulator reduced membrane oxidation. At a salt concentration of 100 mmol/L, the maximum reduction in MDA was 33.33%. It can be speculated that the application of the growth regulator influenced the salt resistance of soybeans, improving their tolerance to salt stress to a certain extent, with the effect being more pronounced at higher salt concentrations.

CONCLUSIONS

In this study, the "Amino VG-Antistress" regulator was evaluated for its ability to improve the salt tolerance of the Zheng 196 soybean variety at the seedling stage. The regulator enhanced the antioxidant capacity of Zheng 196 soybean seedlings and mitigated the effects of salt stress. The effect was most pronounced at a salt concentration of 100 mmol/L, confirming the regulator's ability to improve soybean salt resistance. However, for large-scale field applications and diverse terrains, the effect of this regulator requires further verification due to environmental variability and other uncertain factors.

The application of the regulator during the seedling stage of the Zheng 196 soybean variety was investigated for its efficacy in mitigating salt stress. This was achieved through the analysis and measurement of antioxidant enzyme activity and malondialdehyde content. The findings indirectly verified the regulator's effectiveness in enhancing the salt tolerance of soybean seedlings. Under salt concentrations of 50 mmol/L, 75 mmol/L, and 100 mmol/L, the activities of superoxide dismutase, ascorbate peroxidase, and catalase all increased. At a salt concentration of 100 mmol/L, superoxide dismutase activity increased by 5.78%, though this increase was not

significant. In contrast, ascorbate peroxidase and catalase activities showed significant increases of 30% and 35.96%, respectively, at the same salt concentration, while malondialdehyde content notably decreased by 33%. This demonstrated that under high salt concentrations, the regulator significantly enhanced the antioxidant capacity of soybean seedlings and reduced membrane oxidation. These findings indirectly reflect the regulator's slow-release effect under salt stress during the soybean seedling stage. The proven enhancement of enzyme activity by the regulator contributed to increased soybean resistance to salinity.

According to the results of the research, it was determined that the use of the growth regulator had a significant impact on changes in enzymatic activity. The main compounds (enzymes) that are indicators of the resistance of plant organisms to increased salinity were studied. The key findings demonstrated the effectiveness of the regulator and highlighted its potential for increasing resistance. Along with this, the results are relevant for scientists seeking to develop substances with similar compositions to create newer, more effective growth regulators with anti-stress properties.

Despite earlier reports on the exogenous application of melatonin in soybean, no studies, to the best of the authors' knowledge, have specifically addressed its effects under salt stress

conditions. Furthermore, salt stress can be precisely and reproducibly simulated in laboratory settings. The use of NaCl is a widely accepted approach in studies exploring the impacts of salt stress. The results offer additional evidence supporting the physiological role of melatonin and provide a theoretical foundation for its application in enhancing salt tolerance in agricultural practices. These findings are crucial for advancing the development of new growth regulators and provide scientific evidence supporting their feasibility in addressing the growing challenge of soil salinity. To further build upon these findings, future research should explore the long-term effects of the growth regulator on soybean growth and yield under diverse environmental conditions and examine its potential interactions with other stress factors to develop comprehensive strategies for enhancing crop resilience.

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CONFLICT OF INTEREST

None.

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Вплив регулятора росту на солестійкість сої сорту Чжен 196 на стадії проростання

Анотація. Засолення ґрунтів стає все більш актуальною проблемою для світового сільського господарства. З 230 мільйонів гектарів зрошуваних сільськогосподарських угідь у світі 20 % зазнають впливу засолення, і цей відсоток продовжує щорічно зростати через неправильну практику зрошення. Таким чином, поглиблення досліджень солестійкості є привабливим і економічно ефективним рішенням цієї проблеми. Основна мета роботи – дослідити ефективність впливу регулятора росту на підвищення солестійкості на стадії проростання сої. Гідропонний експеримент із соєю було проведено в Хенанському інституті науки і технологій (м. Сінсян, Китай). Розсаду вирощували в камері зі штучним кліматом. Було проведено вимірювання рівня супероксиддисмутази в експерименті з використанням нітротетразолію синього хлориду, аналіз пероксидази аскорбінової кислоти. Застосування

регуляторів здатне зменшити шкоду сольового стресу для рослин. Після використання регулятора на стадії проростання сої сорту Чжен 196 було досліджено здатність регулятора зменшувати сольовий стрес шляхом аналізу та вимірювання антиоксидантних ферментів та малонового діальдегіду, а також опосередковано підтверджено його позитивний вплив на солестійкість проростків сої. Активність аскорбатпероксидази та каталази значно зростала за концентрації солі 100 ммоль/л, досягаючи 30 % та 35,96 %, тоді як вміст малонового діальдегіду значно знижувався на 33 % за концентрації солі 100 ммоль/л. Ці результати є важливими для розробки нових регуляторів росту та науково доводять доцільність їх застосування за сучасних тенденцій до підвищення засолення ґрунтів

Ключові слова: *Glycine max.* (L.); амінорегулятор; сольовий стрес; активність ферментів; листки проростків

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Combining ability of inbred maize lines in breeding for grain quality

Abstract. The selection of the initial material (inbred lines) and the assessment of its combining ability are key tasks in developing maize hybrids. This study aimed to select the initial material for the creation of heterotic maize hybrids with enhanced grain quality and yield. The article presented the results of research on eight lines bred by the Genetics, Breeding and Seed Production named after Prof. M.O. Zelensky at the National University of Life and Environmental Sciences of Ukraine (lines: AK157 and AK159), as well as lines obtained from the National Centre for Plant Genetic Resources

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of Ukraine (lines: HLG1203, VK19, VK32, AE800, AE746, UHK686, UHK37, CO255, FV243, Q170). Additionally, four tester lines carrying mutant endosperm structure genes were analysed (lines VK13 and VK69 carrying the *wx* mutant endosperm structure gene, and lines AE801 and AE392 carrying the *ae* mutant endosperm structure gene). To assess combining ability, crosses were performed using the incomplete topcross method, resulting in 24 experimental hybrids. Field trials were conducted in 2020–2021. The article presented the results of an assessment of the obtained hybrid combinations based on grain quality and yield indicators. The highest protein content during the study years was recorded in the combination VK13 × CO255; the highest starch content in VK13 × UHK686 and VK69 × FV243; the highest oil content in AE801 × AE746 and AE801 × VK19; and the highest yield in VK13 × UHK686 and AE801 × UHK37. The effects of general combining ability, constants, and variances of specific combining ability were determined for protein, starch, and oil content in the grain, as well as for yield. The findings indicated that lines VK19, AE800, CO255, FV243, Q170, VK13, and AE801 exhibited increased protein content in the grain; lines VK19, AE746, UHK686, CO255, VK13, and AE392 showed increased oil content; and lines AK157, UHK37, and AE746 demonstrated superior yield performance. Based on the results, specific inbred lines are recommended for developing maize hybrids with improved grain quality characteristics

Keywords: trait source; heterosis; inbred line; hybrid; topcross; quality indicators

INTRODUCTION

The chemical composition of maize grain can vary significantly depending on growing conditions. High temperatures promote more intensive protein accumulation. Late-maturing varieties contain more protein in their grain during dry years compared to years with sufficient moisture. However, the primary role in improving maize grain quality belongs to breeding, which highlights the relevance of studying the general and specific combining ability of inbred lines when creating hybrid maize varieties with improved grain quality traits. The sharp increase in market demand for specific types of maize, such as high-lysine, super-sweet, waxy, and high-amylose, determines the direction of work for breeding and genetic institutions to expand genetic diversity, identify genetic regulation systems, and identify reliable sources and donors of these traits, as noted by D. Tymchuk *et al.* (2020).

According to the evaluation of 140 cross combinations using a tester scheme with 5 testers and 28 inbred lines, H. Kustanto & F. Hendrayana (2023) note that selecting the right inbred lines as parental forms is a challenge in maize hybrid development. The chosen inbred lines should form a hybrid pair with excellent characteristics, such as high yield. In addition to limited genetic resources, maize hybrid breeding also requires a lot of time. To address this

challenge, M. Ali *et al.* (2019) recommend analysing and evaluating the combining ability of inbred lines during their creation, starting from the third generation of self-pollination. Combining ability analysis is an invaluable tool for assessing the compatibility of maize inbred lines with testers. In the research of M. Alam *et al.* (2022), the focus was on determining combining ability for traits related to yield potential. As a result of the research, they concluded that combining ability analysis is essential for determining the interaction of inbred lines and testers not only in maize but also for all cross-pollinated crops, as it helps identify parental forms that can be used to create hybrids and synthetic populations.

Investigating inbred lines and maize hybrids with elevated levels of provitamin A and resistance to contrasting stresses, O. Oluwaseun *et al.* (2022) concluded that both additive and nonadditive gene actions are important for the inheritance of grain yield and other quantitative traits. However, additive gene action predominated over non-additive gene action. This supports the assertion that analysing the combining ability of different maize inbred lines is the most effective tool for developing programs to create and compare the performance of hybrids derived from inbred lines. Information about

the combining ability of inbred lines in hybrid combinations is also crucial for the successful improvement of maize hybrids.

The research of G. Sprague & L. Tatum (1942) was fundamental to the breeding of heterotic hybrids, as it was the first to distinguish between general and specific combining abilities, laying the groundwork for further methodological advancements in evaluating initial breeding material. General combining ability is defined as the average performance of a parental form in a hybrid combination and is primarily attributed to the additive effects of genes. Specific combining ability describes instances where certain hybrid combinations perform relatively better or worse than expected based on the average performance of the parental forms and is considered an estimate of non-additive gene action, such as dominance and epistasis. T. Begna (2021) notes that combining ability analysis allows for the identification of parental forms with high general combining ability (GCA) and hybrid combinations with high specific combining ability (SCA) for the commercial exploitation of heterosis.

When creating hybrids, it is necessary to select an appropriate crossing scheme (Dokukina *et al.*, 2021). Although several crossing models are used in plant breeding, they all have certain advantages and disadvantages. The most comprehensive and reliable is the diallel cross scheme. However, using this scheme for analysing a large number of inbred lines is

very timeconsuming. In such cases, W. Mogesse *et al.* (2020) recommend using a tester cross scheme, which evaluates both general and specific combining ability and is widely used in research. All of the above highlights the need for the continuous search, creation, and evaluation of inbred lines with high protein, starch, oil content, and yield based on combining the ability to ensure food security and intensify agricultural production. The aim and objectives of this experiment were to determine the general and specific combining ability of maize inbred lines in an incomplete topcross system based on grain protein, starch, oil content, and yield.

MATERIALS AND METHODS

The study involved 24 experimental maize hybrids obtained through the crossing of lines L₁ (HLG 1203), L₂ (AK 157), L₃ (AK 159), L₄ (VK 19), L₅ (VK 32), L₆ (AE 800), L₇ (AE 746), L₈ (UHK 686), L₉ (UHK 37), L₁₀ (CO 255), L₁₁ (FV 243), and L₁₂ (Q 170) with testers T₁ (VK 13) and T₂ (VK 69) (sources of the *wx* endosperm structure gene mutation), as well as T₃ (AE 801) and T₄ (AE 392) (sources of the *ae* endosperm structure gene mutation) (Table 1). The inbred lines used in the crosses were developed by the Genetics, Breeding and Seed Production named after Prof. M.O. Zelensky at the National University of Life and Environmental Sciences of Ukraine, as well as lines obtained from the National Centre for Plant Genetic Resources of Ukraine.

Table 1. Scheme of incomplete topcross hybridisation, 2020-2021

	T ₁	T ₂	T ₃	T ₄
L ₁		+	+	
L ₂	+			+
L ₃		+	+	
L ₄	+			+
L ₅		+	+	
L ₆	+			+
L ₇		+	+	
L ₈	+			+
L ₉		+	+	
L ₁₀	+			+
L ₁₁		+	+	
L ₁₂	+			+

Source: compiled by the authors

Field trials were conducted in 2020-2021 on experimental plots of the Genetics, Breeding and Seed Production named after Prof. M.O. Zelensky at the NULES of Ukraine, a separate subdivision of the NULES of Ukraine "Agronomic Research Station", located in the Bila Tserkva District of Kyiv Region, Ukraine. The soils of the experimental plots were typical,

low-humus chernozems (Ridey *et al.*, 2009). During the 2020 growing season, which lasted from 29 April to 10 October, 501 mm of precipitation fell. During the 2021 growing season, which lasted from 12 April to 8 October, 430 mm of precipitation fell (Fig. 1). The groundwater table was at a depth of 1.5-2 m, so additional irrigation was not applied.

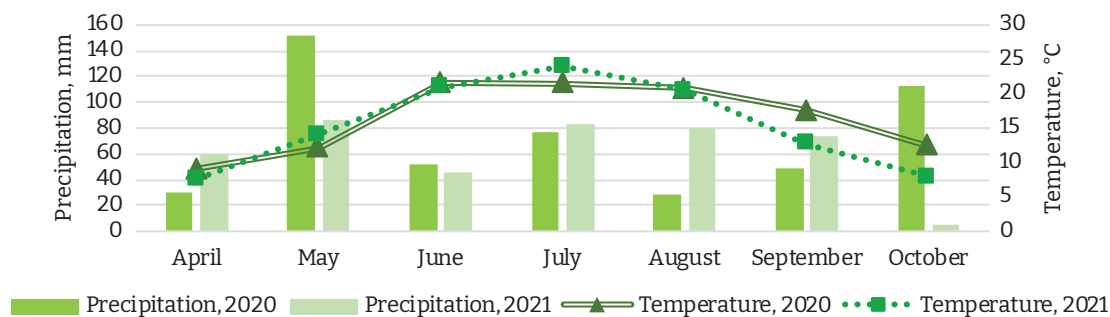


Figure 1. Average monthly precipitation (mm) and temperature (°C), 2020-2021

Source: developed by the authors based on data from Meteopost (n.d.)

Sowing was carried out at a stable soil temperature of 10-12°C at the sowing depth. The predecessor in all years of the study was winter soft wheat. Chemical plant protection products, mineral and organic fertilisers were not used. Weed control was performed using organotropic methods. Experimental plots were arranged according to a Randomized Complete Block Design (RCBD) with three replications. Plot units: two central rows of the experimental plot, 7 m long, with a row spacing of 70 cm and a plant spacing in the row of 17 cm. Phenological observations, grouping by the magnitude of the average manifestation of traits, and comparison with standard samples were performed according to the classifier-directory of the species *Zea mays* L. (Kyrychenko *et al.*, 2009)

The content of protein, starch, and oil in the grain was determined using a FOSS Infratec 1241 Grain Analyzer. For this purpose, 5 random cobs were taken from each experimental plot, dried in the laboratory, threshed manually, and cleaned of impurities on sieves. The content of protein, starch, and oil is indicated in terms of standard grain moisture (Tkachyk, 2016). The general (GCA) and specific (SCA) combining ability of the studied maize inbred lines were

determined using the method of incomplete topcrosses (Sych *et al.*, 2004). Statistical data processing was carried out using Microsoft Excel 2016 in combination with XLSTAT. The error of statistical analysis $P < 0.05$. The research was conducted following the ethical standards of the Convention on Biological Diversity (1992) and the Convention on the Trade in Endangered Species of Wild Fauna and Flora (1973).

RESULTS AND DISCUSSION

Results of trials of experimental hybrids in 2020-2021 showed that the protein content in the grain varied from 7.99% to 13.57% in 2020 and from 7.64% to 12.57% in 2021. It was characteristic that the highest protein content in the grain in the years of the study was in the cross combination $T1 \times L_{10}$, indicating a significant influence of the genotype on the control of this trait. When improving the quality of maize grain, it should be taken into account that its main criteria for hybrids of different uses vary. X. Yu *et al.* (2015) draw attention to the need to identify sources and donors of traits for improved grain quality. In particular, J. BeMiller & R. Whistler (2009) note that for food-grade hybrids, they consider protein content and fatty acid composition

of oil, while for feed purposes and processing into bioethanol, protein content, starch content, and its fractional composition are important. G. Mingramm *et al.* (2024) recommend using genetically contrasting parental forms in breeding programs to obtain high-yielding maize hybrids with improved grain quality traits.

Significant variability in grain starch content was observed, both when comparing genotypes and between years. The range of variation for this trait in 2020 was from 64.62% (combination $T_3 \times L_9$) to 73.51% (combination $T_1 \times L_8$); in 2021, it was from 64.24% (combinations $T_3 \times L_1$ and $T_3 \times L_7$) to 73.80% (combination $T_2 \times L_{11}$). The obtained data indicate the low suitability of forms T_3 and T_4 for creating maize hybrids with high starch content. The amplitude of variation in oil content in the grain of the studied combinations ranged from 3.61% (combination $T_2 \times L_3$) to 5.13%

(combination $T_3 \times L_7$) in 2020; from 3.39% (combination $T_2 \times L_3$) to 6.43% (combination $T_3 \times L_4$). It should be noted that the highest stability was observed for the trait of oil content in the grain over the years of the study. The yield of the studied samples varied significantly among cross combinations and years of the study. In 2020, yield ranged from 2.82 t/ha in the combination $T_3 \times L_7$ to 8.74 t/ha in the combination $T_1 \times L_8$; in 2021, it ranged from 2.99 t/ha in the combination $T_4 \times L_4$ to 8.78 t/ha in the combination $T_3 \times L_9$ (Table 2). As noted in previous research by R. Sprizhka *et al.* (2022), a portion of combinations typically exhibit high values for several traits simultaneously. Y. Long *et al.* (2024) obtained similar results when studying valuable economic traits of maize. Based on the research of L. Shah *et al.* (2015), such combinations are recommended for inclusion in breeding programs.

Table 2. Protein, starch, oil content, and yield of experimental maize hybrids, 2020-2021

Combination		Grain content, %						Yield, t/ha	
		Protein		Starch		Oil		2020	2021
		2020	2021	2020	2021	2020	2021		
T_1	L_2	10.09	9.95	71.58	70.84	4.40	4.39	4.40	5.23
	L_4	11.21	11.13	64.86	70.34	4.93	5.01	4.01	4.87
	L_6	10.56	10.40	70.67	70.63	4.28	4.32	4.46	5.84
	L_8	8.30	7.64	73.51	71.63	5.01	5.13	8.74	6.52
	L_{10}	13.57	12.57	69.17	65.89	4.54	4.40	6.85	6.75
	L_{12}	10.46	10.94	67.67	68.99	4.17	4.23	4.72	4.56
T_2	L_1	8.27	8.39	70.51	68.09	4.78	5.02	5.52	4.22
	L_3	8.60	7.80	72.30	70.42	3.61	3.39	4.97	5.47
	L_5	8.61	8.46	71.97	69.50	3.85	4.15	5.92	5.46
	L_7	9.15	8.79	66.27	68.73	4.57	4.16	5.59	7.18
	L_9	8.53	8.87	71.26	69.60	3.89	3.77	8.19	6.73
	L_{11}	8.39	8.35	70.80	73.80	4.58	4.50	4.39	5.77
T_3	L_1	9.99	9.74	70.56	64.24	4.18	4.56	4.32	5.75
	L_3	10.21	9.25	69.21	66.79	4.16	3.97	5.53	5.75
	L_5	8.81	9.32	66.63	72.04	3.83	3.84	5.14	4.42
	L_7	11.02	11.51	66.69	64.24	5.13	5.14	2.82	3.18
	L_9	9.86	9.21	64.62	68.98	4.00	4.34	6.59	8.78
	L_{11}	11.62	10.74	67.14	69.60	4.67	4.23	6.27	6.09
T_4	L_2	9.73	10.07	67.68	68.85	5.08	4.99	6.55	5.90
	L_4	10.51	10.55	65.07	69.13	5.11	6.43	3.31	2.99
	L_6	10.42	10.72	69.79	65.34	4.30	4.43	3.75	3.72
	L_8	7.99	8.05	66.02	68.76	4.15	4.25	3.91	3.17
	L_{10}	10.41	11.19	68.27	67.73	4.80	5.06	4.65	3.70
	L_{12}	10.46	9.67	70.37	66.03	4.56	4.24	2.75	3.55

Source: developed by the authors

The results of the study revealed insignificant variability in protein and oil content in the grain over different years of the study. At the same time, a significant influence of year-to-year conditions on starch content in the grain and yield was observed, as evidenced by significant differences in these indicators in different years of the study.

Evaluation of the GCA effect of parental material based on grain quality and yield is a leading criterion in breeding work. It is important to note that combining ability, to some extent, depends on the location of the study and climatic conditions. S. Vasal (1998) notes that the correct selection of parental material for creating maize hybrids is a decisive factor in the effectiveness of

the selection process, which was later confirmed by A. Karim *et al.* (2022). In the current study, positive, significantly significant, and stable GCA effects over the years for grain protein content were observed in lines L₄, L₆, L₁₀, and L₁₂. However, in 3 of the 4 mentioned lines, significantly negative GCA effects were recorded for yield. For the yield trait, significantly high GCA values were established for lines L₂ and L₉; however, line L₉ was characterised by a significantly negative GCA effect for grain protein content, indicating an inverse correlation between these traits ($r = -0.19$ in 2020 and $r = -0.21$ in 2021). In lines L₄ and L₁₀, in addition to high GCA values for protein content, a significantly positive GCA effect for oil content in the grain was also noted (Table 3).

Table 3. General combining ability effects of inbred lines for grain protein, starch, oil content, and yield, 2020-2021

Inbred line	Protein, %		Starch, %		Oil, %		Yield, t/ha	
	2020	2021	2020	2021	2020	2021	2020	2021
L ₁	-0.74**	-0.66**	1.55	-2.59*	0.04	0.29**	-0.22*	-0.25*
L ₂	0.04	0.29	0.65	1.09	0.30**	0.19*	0.34**	0.33**
L ₃	-0.46*	-1.20**	1.77	-0.15	-0.56**	-0.82**	0.11	0.38**
L ₄	0.99**	1.12**	-4.02**	0.98	0.58**	1.22**	-1.48**	-1.30**
L ₅	-1.16**	-0.83**	1.82	2.01	-0.60**	-0.50**	0.39**	-0.29*
L ₆	0.62**	0.84**	1.25	-0.77	-0.15*	-0.12	-1.03**	-0.45**
L ₇	0.22	0.43*	-2.50*	-2.27*	0.41**	0.15	-0.93**	-0.05
L ₈	-1.72**	-1.88**	0.78	1.44	0.14*	0.19*	1.19**	-0.39**
L ₉	-0.67**	-0.68**	-1.04	0.53	-0.50**	-0.44**	2.25**	2.52**
L ₁₀	2.12**	2.16**	-0.26	-1.95	0.23**	0.23**	0.61**	-0.01
L ₁₁	0.14	-0.18	-0.01	2.94**	0.18*	-0.13	0.19	0.70**
L ₁₂	0.59**	0.58**	0.04	-1.25	-0.08	-0.26**	-1.40**	-1.18**
SE _{dgi}	0.17	0.15	1.02	0.99	0.07	0.07	0.10	0.10
LSD _{0.05}	0.36	0.31	2.17	2.09	0.14	0.16	0.21	0.22
LSD _{0.01}	0.49	0.43	2.99	2.88	0.19	0.21	0.29	0.30

Note: * and ** indicate significance at 5% and 1% levels, respectively

Source: developed by the authors

Using the tester-cross scheme, M. Mur-tadha *et al.* (2018) noted a significant influence of testers on the formation of grain quality traits. Analysis of the GCA effect of testers showed that testers T₁ and T₃ had a stable, significantly high positive effect on grain protein content over the years. A stable significant trend towards increasing oil content in the grain was found in tester T₄, and for yield – in testers T₁ and T₂. It should be

noted that no trend towards a decrease in any of the studied traits was recorded for tester T₁, which characterises the possibility of its wide use (Table 4). The effectiveness of breeding programs depends on the level of genetic variability inherent in inbred lines, testers, and their combining ability, as indicated in their research by M. Alam *et al.* (2024). Since the focus is on lines, testers, and cross combinations, the identified

lines, testers, and combinations have a clear potential for strategic integration into breeding programs to create high-yielding maize hybrids with improved quality traits.

Table 4. General combining ability effects of testers for grain protein, starch, oil content, and yield, 2020-2021

Inbred line	Protein, %		Starch, %		Oil, %		Yield, t/ha	
	2020	2021	2020	2021	2020	2021	2020	2021
T ₁	0.83**	0.72**	0.59	0.96	0.11**	0.08	0.39**	0.40**
T ₂	-1.27**	-1.28**	1.53*	1.27*	-0.23**	-0.33**	0.62**	0.57**
T ₃	0.39**	0.24**	-1.01*	-1.11*	-0.11**	-0.15**	-0.03	0.43**
T ₄	0.05	0.32**	-1.12*	-1.12*	0.23**	0.40**	-0.99**	-1.40**
SE _{d_gj}	0.09	0.08	0.53	0.51	0.03	0.04	0.05	0.05
LSD _{0.05}	0.19	0.16	1.13	1.09	0.07	0.08	0.11	0.11
LSD _{0.01}	0.26	0.23	1.56	1.51	0.10	0.11	0.15	0.16

Note: * and ** indicate significance at 5% and 1% levels, respectively
Source: developed by the authors

The values of SCA constants for grain protein content varied from -1.63 (T₄ × L₁₀) to 1.23 (T₃ × L₁₁) in 2020, and from -1.01 (T₄ × L₁₀) to 1.12 (T₃ × L₇) in 2021. A characteristic feature of the studied cross combinations is the relative stability of the SCA effect over the years of the study. Significantly high, positive, and stable SCA effects were recorded in crosses T₂ × L₅, T₂ × L₉, T₃ × L₇, and T₃ × L₁₁. High values of SCA variances, even with low or negative GCA values, indicate the

possibility of obtaining individual highly heterozygous combinations involving such parental forms, as noted by O. Haydash (2016). Analysis of line variances (δ^2_{si}) compared to their mean ($\bar{x}\delta^2_{sj}$) indicates that lines L₁₀ and L₁₁ tend to increase protein content in the grain. The values of tester variances (δ^2_{st}) compared to their mean ($\bar{x}\delta^2_{sj}$) were inverse over the years of the study, suggesting a low impact of the testers used on the inheritance of grain protein content in maize (Table 5).

Table 5. Constants and variances of specific combining ability for grain protein content, 2020-2021

Combination of crosses	2020					2021				
	T ₁	T ₂	T ₃	T ₄	δ^2_{si}	T ₁	T ₂	T ₃	T ₄	δ^2_{si}
L ₁		0.41	0.47		0.46		0.60	0.43		0.60
L ₂	-0.65			-0.23	0.54	-0.78			-0.26	0.72
L ₃		0.47	0.42		0.46		0.55	0.48		0.59
L ₄	-0.48			-0.40	0.46	-0.43			-0.61	0.60
L ₅		1.17	-0.29		1.52		0.85	0.19		0.80
L ₆	-0.76			-0.12	0.66	-0.88			-0.16	0.84
L ₇		0.34	0.55		0.48		-0.08	1.12		1.31
L ₈	-0.68			-0.21	0.57	-0.92			-0.12	0.91
L ₉		0.61	0.28		0.51		1.11	-0.07		1.28
L ₁₀	0.75			-1.63	3.29	-0.03			-1.01	1.07
L ₁₁		-0.34	1.23		1.69		0.08	0.95		0.97
L ₁₂	-0.83			-0.05	0.76	-0.08			-0.96	0.97
$\bar{\delta}^2_{sj}$	0.54	0.42	0.42	0.54		0.44	0.49	0.49	0.44	
LSD _{0.05}	0.37					0.33				
LSD _{0.01}	0.52					0.45				
$\bar{x}\delta^2_{st}$	0.95					0.89				
$\bar{x}\delta^2_{sj}$	0.48					0.47				

Source: developed by the authors

Typically, the influence of the general combining ability of parental forms is also reflected in the specific combining ability for most traits studied, as noted by M. Matin *et al.* (2017). SCA consists of positive and negative values. H. Kustanto & F. Hendrayana (2023) determined that the results of the SCA effect analysis often demonstrate differences between observed crosses and traits. Inbred lines with significant positive GCA effects, when forming significantly positive SCA values, are recommended by D. Gurjar *et al.* (2022) not only for

the creation of simple interline hybrids but also for developing new inbred lines through reciprocal recurrent selection.

A significantly high, positive SCA effect for grain starch content was established only in the combination of $T_1 \times L_8$ in 2020. In 2021, this combination was also characterised by a positive value of the SCA constant, but this excess was not significant. When evaluating the variances of the studied lines and testers, a stable, positive manifestation of the trait of increasing starch content in the grain was not detected (Table 6).

Table 6. Constants and variances of specific combining ability for grain starch content, 2020-2021

Combination of crosses	2020					2021				
	T_1	T_2	T_3	T_4	δ_{sj}^2	T_1	T_2	T_3	T_4	δ_{sj}^2
L_1		-1.56	1.03		5.78		0.66	-0.82		3.23
L_2	1.36			-0.83	4.82	0.03			0.12	2.14
L_3		0.01	-0.54		2.57		0.55	-0.71		2.93
L_4	-0.70			1.22	4.26	-0.36			0.51	2.52
L_5		-0.36	-0.16		2.44		-2.54	2.38		14.22
L_6	-0.15			0.68	2.76	1.68			-1.53	7.29
L_7		-1.74	1.22		6.81		0.98	-1.14		4.37
L_8	3.15			-2.63	19.12	0.47			-0.32	2.45
L_9		1.79	-2.31		10.81		-0.96	0.80		3.68
L_{10}	-0.14			0.67	2.75	-1.88			2.04	9.82
L_{11}		0.30	-0.82		3.04		0.83	-0.99		3.80
L_{12}	-1.94			2.47	12.14	0.52			-0.36	2.52
δ_{sj}^2	1.39	-0.05	-0.05	1.39		-0.30	0.25	0.25	-0.30	
LSD _{0.05}	2.26					2.19				
LSD _{0.01}	3.12					3.01				
$\bar{x} \delta_{sj}^2$	6.44					4.91				
$\bar{x} \delta_{sj}^2$	0.67					-0.03				

Source: developed by the authors

For grain oil content in experimental maize hybrids, SCA constants varied from -0.66 to 0.53 in 2020 and from -0.84 to 0.64 in 2021. Stable, significantly positive SCA effects were recorded in combinations $T_1 \times L_8$, $T_2 \times L_1$, $T_2 \times L_5$, $T_3 \times L_3$, and $T_3 \times L_7$. Lines L_7 and L_8 were characterised by a significant and stable influence on grain oil content, according to variance analysis, with an excess over the mean of 0.01 and 0.38 in 2020 and 0.12 and 0.52 in 2021, respectively. Testers T_1 and T_4 were characterised by a stable influence on grain oil content, in contrast to grain protein and

starch content, where the influence of testers was reversed over the years of the study (Table 7).

The highest differentiation of SCA constants over the years of the study was found for the yield trait, with a variation from -1.56 to 2.06 in 2020 and from -2.43 to 1.73 in 2021. However, combinations $T_1 \times L_8$, $T_1 \times L_{10}$, $T_2 \times L_7$, $T_4 \times L_2$, and $T_4 \times L_4$ were characterised by significantly high and stable positive SCA constants. According to the results of variance analysis, a significant stable influence on hybrid yield was observed when using lines L_2 and L_7 (Table 8).

Table 7. Constants and variances of specific combining ability for grain oil content, 2020-2021

Combination of crosses	2020					2021				
	T ₁	T ₂	T ₃	T ₄	δ^2_{si}	T ₁	T ₂	T ₃	T ₄	δ^2_{si}
L ₁		0.53	-0.19		0.32		0.56	-0.08		0.33
L ₂	-0.45			0.11	0.23	-0.38			-0.10	0.17
L ₃		-0.05	0.39		0.16		0.04	0.44		0.21
L ₄	-0.20			-0.14	0.07	-0.79			0.31	0.73
L ₅		0.24	0.10		0.08		0.49	0.00		0.25
L ₆	-0.12			-0.22	0.07	-0.14			-0.35	0.15
L ₇		-0.05	0.39		0.17		-0.16	0.64		0.45
L ₈	0.32			-0.66	0.54	0.36			-0.84	0.85
L ₉		0.17	0.17		0.07		0.05	0.44		0.20
L ₁₀	-0.24			-0.10	0.08	-0.41			-0.07	0.19
L ₁₁		0.18	0.16		0.07		0.47	0.02		0.23
L ₁₂	-0.31			-0.03	0.11	-0.09			-0.40	0.18
δ^2_{sj}	0.10	0.07	0.07	0.10		0.21	0.15	0.15	0.21	
LSD _{0.05}	0.15					0.16				
LSD _{0.01}	0.20					0.22				
$\bar{x} \delta^2_{si}$	0.16					0.33				
$\bar{x} \delta^2_{sj}$	0.08					0.18				

Source: developed by the authors

Table 8. Constants and variances of specific combining ability for yield, 2020-2021

Combination of crosses	2020					2021				
	T ₁	T ₂	T ₃	T ₄	δ^2_{si}	T ₁	T ₂	T ₃	T ₄	δ^2_{si}
L ₁		-0.02	-0.57		0.35		-1.34	0.34		1.92
L ₂	-1.47			2.06	6.42	-0.73			1.73	3.55
L ₃		-0.90	0.31		0.93		-0.71	-0.29		0.61
L ₄	-0.04			0.64	0.43	0.55			0.46	0.53
L ₅		-0.23	-0.36		0.21		-0.05	-0.95		0.93
L ₆	-0.04			0.63	0.42	0.67			0.34	0.58
L ₇		0.76	-1.36		2.44		1.43	-2.43		7.96
L ₈	2.02			-1.43	6.16	1.28			-0.28	1.74
L ₉		0.18	-0.77		0.65		-1.60	0.60		2.93
L ₁₀	0.71			-0.11	0.54	1.13			-0.13	1.32
L ₁₁		-1.56	0.97		3.40		-0.73	-0.27		0.63
L ₁₂	0.59			0.00	0.37	0.11			0.89	0.83
δ^2_{sj}	1.40	0.77	0.77	1.40		0.82	1.47	1.47	0.82	
LSD _{0.05}	0.22					0.23				
LSD _{0.01}	0.30					0.31				
$\bar{x} \delta^2_{si}$	1.86					1.96				
$\bar{x} \delta^2_{sj}$	1.09					1.14				

Source: developed by the authors

The results of the study demonstrate significant differentiation in general and specific combining ability for grain protein, starch, and oil content, and yield in the incomplete top-cross mating system. A similar conclusion was reached by O. Makarchuk & V. Zhemoida (2009) in their research. Similarly to grain protein and starch content, tester variances for the yield trait were reversed over the years, indicating an unstable influence of testers on the formation of these traits.

CONCLUSIONS

Results of testing experimental hybrids revealed a significant impact of genotype on controlling the protein content trait in the grain of combination $T_1 \times L_{10}$. The highest starch content in the grain (over 73%) was recorded in combination with $T_1 \times L_8$ in 2020 and in combination with $T_2 \times L_{11}$ in 2021. A low suitability of inbred lines T_3 and T_4 for creating maize hybrids with high starch content was determined. A high oil content in the grain is characteristic of combinations $T_3 \times L_7$ and $T_3 \times L_4$. The highest stability for this trait was observed across the years of the study. A yield of over 8 t/ha was recorded in combination with $T_1 \times L_8$ in 2020 and $T_3 \times L_9$ in 2021. When selecting maize hybrids with improved quality traits and high heterosis, it is recommended to use parental forms (inbred lines) with significantly high, stable positive effects of general and specific combining ability over the years. The obtained data on GCA and SCA indicate that the selected samples are

valuable sources of traits for improved grain quality and yield, specifically for protein content in the grain: lines L_4 , L_6 , L_{10} , L_{12} , T_1 , and T_3 with stable significant high GCA effects; lines L_{10} and L_{11} – according to SCA variance analysis; for oil content in the grain: lines L_4 , L_{10} , and T_4 with stable significant high GCA effects; lines L_7 , L_8 , T_1 , and T_4 – according to SCA variance analysis; for yield: lines L_2 , L_9 , with high significant and stable GCA effects; lines L_2 , L_7 – according to SCA variance analysis; for a complex of traits: lines L_4 , and L_{10} – with stable significant high GCA effects for protein and oil content; line L_7 – according to SCA variance analysis for oil content and yield.

The exploitation of heterosis provides a significant increase in the yield of agricultural crops, which is the basis for global food security in the face of the ever-growing world population. In turn, determining the effects of the general and specific combining ability of maize inbred lines for a complex of valuable traits, including grain protein, starch, oil content, and yield, is the basis for creating hybrid varieties, which determines the prospects for further research in this direction.

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CONFLICT OF INTEREST

None.

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Комбінаційна здатність інбредного матеріалу кукурудзи при селекції на якість зерна

Анотація. Підбір вихідного матеріалу (інбредних ліній) та оцінка його комбінаційної здатності є ключовим завданням при створенні гібридів кукурудзи. Метою досліджень був підбір вихідного матеріалу для створення гетерозисних гібридів кукурудзи з підвищеними показниками якості зерна та урожайності. У статті представлено результати досліджень 8 ліній селекції кафедри генетики, селекції і насінництва ім. М.О. Зеленського Національного університету біоресурсів і природокористування України (лінії: AK157 та AK159), ліній, отриманих з Національного центру генетичних ресурсів рослин України (лінії: HLG1203, VK19, VK32, AE800, AE746, УНК686, УНК37, CO255, FV243, Q170), а також 4 ліній-тестерів – носіїв мутантних генів структури ендосперму (лінії VK13 та VK69 – носії мутантного гену структури ендосперму wx та лінії AE801 і AE392 – носії мутантного гену структури ендосперму ae). Для визначення комбінаційної здатності було проведено схрещування за методом неповних топкросів, за результатом яких отримано 24 експериментальних гібриди. Польові дослідження проводили у 2020-2021 роках. У статті наведено результати оцінки отриманих комбінацій схрещувань за показниками якості зерна та урожайності. Зокрема, найвищий вміст білка в роки досліджень зафіксовано у комбінації VK13 × CO255; крохмалю – у комбінацій VK13 × УНК686 та VK69 × FV243; олії – AE801 × AE746 та AE801 × VK19; урожайність – у комбінацій VK13 × УНК686 та AE801 × УНК37. Встановлено ефекти загальної комбінаційної здатності, константи і варіанси специфічної комбінаційної здатності за показниками вмісту у зерні білка, крохмалю, олії та урожайності. Отримані дані свідчать, що лінії VK19, AE800, CO255, FV243, Q170, VK13 та AE801 мали підвищений вміст білка у зерні; лінії VK19, AE746,

УНК686, СО255, ВК13 та АЕ392 – підвищений вміст олії; АК157, УНК37, АЕ746 були найкращими за урожайністю. За результатами досліджень рекомендовано інбредні лінії для створення гібридів кукурудзи з поліпшеними показниками якості зерна

Ключові слова: джерело ознаки; гетерозис; інбредна лінія; гібрид; топкрос; показники якості

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Optimisation of plant nutrition under the influence of biopreparations in integrated sunflower cultivation technologies

Abstract. The increasing production of sunflowers is driven by multiple factors, including the introduction of high-intensity hybrids, an advanced fertilisation system, a balanced plant protection strategy, and modern high-efficiency machinery. Further technological improvements

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require identifying new approaches based on factor interactions or their combined application. One such approach involves the use of biopreparations, which not only enhance productivity but also reduce pesticide load, a crucial consideration in the current ecological context. This study aimed to determine the impact of mineral fertilisers and foliar application of a biopreparation on sunflower seed productivity. The effects of mineral fertilisers and biopreparations on seed productivity formation were examined. The influence of biopreparations on sunflower growth, development, and productivity under varying mineral nutrition conditions was established. It was found that the application of biopreparations during pre-sowing cultivation positively affected crop yield, both in the absence and presence of mineral fertilisers. The use of biopreparations contributed to increased sunflower yield, with one of the tested biopreparations leading to a 6.9% increase, while the combined application of two biopreparations resulted in a 10.7% increase compared to the unfertilised control. Under mineral nutrition conditions ($N_{60}P_{60}K_{60}$), the yield increase resulting from biopreparation application ranged from 0.21 to 0.17 t/ha, depending on the fertiliser dosage. The study also revealed positive changes in the microbiological state of the soil under the influence of biopreparations. The reduction in pathogenic fungi during the ripening phase reached 10.1% when a biopreparation was applied in the absence of fertilisers. The use of biopreparations also reduced the intensity of mineralisation processes, facilitating more efficient nitrogen uptake by plants. The dynamics of alkaline-hydrolysable nitrogen, available phosphorus, and potassium content confirmed the beneficial effects of biopreparations on the soil biogenic regime and plant nutrition. The findings support the feasibility of incorporating biopreparations into sunflower cultivation technologies, contributing to increased crop yields and improved soil ecological conditions. The results can be utilised to maximise the genetic potential of sunflower plants and ensure stable productivity

Keywords: yield; alkaline-hydrolysable nitrogen; available phosphorus; available potassium; soil microbiological state

INTRODUCTION

The high saturation of agrocenoses with sunflowers and the disruption of crop rotation intervals lead to an accumulation of toxic compounds in the soil, increased pathogen load, and intensified degradation processes, resulting in the deterioration of soil water, air, and nutrient regimes. This necessitates the search for alternative methods to improve soil fertility in agrocenoses (Kovalenko *et al.*, 2020). In modern plant nutrition strategies, the use of biopreparations is gaining prominence. These products aim to restore beneficial soil microorganisms, stimulate soil biological processes, and improve the uptake of macro- and micronutrients from fertilisers and the soil itself (Tsytsiura & Didur, 2021). As noted in the study by V. Tsyhanskyi (2020), in the context of modern agricultural production, where fertilisers represent one of the most costly components of crop cultivation technologies, optimising plant nutrition systems is critically important. The author highlights the growing

popularity of biological products that stimulate the development of beneficial soil microorganisms, enhance soil fertility, increase its suppressiveness against phytopathogens, and improve crop productivity and yield, including sunflower.

X. Wei *et al.* (2024) explain that microbial fertilisers not only enrich the soil with beneficial microorganisms but also actively attract specific fungal microorganisms through substances released by plant root systems. These recruited microorganisms, in turn, contribute to the physical strengthening of the soil, improving its structure and reducing compaction. They also enhance nutrient uptake by plants and protect them from pathogens. In essence, microbial fertilisers initiate a complex and targeted interaction between plants and microorganisms to optimise soil fertility and plant health. According to T. Wang *et al.* (2024), microorganisms introduced via biopreparations, due to their rapid reproduction and population changes, engage a

substantial amount of nutrients in the biological cycle. This prevents the migration of compounds to lower soil horizons, reducing environmental pollution. They also play a crucial role in optimising ecological balance in agricultural landscapes under various farming intensities. Furthermore, they improve the efficiency of mineral fertiliser and pesticide use, increase crop productivity, and enhance product quality (Karpenko, 2019).

According to A. Pavlichenko *et al.* (2023), the prolonged use of mineral fertilisers and chemical soil improvement methods (such as liming) significantly affects the dynamics of humus in grey forest soils. This alters the amount, quality, and processes of humus formation. Research indicates that only combining organic matter with moderate doses of mineral fertilisers, along with liming, promotes humus regeneration and increases the proportion of stable humic acids. This brings humus formation closer to that typical of the region's natural forest soils. Microorganisms play a crucial role by mobilising atmospheric nitrogen, breaking down organic nitrogen compounds, and converting them into plant-available forms. This reduces the need for nitrogen fertilisers, which are the most costly to produce (Potapenko & Horbachenko, 2021).

As highlighted by O. Litvinova *et al.* (2023), while most soils contain an abundance of phosphorus, approximately 40% of arable land experiences a deficiency in its available forms. The issue of phosphorus depletion due to crop harvests is becoming increasingly pressing, as phosphorus accumulation is primarily concentrated in grains and fruits rather than in vegetative biomass, which replenishes soil nutrient reserves after harvest. Consequently, the challenge lies in ensuring the availability of phosphorus compounds to plants by enhancing their mobility, specifically by converting insoluble forms into soluble ones (Kovalenko *et al.*, 2022). Microorganisms within biopreparations, by producing organic acids and enzymes, interact with minerals, releasing available potassium and silicon. This enhances the accessibility of these elements to plants. V. Hamayunova *et al.* (2021) highlight that in the face of climate change, particularly prolonged droughts, optimising crop nutrition, especially micronutrients, is crucial for achieving high-quality

yields. While the activation of beneficial soil biological processes by microorganisms in biopreparations has been extensively studied, the combined impact of microorganisms on the soil ecosystem in agricultural settings remains under-researched. Therefore, this study aimed to determine the changes in soil nutrient status and sunflower yield in response to the application of biopreparations.

MATERIALS AND METHODS

The study was conducted in 2022-2023 under agreements No. 04-2022-NP/98 dated 21 July 2022 and No. 04-2023-NP/62 dated 05 April 2023 on an agricultural land plot located in the administrative units of Kropyvnytskyi District, Kirovohrad Region, Ukraine. The land is managed by the Institute of Steppe Agriculture of the National Academy of Agrarian Sciences of Ukraine. The soil is an ordinary deep medium-humus heavy loam chernozem. In the arable soil layer where the study was conducted, the humus content was 3.97%, hydrolysable nitrogen – 10.8 mg per 100 g of soil, available phosphorus and potassium – 6.9 mg and 14.4 mg per 100 g of soil, respectively, with a pH of 5.9. Sunflower cultivation technology in the experimental plots followed standard practices for the Steppe zone. The preceding crop was spring barley after soybean. The fertilisation background was the natural soil fertility level. The experiment was conducted in triplicate. The sunflower hybrid used was LG 5555 CLP, with an established plant density of 55,000 plants per hectare at emergence.

During the flowering and ripening phases of sunflower growth, soil samples were collected from the 0-20 cm layer according to the variants of the experiment, following the DSTU 4287:2004 (2005) standard. The content of alkali-hydrolysable nitrogen was determined using the Kornfeld method, following DSTU 7863:2015 (2016). The available phosphorus and potassium compounds were analysed using the Kirsanov method, modified by the NSC ISA, in compliance with DSTU 4405:2005 (2006). The study investigated the effects of applying biopreparations with fertiliser properties during pre-sowing cultivation under different nutrient backgrounds:

- 1) No fertiliser (control 1);
- 2) $N_{60}P_{60}K_{60}$ applied during cultivation (control 2);
- 3) Groundfix, 5 L/ha applied during cultivation;
- 4) Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha applied during cultivation;
- 5) Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha + $N_{60}P_{60}K_{60}$ applied during cultivation;
- 6) Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha + $N_{42}P_{42}K_{42}$ applied during cultivation.

Groundfix is a soil-acting biopreparation. Its primary function is to enhance phosphorus and potassium nutrition in plants by converting phosphates into a soluble form, releasing retrograded phosphorus bound by secondary minerals, and mobilising potassium from hydromicas and montmorillonite-group minerals. Additionally, it increases the mobility and availability of silicon for plants throughout the entire growing season. Due to the complex of bacteria that produce organic acids, amino acids, enzymes, phytohormones, antibiotics, and other substances that positively impact plants, enhancing their adaptive and immune capabilities. A key advantage of this biopreparation is its ability to improve soil structure, aeration, and moisture retention. Composition: live bacterial cells of

Bacillus subtilis, *Bacillus megaterium* var. *phosphaticum*, *Azotobacter chroococcum*, *Enterobacter* sp., *Paenibacillus polymyxa*; other beneficial microflora (lactic acid bacteria, enzyme producers); vitamins, phytohormones, amino acids, and other physiologically active substances. The total number of viable cells is $(0.5-1.5) \times 10^9$ CFU/cm³.

Azotofit is a natural growth stimulant that actively fixes molecular atmospheric nitrogen, enriching the soil by up to 60 kg/ha (on average, 20 kg/ha). It synthesises growth-promoting substances that enhance root system development and vegetative biomass, improve seed germination, increase plant resilience to stress factors, optimise nutrient uptake, and boost crop yields. Composition: live cells of associative nitrogen-fixing bacteria.

RESULTS AND DISCUSSION

The research results demonstrated that applying biopreparations during pre-sowing cultivation positively influenced sunflower plant growth, development, and yield. Specifically, under conditions without mineral fertilisers, applying Groundfix during pre-sowing cultivation increased sunflower yield by 0.22 t/ha, or 6.9%, compared to the control (no fertiliser) yield of 3.18 t/ha (Fig. 1).

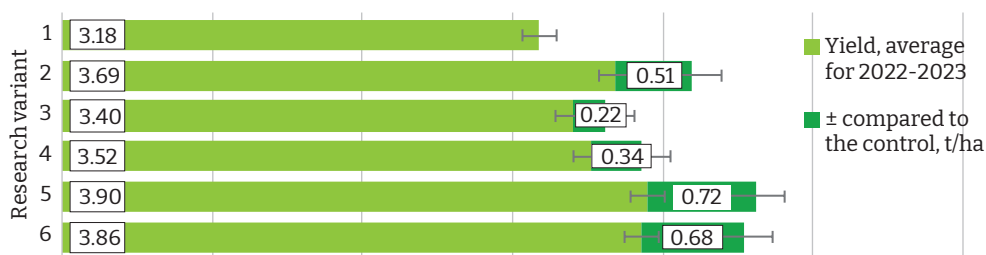


Figure 1. Sunflower yield depending on the application of biopreparations

Note: fertilisation treatments – 1. No fertiliser (control 1); 2. $N_{60}P_{60}K_{60}$ applied during cultivation (control 2); 3. Groundfix, 5 L/ha applied during cultivation; 4. Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha applied during cultivation; 5. Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha + $N_{60}P_{60}K_{60}$ applied during cultivation; 6. Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha + $N_{42}P_{42}K_{42}$ applied during cultivation

Source: developed by the authors

The combined application of Groundfix + Azotofit under these conditions increased crop productivity by 0.34 t/ha, or 10.7%, compared to control 1. It is worth noting that the application of mineral fertilisers at a dose of $N_{60}P_{60}K_{60}$

(control 2) had a significant impact on sunflower yield, with an increase of 0.51 t/ha, resulting in a yield of 3.69 t/ha. The combined use of the biopreparations Groundfix + Azotofit, with a full dose of mineral fertilisers ($N_{60}P_{60}K_{60}$) and a 30%

reduced dose ($N_{42}P_{42}K_{42}$), positively influenced crop productivity, with increases of 0.21 t/ha and 0.17 t/ha, respectively, compared to control 2, and 0.72 t/ha and 0.68 t/ha compared to control 1, yielding 3.9 t/ha and 3.86 t/ha, respectively. These results are supported by the research of V. Hama-yunova & V. Kudrina (2018) and Ye. Yurkevych *et al.* (2023), found that the application of Groundfix at a dose of 5 L/ha increased sunflower yield by 11.5% and 19.5%, respectively, compared to the treatment without the preparation.

When comparing the combined application of the biopreparations Groundfix + Azotofit with $N_{60}P_{60}K_{60}$ and $N_{42}P_{42}K_{42}$, a higher effectiveness of the biopreparations was observed when applied with a 30% reduced dose of mineral fertilisers, with the yield difference between them being minimal at 0.04 t/ha. Research conducted by Yu. Soroka *et al.* (2017) and Ye. Domarat-skyy (2018) suggests that the combined action of mineral fertilisers and biopreparations exceeded the combined effect of the two individual factors, thus demonstrating a synergistic effect: an increase in yield of 0.17 t/ha with the application of $N_{60}P_{90}$, 0.23 t/ha with the application of the biopreparation, and a 0.42 t/ha increase with the combined application.

Analysis of the yield data showed that the use of biopreparations in pre-sowing cultivation positively influenced sunflower productivity, both when no mineral fertilisers were applied and when they were used. It should be noted that the increase in sunflower productivity following the application of biopreparations to the soil occurred against the backdrop of positive changes within the soil, brought about by the action of the microbial complex. This was reflected in a reduction in the number of pathogenic fungi in the soil and the intensity of mineralisation processes, as well as a positive trend in the content of key biogenic nutrients. The findings of V. Volkogon (2018) also confirm that, under the influence of microorganisms present in biopreparations, there is mobilisation of unavailable phosphorus in the soil, particularly in the rhizosphere zone, where a gradient exists for the movement of nutrients from the soil to the roots. The application of biopreparations in pre-sowing cultivation in the current study reduced the number of pathogenic fungi in the soil, depending on the experimental variant. During the flowering phase, this reduction ranged from 2.55% to 8.6%, and during the maturation phase of the sunflower, it ranged from 5.65% to 10.1% (Fig. 2).

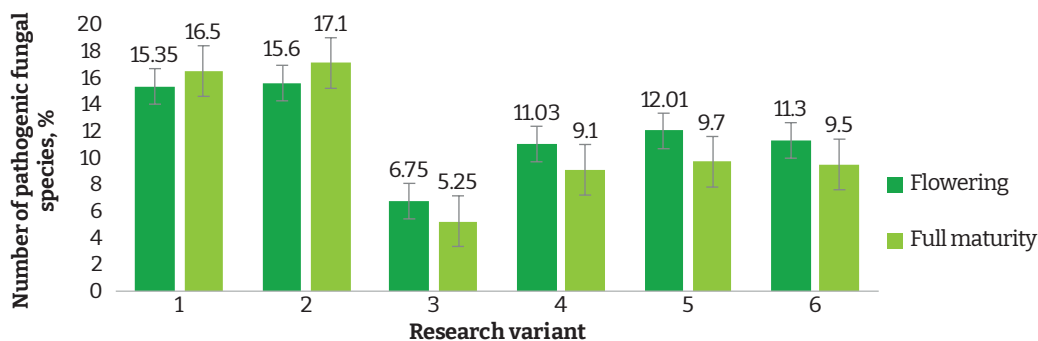


Figure 2. Number of pathogenic fungal species in the soil (%) in sunflower crops with the application of biopreparations

Note: fertilisation treatments – 1. No fertiliser (control 1); 2. $N_{60}P_{60}K_{60}$ applied during cultivation (control 2); 3. Groundfix, 5 L/ha applied during cultivation; 4. Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha applied during cultivation; 5. Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha + $N_{60}P_{60}K_{60}$ applied during cultivation; 6. Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha + $N_{42}P_{42}K_{42}$ applied during cultivation

Source: developed by the authors

Thus, the application of Groundfix in pre-sowing cultivation, with no fertilisers, resulted in a reduction of pathogens in the soil by

8.6% during the flowering phase and 10.1% during the maturation phase. When biopreparations Groundfix + Azotofit were applied together

on different nutrient backgrounds, the number of pathogenic fungi decreased by 3.34-4.32% during flowering and 5.65-6.25% during maturation. The combined use of biopreparations was less effective against pathogenic fungi in the soil, which can be attributed to the lower application rate of Groundfix, which contains microorganisms with high fungicidal activity. On the full rate of mineral fertilisers and a 30% reduced rate, with the application of Groundfix + Azotofit, the number of pathogenic fungi was slightly higher than with no fertilisers. This suggests that the application of mineral fertilisers promoted the growth of pathogenic fungi in the soil, both with the application of fertilisers alone and in combination with biopreparations. Ye. Domratskiy & O. Kozlova (2018) emphasise that the timely and rational use of biological growth

stimulants, combined with a biological fungicide, will ensure the expected sunflower yield and reduce plant infection by fungal diseases.

Changes in the soil microbiome also occurred under the influence of biopreparations. It is known that mineralisation-immobilisation ratios lower than 1.0 indicate a decrease in the rate of these processes while exceeding this value suggests undesirable intensity in mineralisation-immobilisation. When mineral fertilisers were applied (control 2), the intensity of the mineralisation processes of organic matter in the soil during the flowering phase of sunflower was the highest, with the mineralisation-immobilisation ratio at 1.45. This indicates the active use of mineral nitrogen forms from fertilisers by microorganisms, resulting in a reduction of nitrogen content in the soil and its uptake by plants (Fig. 3).

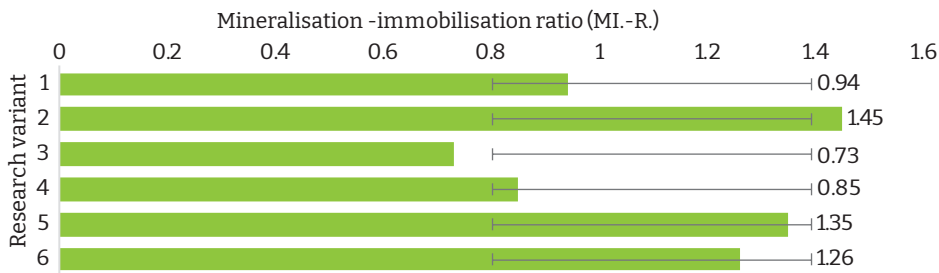


Figure 3. Mineralisation-immobilisation ratio during the flowering phase of sunflower depending on the application of biopreparations

Note: fertilisation treatments – 1. No fertiliser (control 1); 2. $N_{60}P_{60}K_{60}$ applied during cultivation (control 2); 3. Groundfix, 5 L/ha applied during cultivation; 4. Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha applied during cultivation; 5. Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha + $N_{60}P_{60}K_{60}$ applied during cultivation; 6. Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha + $N_{42}P_{42}K_{42}$ applied during cultivation

Source: developed by the authors

Such conditions result in the loss of soil organic matter. Biopreparation application reduced the intensity of mineralisation processes in the soil, improving nitrogen availability for plants. For example, the mineralisation-immobilisation ratio with Groundfix application without fertilisers was 0.73, and with the combined Groundfix + Azotofit application, it was 0.85. The positive effect of biopreparations Groundfix + Azotofit was also observed with full and 30% reduced mineral fertiliser doses, where the mineralisation-immobilisation ratio decreased to 1.26 and 1.35, respectively, compared to mineral

fertilisers alone. This indicates that biopreparations reduced competition for nitrogen between microorganisms and plants, contributing to increased yields as previously discussed. These findings are supported by research conducted by O. Tonkha *et al.* (2019). The study revealed that the level of alkaline hydrolysed nitrogen in the soil decreased throughout the growing season (from sunflower flowering to maturity) across all treatments. However, the variability of this indicator was low ($V = 4\%$). Changes in soil microbial processes, induced by the microorganisms in the biopreparations, affected soil nitrogen

dynamics and its uptake by plants. For example, in the unfertilised treatment and with Groundfix application, the alkaline hydrolysed nitrogen

content at flowering was 101 mg/kg soil (Table 1). However, the rate of nitrogen uptake by plants increased with biopreparation use.

Table 1. Changes in soil agrochemical indicators with biopreparation application in sunflower crops

Research variant	Hydrolysed nitrogen, mg/kg		Mobile phosphorus, P ₂ O ₅ , mg/kg		Mobile potassium, K ₂ O, mg/kg	
	Flowering	Full maturity	Flowering	Full maturity	Flowering	Full maturity
1. No fertiliser (control 1)	101	97	132	140	158	150
2. N ₆₀ P ₆₀ K ₆₀ applied during cultivation (control 2)	109	103	145	152	165	157
3. Groundfix, 5 L/ha applied during cultivation	101	94	142	150	161	154
4. Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha applied during cultivation	103	96	140	149	161	154
5. Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha + N ₆₀ P ₆₀ K ₆₀ applied during cultivation	109	102	151	158	172	160
6. Groundfix, 2.5 L/ha + Azotofit, 1.5 L/ha + N ₄₂ P ₄₂ K ₄₂ applied during cultivation	106	98	147	156	165	156

Source: developed by the authors

Applying Groundfix in combination with Azotofit improved the soil nitrogen status by 2 mg/kg compared to using Groundfix alone. This is attributed to the high nitrogen-fixing capacity of the bacteria in Azotofit. In treatments with mineral fertilisers alone (N₆₀P₆₀K₆₀) and in combination with biopreparations Groundfix + Azotofit, the alkaline hydrolysed nitrogen level in the soil increased to 109 mg/kg. Applying Groundfix + Azotofit with N₄₂P₄₂K₄₂ resulted in a slight decrease in nitrogen. According to the authors, this is due to the increased mineralisation processes in the soil and the enhanced nitrogen uptake during sunflower vegetative growth.

During sunflower maturity, there was a slight decrease in alkaline hydrolysed nitrogen content in the soil with biopreparation use, both with and without mineral fertilisers. This indicates increased nitrogen uptake by sunflower plants and the inability of nitrogen-fixing microorganisms to fully compensate for nitrogen demand. The available phosphorus content in the soil increased throughout the growing season, with low variability (V=4-5%) observed during this period.

The complex of microorganisms introduced with the Groundfix biopreparation facilitated the more efficient conversion of unavailable phosphorus compounds, both organic

and mineral, into plant-available forms. This is evidenced by the increased levels of available phosphorus in the soil and its uptake in the crop yield. Applying Groundfix during pre-sowing cultivation increased available phosphorus in the soil by 10 mg/kg during flowering, reaching 145 mg/kg, compared to the control level of 132 mg/kg. Applying Groundfix in combination with Azotofit during presowing cultivation reduced available phosphorus in the soil by 2 mg/kg compared to using Groundfix alone, resulting in 140 mg/kg. This is attributed to the lower dose of Groundfix and the primary function of Azotofit, which focuses on nitrogen fixation and plant stimulation. Applying Groundfix + Azotofit with N₆₀P₆₀K₆₀ and N₄₂P₄₂K₄₂ increased available phosphorus in the soil by 6 and 2 mg/kg, respectively, compared to applying the full mineral fertiliser dose alone (control 2).

Analysis of available phosphorus in the soil during sunflower maturity indicates the continued activity of the Groundfix biopreparation's microorganisms in decomposing organic matter and converting sparingly soluble phosphates into available forms. For example, applying Groundfix at 5 L/ha during pre-sowing cultivation increased available phosphorus by 10 mg/kg compared to the control, reaching 150 mg/kg (Table 1). With the combined

application of Groundfix + Azotofit, available phosphorus decreased by 1 mg/kg compared to Groundfix alone, resulting in 149 mg/kg. This slight reduction is within a trend and is attributed to higher phosphorus uptake by the crop yield. Applying Groundfix + Azotofit with $N_{60}P_{60}K_{60}$ and $N_{42}P_{42}K_{42}$ increased available phosphorus by 8 and 6 mg/kg, respectively, compared to mineral fertiliser application alone (control 2), reaching 158 and 156 mg/kg. It is important to note that available phosphorus in the soil increased with the application of Groundfix + Azotofit under different nutrient conditions, particularly with mineral fertiliser application.

Sunflower is a potassium-demanding crop, making potassium crucial for its nutrition. A deficiency in this element significantly impacts yield, oil content in the seeds, and plant susceptibility to diseases. Analysing the seasonal dynamics of available potassium in the typical medium-humus, deep, heavy loam chernozem soil, a decrease in potassium content was observed across all treatments throughout the growing season. From flowering to maturity, available potassium levels declined, although they remained within high sufficiency ranges. The decrease in available potassium in the soil is due to its uptake by plants. As plants grow and develop, nutrient absorption naturally leads to a reduction in potassium concentration in the soil solution.

As indicated by the soil sample analysis from the sunflower flowering stage, available potassium increased from 158 mg/kg in the control to 161 mg/kg with Groundfix application. Adding Azotofit (variant 4) did not affect the available potassium in the soil, remaining at 161 mg/kg. The combined application of Groundfix + Azotofit with $N_{60}P_{60}K_{60}$ increased available potassium in the soil by 7 mg/kg (172 mg/kg) compared to applying $N_{60}P_{60}K_{60}$ alone (control 2). With $N_{42}P_{42}K_{42}$, the potassium level was similar to control 2 (165 mg/kg). Samples taken at sunflower maturity showed a similar trend in available potassium dynamics. Changes in available potassium in sunflower crop soil are directly influenced by the complex of microorganisms introduced into the soil, alterations in microbial processes, and plant uptake. Nutrient absorption naturally leads to a decrease in potassium concentration in the soil solution.

CONCLUSIONS

This study found that using biopreparations during pre-sowing cultivation positively influenced sunflower growth, development, and yield. Applying biopreparations without mineral fertilisers increased sunflower yield: Groundfix increased yield by 0.22 t/ha (6.9%), and the combined application of Groundfix + Azotofit increased yield by 0.34 t/ha (10.7%) compared to the control. With mineral fertiliser application at $N_{60}P_{60}K_{60}$, sunflower yield increased by 0.51 t/ha (3.69 t/ha total), and when combined with biopreparations, yield increased by an additional 0.17-0.21 t/ha. Biopreparations were more effective with a 30% reduced mineral fertiliser dose, with only a 0.04 t/ha yield difference between the full and reduced doses.

Biopreparation application reduced pathogenic fungi in the soil by 2.55-8.6% during flowering and by 5.65-10.1% during sunflower maturity. Groundfix was most effective without mineral fertilisers, while the combined biopreparation application was less effective due to the reduced Groundfix dose. Biopreparations reduced the intensity of mineralisation processes in the soil, enhancing nitrogen availability for plants. The mineralisation-immobilisation ratio was 1.45 with mineral fertilisers alone, but only 0.73 with Groundfix application, indicating reduced soil organic matter loss and improved nitrogen availability.

The application of biopreparations positively influenced the content of essential nutrients in the soil. The level of alkaline hydrolysed nitrogen at flowering increased to 101 mg/kg with Groundfix, and by an additional 2 mg/kg with the combined application of Groundfix + Azotofit. Groundfix application increased available phosphorus at flowering by 10 mg/kg (to 145 mg/kg), and the combined application with Azotofit reached 140 mg/kg. In treatments with mineral fertilisers, biopreparations further increased available phosphorus by 6-8 mg/kg compared to mineral fertilisers alone. Analysis of potassium dynamics showed a decrease in soil potassium content throughout the growing season, attributed to active sunflower uptake. However, sufficiency levels remained high, indicating effective potassium mobilisation by the biopreparation microorganisms.

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None.

CONFLICT OF INTEREST

None.

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Оптимізація живлення рослин під дією біопрепаратів в інтегрованих технологіях вирощування соняшнику

Анотація. Феномен зростання виробництва соняшнику має поліфакторіальне походження – нові високоінтенсивні гібриди, довершена система удобрення, збалансована система захисту рослин, сучасна високоефективна техніка. Тому для подальшого удосконалення технології потрібно знаходити нові шляхи, пов'язані із взаємодією факторів, або комбінативне їх використання. Одним з напрямів такої взаємодії є застосування біопрепаратів, які водночас вирішують не лише питання росту продуктивності, але й зменшують пестицидне навантаження, що є своєчасним і актуальним в сучасних екологічних умовах. Метою досліджень було встановлення впливу мінеральних добрив та позакореневого підживлення біопрепаратом на продуктивність насіння соняшнику. Досліджувався вплив застосування мінеральних добрив і біопрепаратів на формування продуктивності насіння соняшнику. Встановлено вплив біопрепаратів на ріст, розвиток та продуктивність рослин соняшника за різних фонових умов мінерального живлення. Встановлено, що внесення біопрепаратів під передпосівну культивування позитивно впливало на врожайність культури як за відсутності мінеральних добрив, так і за їх внесення. Використання біопрепаратів сприяло підвищенню врожайності соняшника, зокрема, внесення одного з досліджуваних біопрепаратів забезпечило приріст на 6,9 %, а сумісне застосування двох – на 10,7 % порівняно з контролем без добрив. На фоні мінерального живлення ($N_{60}P_{60}K_{60}$) приріст врожаю від застосування біопрепаратів становив 0,21-0,17 т/га залежно від дози добрив. Дослідження також виявили позитивні зміни в мікробіологічному стані ґрунту під дією

біопрепаратів. Зниження кількості патогенних грибів у фазу дозрівання досягало 10,1 % за застосування біопрепарату на фоні без добрив. Використання біопрепаратів також знижувало інтенсивність мінералізаційних процесів, що сприяло ефективнішому використанню азотурослинами. Динаміка вмісту лужногідролізованого азоту, рухомого фосфору та калію підтверджувала позитивний вплив біопрепаратів на біогенний режим ґрунту та живлення рослин. Дослідження підтвердили доцільність застосування біопрепаратів у технології вирощування соняшника, що сприяло підвищенню врожайності культури та поліпшенню екологічного стану ґрунтів. Результати дослідження можуть бути використані для реалізації генетичного потенціалу рослин соняшнику з метою формування стабільної його продуктивності

Ключові слова: урожайність; лужногідролізований азот; рухомий фосфор; рухомий калій; мікробіологічний стан ґрунту

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