Effect of Plant Growth Regulators on Regulating of the Expression of Genes Encoding Seed Traits in Soybean (*Glycine max* L.)

Ghofran Mohammed Abbass [1], Ayoob Obaid Alfalahi,[2]

1Department of Field Crops, College of Agriculture, University of Anbar, Anbar 55431, Iraq

2Department of Plant Protection, College of Agriculture, University of Anbar, Anbar 55431, Iraq

Abstract

This study was conducted to investigate the efficacy of plant growth regulators (PGRs) and their application stages in regulating the expression of genes encoding seed traits in soybean. The field experiment evaluated three selected PGRs—Gibberellic acid (GA), Abscisic acid (ABA), and Brassinolide (BR)—applied at a concentration of 100 mg L^{-1} across four distinct growth stages: the late vegetative stage (V3), flowering (R1), pod setting (R3), and seed filling (R6). The treatments were arranged in a factorial experiment using a Randomized Complete Block Design (RCBD) with three replications.

The study evaluated the efficacy of these factors in regulating the expression levels of the *GmCKX3* and *GmCIF1* genes, which are responsible for seed traits. The results revealed a significant down-regulation of gene expression levels influenced by the PGRs and application stages, with the BR treatment and the V3 stage exhibiting the most pronounced effects. Regarding phenotypic traits, foliar application of GA and BR significantly increased the 100-seed weight, reaching 13.46 g and 13.11 g, respectively, whereas the ABA treatment recorded the lowest average (11.85 g). Among the application stages, the V3 stage achieved the highest 100-seed weight average of 13.29 g. Furthermore, BR application led to a significant reduction in the percentage of severely wrinkled seeds (9.17%) while simultaneously increasing the percentage of non-wrinkled seeds (78.50%). This improvement positively reflected on the total seed yield, which peaked under the BR treatment (4.376 t ha^{-1}) and the V3 application stage (4.170 t ha^{-1}).

Keywords: Soybean, Plant Growth Regulators, Brassinolide, Gene Expression

Introduction

Soybean (*Glycine max L.*) is one of the most vital oilseed crops worldwide, holding substantial economic significance due to its diverse applications and high market value. Soybean seeds are classified as a rich, cost-effective source of protein for human consumption, in addition to containing high percentages of oil, carbohydrates, and fats, making them an essential dietary component and energy source [8,3]. Soybean is widely used as a raw material in numerous industries, particularly in nutrition, serving as a core component in animal protein feed formulations. It is also considered one of the primary sources of vegetable oil globally, ranking alongside major oilseed crops such as sunflower and maize.

Most soybean breeding and improvement programs have successfully developed modern cultivars with enhanced seed characteristics compared to their wild ancestors, primarily focusing on traits such as seed color, shape, size, and weight [16]. Moreover, crop seeds represent the primary reproductive organs in seed plants, playing a critical role in preserving species, propagation, and productivity. However, seed traits frequently deteriorate

ISSN 2072-3857

due to exposure to various biotic and abiotic stresses during development, which negatively affect both qualitative and quantitative attributes. On the other hand, the quality of soybean seeds is evaluated through several indices, including size, shape, weight, and the degree of wrinkling. These traits are crucial not only for the market value of the seeds but also for their agricultural importance in germination and field establishment. Given their sensitivity to complex interactions between genetic and environmental factors, these traits have become primary targets in soybean breeding programs aimed at improving crop potential and environmental adaptation [20, 7].

Locally, the crop suffers from several production challenges, foremost among which is the deterioration of seed characteristics at harvest, particularly the issue of seed wrinkling and irregular growth dynamics. This phenomenon typically occurs when plants prolong vegetative growth and fail to transition smoothly into the reproductive phase. Consequently, this leads to a rapid rate of moisture loss accompanied by a sharp decline in dry matter accumulation due to unfavorable maturation conditions during the final stages of crop development.

In this context, in terms of crop yield, soybean seed size is strongly correlated with subsequent seed viability and early-stage plant growth. Larger seeds generally possess greater nutrient reserves, which enhances seed vigor, germination percentage, and tolerance to environmental stresses during the initial post-germination phases. Seed size also directly contributes to seed weight; however, an increase in seed size can sometimes be accompanied by a reduction in the total number of produced seeds, indicating a physiological trade-off between seed size and number [28]. As seed weight constitutes one of the primary determinants of soybean productivity, it reflects the efficiency of nutrient accumulation during the seed-filling period, making it a widely adopted selection criterion in soybean management and improvement programs.

During the final stages of the crop life cycle, soybean seeds are susceptible to shrinkage (wrinkling), which is a major defect that severely impairs seed yield and quality traits. Wrinkling occurs due to incomplete seed filling triggered by sudden environmental stresses at the end of the growing season—such as drought, elevated temperatures, nutrient deficiencies, or pathogen infections—in addition to genetic factors. Wrinkled seeds are characterized by reduced size and weight, low oil and protein accumulation, poor germination, decreased vigor, and diminished market value [18,2].

Numerous studies have employed modern molecular techniques, including genomics, proteomics, and transcriptomics, to identify the key genes associated with seed development in legumes, including soybean [6,1,11]. Indeed, a set of genes associated with quantitative traits regulating soybean seed yield has been identified through their influence on vegetative growth attributes—such as the number of branches on the main stem, plant height, node number, pod number, and seed number—as well as seed weight [22].

The exogenous foliar application of various plant growth regulators (PGRs)—including auxins, cytokinins, gibberellins, abscisic acid (ABA), and ethylene—presents a promising approach for enhancing agricultural production. This has led to an increasing interest in studying the physiological changes and endogenous plant responses resulting from their application [15]. The use of these regulators, whether stimulatory or inhibitory, exerts significant effects on the

ISSN 2072-3857

plant's enzymatic system, thereby rearranging internal metabolic activities and altering plant morphology [13]. Accordingly Several studies have focused on utilizing these regulators to improve soybean seed traits, following verified evidence of their positive impact on elevating crop yield and quality, especially when synchronized with a clear understanding of the regulator's mode of action and its critical application timing [26,4,14]. Study Objectives

The objectives of this study were as follows:

To evaluate the effects of three selected plant growth regulators (GA, ABA, and BR) and their four application timings [late vegetative stage (V3), flowering (R1), pod setting (R3), and seed filling (R6)] on seed traits, growth, and yield of soybean.

To investigate the influence of these two studied factors on the expression levels of the *GmCKX3* (Cytokinin oxidase) and *GmCIF1* (Cell wall invertase inhibitor) genes and their role in regulating seed maturation and filling in soybean plants.

Material and Methods

Prepare samples:

Experimental Site and Plant Material

A field experiment was conducted during the summer season at the Abu Ghraib Research Station, Department of Agricultural Research, Ministry of Agriculture. Experimental Design and Treatments

The experimental design evaluated two main factors The study evaluated two main factors:

Plant Growth Regulators (PGRs): Three selected growth regulators, namely Gibberellic acid (GA), Abscisic acid (ABA), and Brassinolide (BR), were applied as a foliar spray at a uniform concentration of 100 mg L^{-1}.A control treatment (sprayed with distilled water) was included for comparison. Application Stages: The foliar treatments were synchronized with four critical growth stages of the soybean crop: Late vegetative stage (V3), Flowering stage (R1), Pod setting stage (R3), and Seed filling stage (R6). All standard agricultural practices, including soil preparation, fertilization, irrigation, and weed control, were applied uniformly across all experimental units in accordance with local production recommendations.



Figure 1: The four growth stages for applying plant growth regulators in soybean (Al-Shaimaa cultivar). V3: Flower bud stage R1: Flowering stage R3: Pod set stage R6: Seed filling stage.

ISSN 2072-3857

Phenotypic and Yield Measurements

The following agricultural, phenotypic, and molecular parameters were measured and evaluated to determine the impact of the treatments:

100-Seed Weight (g): Determined by counting and weighing 100 random seeds from each experimental unit.

Seed Wrinkling Percentages (%): Harvested seeds were visually inspected and classified based on the degree of surface shrinkage into three distinct categories: Severe wrinkling percentage (%). Moderate wrinkling percentage (%). Non-wrinkled seeds percentage (%).

Total Seed Yield t/ha^{-1}: Harvested from the central rows of each plot, adjusted to standard moisture content, and converted to total yield per hectare.

Gene Expression Measurement (Molecular Analysis): The relative expression levels of the target genes responsible for seed development and trait regulation, namely *GmCKX3* (Cytokinin oxidase) and *GmCIF1* (Cell wall invertase inhibitor), were quantified using Quantitative Real-Time PCR (qRT-PCR) and normalized against the Actin11 housekeeping gene.

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

To assess the molecular regulation of seed traits, fresh tissue samples were collected from the developing seeds following the respective treatment stages. Total RNA was extracted using a specialized plant RNA extraction kit according to the manufacturer's protocol. The quality and concentration of the extracted RNA were verified using a Nanodrop spectrophotometer. Complementary DNA (cDNA) synthesis was performed using a reverse transcription kit.

Quantitative Real-Time PCR (qRT-PCR) was executed to quantify the expression levels of the target genes: *GmCKX3* (encoding Cytokinin oxidase) and *GmCIF1* (encoding Cell wall invertase inhibitor). The Actin11 gene was utilized as the internal reference (housekeeping) gene to normalize the expression data.

Gene Expression Calculations

Following the determination of the mean cycle threshold (C_T) values for the targeted genes (*GmCKX3* and *GmCIF1*) across all treatments involving different plant growth regulators (PGRs) and foliar application stages, relative gene expression levels were calculated according to the method described by Livak and Schmittgen [23]. as follows:

-ΔCt = Ct gene – Ct reference gene (*Actin11*)

ΔΔCt = ΔCt of each sample – ΔCt of Control (not treated sample)

Exppression fold change = 2- ΔΔCt

Depending on that the expression fold for Control should be 2^{-0} =1

Consequently, the expression fold change for the control treatment was normalized to 2^0 = 1.

Statistical Analysis

Data were collected, tabulated, and subjected to analysis of variance (ANOVA) for factorial experiments using a Randomized Complete Block Design (RCBD) with three replications. Significant differences between treatment means were determined using the Least Significant Difference (LSD) test at a significance level of P \le 0.05 via the GenStat statistical software.

Results and Discussion(

1-100-Seed Weight (g)

ISSN 2072-3857

The results presented in Table (1) indicate significant differences among the three PGR types, their four application stages, and the binary interaction between them regarding the 100-seed weight of soybean. Foliar application of Gibberellic Acid (GA at 100 mg L^{-1}) achieved the highest 100-seed weight 13.46 g, which did not differ significantly from the Brassinolide (BR at 100 mg L^{-1}) treatment 13.11g. Conversely, the Abscisic Acid (ABA at 100 mg L^{-1}) treatment significantly reduced the trait to its lowest mean value 11.85 g, compared to the control treatment (Co), which recorded 11.31g.

Regarding the application stages, the late vegetative / flower bud stage (V3) produced the highest 100-seed weight 13.29 g, whereas the seed filling stage (R6) recorded the lowest mean 11.84 g. The flowering stage (R1) yielded a mean value of 12.47 g, showing no significant difference from the pod setting stage (R3), which achieved 12.13 g.

The significant interaction between PGRs and application stages Showed variations across different PGR types. The interaction between BR (100 mg $^{L-}$1) and the V3 stage (BR and V3) exhibited the highest 100-seed weight 14.32 g. In contrast, the interaction between ABA (100 mg L^{-1}) and the pod setting stage (ABA and R3) resulted in the lowest mean value 11.12 g.

Soybean yield is substantially influenced by seed weight, which represents the final yield component of the crop. Seed weight is directly correlated with seed size and is regulated by both endogenous growth factors and exogenous conditions. Applying PGRs at a uniform concentration of 100 mg L^{-1} at specific developmental stages enhances seed weight and size, thereby optimizing the total seed yield of soybean. Since the V3 stage precedes critical reproductive events in flowering plants and is tightly linked with subsequent reproductive phases, its optimization contributes to maximizing crop productivity. Seed weight and size are not only crucial for plant evolutionary fitness but are also dynamically linked to seed number; both factors concurrently dictate total seed yield [12].

The superiority of the BR treatment (100 mg L^{-1}) at a concentraction of 100 mg L^{-1} in increasing the 100-seed weight can be attributed to its capacity to directly stimulate dry matter accumulation within the seeds. This stimulation is mediated by enhanced sucrose synthase activity, which increases the sink strength of the seeds, allowing them to efficiently import sugars and amino acids from the source leaves and convert them into starch, proteins, and oils within the maturing seed. Furthermore, BR applied at 100 mg L^{-1} prolongs the seed-filling duration by delaying leaf senescence (extending the green-filled period). This is supported by the observed significant superiority of the BR treatment across most vegetative growth parameters and yield attributes.

Molecularly, the enhanced dry matter accumulation under BR treatment (100 mg L^{-1}) corresponds to the down-regulation or inhibition of *GmCKX3* and *GmCIF1* gene activities, which increases the rate and duration of assimilate translocation into the seeds. BR application at 100 mg L^{-1} negatively regulated the expression of the *GmCKX3* gene, which is responsible for degrading active cytokinins in flower buds. Suppressing this gene promotes robust cell division, thereby expanding the sink capacity of the developing seed to accommodate greater carbohydrate assimilation, which ultimately elevates seed weight. Concurrently, the down-regulation of *GmCIF1* enhances the activity of enzymes responsible for developing the vascular tissues within the pod walls and developing seeds, ensuring an uninterrupted flow of metabolites into the seeds.

ISSN 2072-3857

Table 1. Effect of plant growth regulators and application stages on the 100-seed weight (g) of soybean.

Average	Adding stage				Growth regulator
	R6	R3	R1	V3	
11.31	10.77	11.14	11.08	12.24	Co
13.46	13.16	13.28	13.66	13.75	GA
11.85	11.64	11.12	11.78	12.86	ABA
13.11	11.77	12.99	13.34	14.32	BR
	11.84	12.13	12.47	13.29	average
Interaction	Adding stage		Growth regulator		LSD0.05
1.13	0.57		0.57		

2-Percentage of Severely Wrinkled Seeds (%)

The results in Table (2) reveal significant effects of the studied factors and their binary interaction on the percentage of severely wrinkled seeds. The BR treatment successfully minimized the occurrence of severely wrinkled seeds, recording the lowest mean value 9.17 %, followed by the ABA treatment 10.00 % and the GA treatment 11.67 %. In contrast, the control treatment sprayed with distilled water only (Co) exhibited the highest incidence of severe seed wrinkling 22.92 %.

Table (2) also indicates significant variations among application stages. The flowering stage (R1) recorded the lowest percentage of severely wrinkled seeds 10.00 %, whereas the seed filling stage (R6) generated the highest incidence 16.17 %. The V3 stage demonstrated a relatively low percentage 12.25 %, while the ratio increased to 15.33 % during the pod setting stage (R3).

Regarding interactions, the combination of ABA and the flowering stage (ABA and R1) achieved the lowest percentage of severely wrinkled seeds 3.33 %, whereas the highest percentage 16.00 % was recorded under the interaction between GA and the seed filling stage (GA and R6).

The commercial and industrial value of seed crops, particularly soybean, depends heavily on harvested seed quality, which is reflected in size uniformity, proper maturation, and a minimal wrinkling percentage [15]. Furthermore, seed quality directly influences subsequent field

performance, particularly seed germination and seedling vigor [17].

The severity of seed wrinkling is closely linked to the translocation rate of metabolic compounds from vegetative source tissues to seed sinks, as well as the ratio of dry matter accumulation relative to moisture loss during the final maturation stages, especially under adverse environmental conditions (Figure 2). Seed wrinkling is also influenced by the duration of seed filling; a truncated maturation period due to cultivar characteristics may restrict the accumulation of metabolites during the active filling window [5,21].

The efficacy of BR (100 mg L^{-1}) in reducing the percentage of severely wrinkled seeds compared to other regulators highlights its regulatory role in

ISSN 2072-3857

down-regulating key genes that govern assimilate transport and sink capacity, thereby extending the seed-filling period. The *GmCKX3* gene modulates cytokinin degradation, maintaining metabolic homeostasis in flowers and developing seeds. Meanwhile, the down-regulation of *GmCIF1* increases sink strength and assimilate accumulation in the seeds, effectively reducing the wrinkling percentage while increasing seed weight [9,29].

Table 2. Effect of plant growth regulators and application stages on the percentage of severely wrinkled seeds (%) of soybean.

Average	Adding stage				Growth regulator
	R6	R3	R1	V3	
22.92	23	22.33	22.67	23.67	Co
11.67	16.00	12.00	10.00	8.67	GA
10.00	13.33	15.33	3.33	8.00	ABA
9.17	12.33	11.67	4.00	8.67	BR
	16.17	15.33	10.00	12.25	average
intraction	Adding stage		Growth regulator		$LSD_{0.05}$
1.68	0.84		0.84		

3-Percentage of Moderately Wrinkled Seeds (%)

The results in Table (3) demonstrate significant differences among the studied factors and their binary interaction regarding the percentage of moderately wrinkled seeds.

A significant variance was observed among the PGR treatments. The BR treatment recorded the lowest percentage of moderately wrinkled seeds 12.33 %, followed by the GA treatment 15.67 %. The ABA treatment increased the percentage to 16.92\%, which was significantly lower than the highest value recorded in the control treatment 29.42 %.

Significant variations were also observed among the four foliar application stages. The flowering stage (R1) yielded the lowest percentage of moderately wrinkled seeds 15.92 %, while the pod setting (R3) and seed filling (R6) stages both recorded 19.67 %. The V3 stage resulted in a moderate wrinkling percentage of 19.08\%.

The interaction between the factors showed significant effects on this trait. The interaction between BR and the V3 stage (BR and V3) minimized the percentage of moderately wrinkled seeds to its lowest value 9.67 %, whereas the interaction between GA and the V3 stage (GA and V3) produced the highest value among the treated groups 19.33 %.

ISSN 2072-3857

Table 3. Effect of plant growth regulators and application stages on the percentage of moderately wrinkled seeds (%) of soybean.

average	Adding stage				Growth regulator
	R6	R3	R1	V3	
29.42	27.33	33.33	27.67	29.33	Co
15.67	16	16.33	11	19.33	GA
16.92	19	16	14.67	18	ABA
12.33	16.33	13	10.33	9.67	BR
	19.67	19.67	15.92	19.08	average
intraction	Adding stage		Growth regulator		$LSD_{0.05}$
1.574	0.787		0.787		

4-Percentage of Non-Wrinkled Seeds (%)

The analysis of variance presented in Table (4) indicates significant effects of the main factors and their interaction on the percentage of non-wrinkled seeds in soybean (cv. Shaima).

The PGR treatments differed significantly; BR application achieved the highest percentage of non-wrinkled seeds 78.50 %, whereas the GA treatment recorded 72.67 %. The ABA treatment resulted in a non-wrinkled seed percentage of 73.08 %, compared to the control treatment, which recorded the lowest value 47.67 %.

Furthermore, the application stages showed significant variation. The flowering stage (R1) was superior, yielding the highest percentage of non-wrinkled seeds 74.08 %, followed by the V3 stage 68.67 %. The percentage declined during the pod setting (R3) and seed filling (R6) stages to 65.00\% and 64.17 %, respectively.

The interaction between the two factors was also significant. The interaction between BR and the flowering stage (BR and R1) achieved the highest percentage of non-wrinkled seeds 85.67 %. Conversely, the lowest percentage among the treated plants was observed under the interaction of ABA and the seed filling stage (ABA and R6), which recorded 67.67 %.

Exogenous application of PGRs during early developmental phases (V3 and R1) promotes seed vigor and growth within an optimal filling duration, facilitating maximum dry matter accumulation and seed expansion, which elevates both crop yield and quality. PGRs can perceive cellular disruptions, modulate metabolic pathways, and facilitate the source-to-sink translocation of assimilates (from leaves to pods and seeds), thereby improving seed filling efficiency and generating well-developed, highly viable seeds.

Seed viability and integrity are directly associated with the capacity to absorb metabolic compounds following successful fertilization, pod setting, and seed formation. Modulating key genes related to seed development, such as *GmCKX3*, directly affects cell division in developing seeds. Because *GmCKX3* encodes the enzyme responsible for degrading endogenous plant cytokinins, suppressing its expression maintains active cytokinin

ISSN 2072-3857

levels, enhancing seed growth and sink capacity [10]. Concurrently, the expression of *GmCIF1* inhibits cell wall invertase (CWI), which cleaves sucrose into glucose and fructose. Suppressing *GmCIF1* enhances sucrose breakdown and sugar transport into the seeds, maximizing seed filling efficiency and overall seed quality [25,27].

Table 4. Effect of plant growth regulators and application stages on the percentage of non-wrinkled seeds (%) of soybean.

Average	Adding stage				Growth regulator
	R6	R3	R1	V3	
47.67	49.67	44.33	49.67	47	**Co**
72.67	68	71.67	79	72	**GA**
73.08	67.67	68.67	82	74	**ABA**
78.5	71.33	75.33	85.67	81.67	**BR**
	64.17	65	74.08	68.67	**average**
Intraction	**Adding stage**		**Growth regulator**		**$LSD_{0.05}$**
3.412	1.706		1.706		

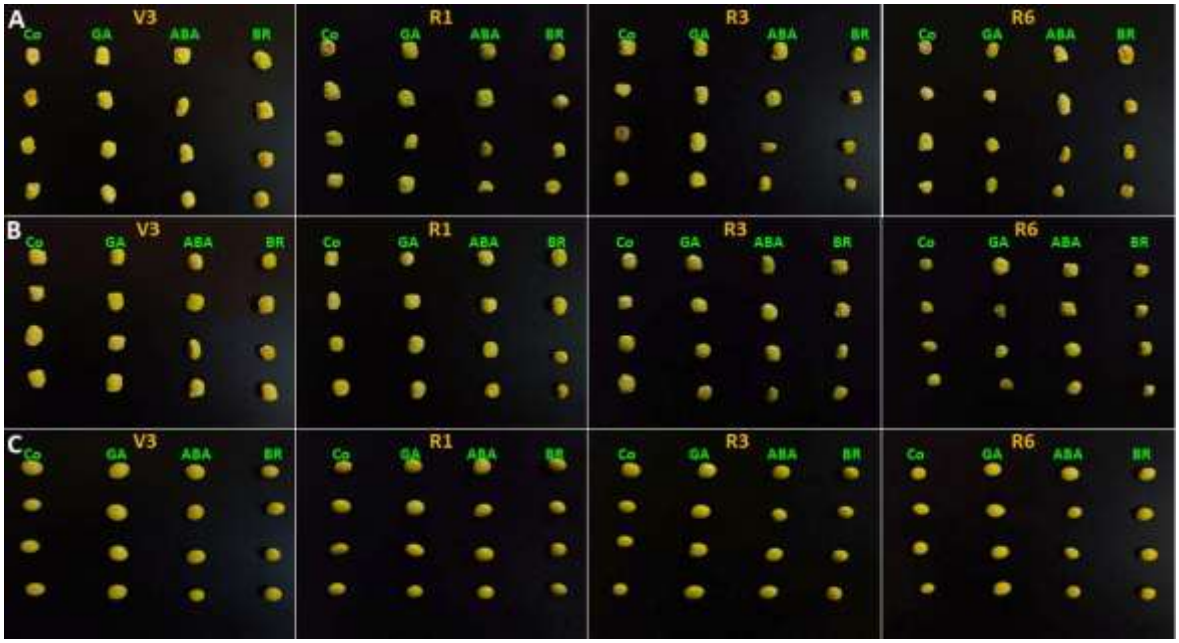


Figure 2: Effects of plant growth regulators (GA: Gibberellic Acid, ABA: Abscisic Acid, BR: Brassinolide, Co: Control) and application stages (V3: late vegetative/flower bud, R1: flowering, R3: pod setting, R6: seed filling) on the wrinkling percentage of soybean seeds (cv. Shaima).

ISSN 2072-3857

5-Total Seed Yield (t/ ha^{-1})

The results in Table (5) indicate that total seed yield was significantly influenced by the foliar application of different PGRs, application stages, and their binary interaction.

The BR treatment achieved the highest total seed yield 4.376 t ha^{-1}, while the ABA treatment recorded the lowest yield 3.244 t ha^{-1}. Foliar application of GA produced a seed yield of 3.943 t ha^{-1}, compared to the control treatment (Co), which yielded 2.232 t/ ha^{-1}.

Regarding the application stages, the V3 stage was superior, achieving the highest mean seed yield (4.170 t ha^{-1}, whereas the yield decreased to 2.573 t ha^{-1} when PGRs were applied at the seed filling stage (R6). Applications at the flowering (R1) and pod setting (R3) stages resulted in yields of 3.725 t ha^{-1} and 3.328 t ha^{-1}, respectively.

The interaction between the two factors significantly altered total seed yield. The combination of BR and the V3 stage (BR \times V3) produced the highest total seed yield (5.726 t ha^{-1} while the control treatment at the R6 stage (Co and R6) generated the lowest yield (1.660 t ha^{-1}.

The increased seed yield under the BR treatment is attributed to its positive impact on vegetative growth parameters, particularly leaf area, which correlates positively with photosynthetic efficiency and prolonged physiological activity of the leaves, thereby enhancing dry matter translocation to the seeds. Furthermore, BR application increased the number of branches per plant, total pods per plant, and seeds per pod, directly translating into higher total seed yield.

BR also optimized source-to-sink relationships, promoting metabolite transport, improving seed traits, and reducing the seed wrinkling percentage relative to the control. This increase coincided with a marked down-regulation in the expression levels of both *GmCKX3* and *GmCIF1*. Suppressing *GmCKX3* expression maintained cytokinin flow and stimulated cell division, increasing the capacity of the seed to accumulate dry matter. This synergy worked alongside the inhibition of *GmCIF1* (cell wall invertase inhibitor), which promoted sucrose-cleaving enzymes to convert sucrose into simple sugars, facilitating smooth translocation into the cotyledons and ensuring high seed filling efficiency.

Table 5. Effect of plant growth regulators and application stages on the total seed yield t/ ha^{-1} of soybean.

Average	Adding stage				Growth regulator
	R6	R3	R1	V3	
2.232	1.660	2.264	2.332	2.673	Co
3.943	3.322	3.905	4.210	4.334	GA
3.244	2.610	2.826	3.595	3.946	ABA
4.376	2.701	4.315	4.763	5.726	BR
	2.573	3.328	3.725	4.170	average
Intraction	Adding stage		Growth regulator		$LSD_{0.05}$
0.117	0.058		0.058		

Gene Expression Analysis **6- *GmCKX3* Gene Expression**

ISSN 2072-3857

The results in Table (6) show significant differences in the relative expression levels of the *GmCKX3* gene as influenced by PGR types, application stages, and their interaction.

GA application caused the most pronounced down-regulation of the targeted gene, reducing its relative expression level to a mean of 0.789 -21.1 % decrease relative to the control). Conversely, the ABA treatment caused a significant up-regulation, increasing gene expression to 1.115 (an 11.5 % increase. The BR treatment also induced a down-regulation, reducing the mean expression level to 0.874 relative to the control.

Regarding the application timings, the V3 and R1 stages recorded the lowest relative expression levels for *GmCKX3* (0.904), representing a 9 % reduction compared to the control. The pod setting stage (R3) recorded the highest relative gene expression (1.123, a 12.3 % increase), followed by the seed filling stage (R6) at 1.080 (an 8\% increase).

The interaction between the two factors significantly affected *GmCKX3* expression. The interaction between BR and the V3 stage (BR and V3) resulted in the lowest relative expression level (0.612),

representing a significant down-regulation of -38.8 %. In contrast, the interaction between ABA and the seed filling stage (ABA and R6) generated the highest up-regulation (1.174, a 17.4 % increase relative to the control).

The *GmCKX3* gene plays a crucial role by encoding the enzyme cytokinin oxidase/dehydrogenase (CKX), which metabolically cleaves active cytokinins, thereby limiting their physiological activity. Consequently, it acts as a negative regulator of growth and assimilate accumulation within sinks, particularly seeds, impacting the rate of pod development and seed filling in soybean. Recent studies have highlighted elevated expression levels of this gene in maternal tissues, flowers, and emerging seeds, emphasizing its critical role in the development of reproductive organs in soybean [8].

Table 6. Effect of plant growth regulators and application stages on the relative expression levels of the *GmCKX3* gene in soybean.

Average	Adding stage				Growth regulator
	R6	R3	R1	V3	
1.00	1.00	1.00	1.00	1.00	Co
0.789	0.876	0.991	0.655	0.634	GA
1.115	1.174	1.011	1.128	1.146	ABA
0.874	1.043	1.052	0.787	0.612	BR
	1.080	1.123	0.904	0.904	average
Intraction	Adding stage		Growth regulator		$LSD_{0.05}$
0.094	0.047		0.047		

7-*GmCIF1* Gene Expression

Table (7) outlines the significant variance in the relative expression levels of the *GmCIF1* gene under the influence of PGR

ISSN 2072-3857

treatments and their interactions, while the main effect of application stages showed statistically significant variance.

BR application led to the greatest down-regulation of *GmCIF1*, reducing its relative expression level by -32.6 % to a mean value of 0.674 relative to the control. This was followed by the GA treatment, which achieved a -22.2 % down-regulation with a mean expression value of 0.778. Conversely, the ABA treatment markedly up-regulated *GmCIF1* expression, increasing it to 1.484 (a 48.4 % up-regulation compared to the control).

Although the application stages displayed no statistically significant variance as a main effect, their interaction with PGR types was highly significant. The interaction between BR and the V3 stage (BR and V3) minimized the relative expression level of *GmCIF1* to 0.312, establishing a significant down-regulation of -68.8 %. In contrast, the interaction between ABA and the V3 stage (ABA and V3) induced a substantial up-regulation, reaching an expression level of 2.196 (a 119.8 % increase relative to the control.

The function of the *GmCIF1* gene is tied to encoding the cell wall invertase inhibitor (CIF) in soybean, which modulates the extracellular hydrolysis of sucrose into simpler monosaccharides (glucose and fructose). Thus, down-regulating this gene enhances cell wall invertase (CWI) activity, which increases sink strength within reproductive structures (flowers and seeds) by adjusting growth rates and dry matter accumulation in emerging pods and seeds. Consequently, this gene directly controls seed characteristics, particularly dimensions, volume, weight, and total crop yield[21].

Table 7. Effect of plant growth regulators and application stages on the relative expression levels of the *GmCIF1* gene in soybean.

Average	Adding stage				Growth regulator
	R6	R3	R1	V3	
1.00	1.00	1.00	1.00	1.00	Co
0.778	0.876	0.991	0.561	0.683	GA
1.484	1.054	1.088	1.596	2.196	ABA
0.674	0.926	0.868	0.588	0.312	BR
	1.045	1.068	1.052	1.051	average
Intraction	Adding stage		Growth regulator		$LSD_{0.05}$
0.124	0.062		0.062		

Principal Component Analysis (PCA)

Principal Component Analysis (PCA) performed on the relative expression values of *GmCKX3* and *GmCIF1* revealed a distinct variation in expression patterns corresponding to the different PGR treatments (GA, ABA, and BR) and the four application stages (V3, R1, R3, and R6). Overall, the PCA results showed a synchronized behavior between both genes in response to the studied factors. The treatments ABA and V3 and ABA and R1 were strongly associated with the highest positive up-regulation values relative to the first principal component (PC1), indicating elevated transcription under ABA application.

Conversely, the treatments BR and V3, GA and V3, and GA and R1 clustered on the opposite sector of the biplot (along PC2), reflecting a distinct profile defined by a lower transcript copy number, confirming gene down-regulation. The close proximity of the *GmCKX3* and *GmCIF1* vectors indicates a strong positive correlation between the expression patterns of both genes across the various application stages and PGR treatments.

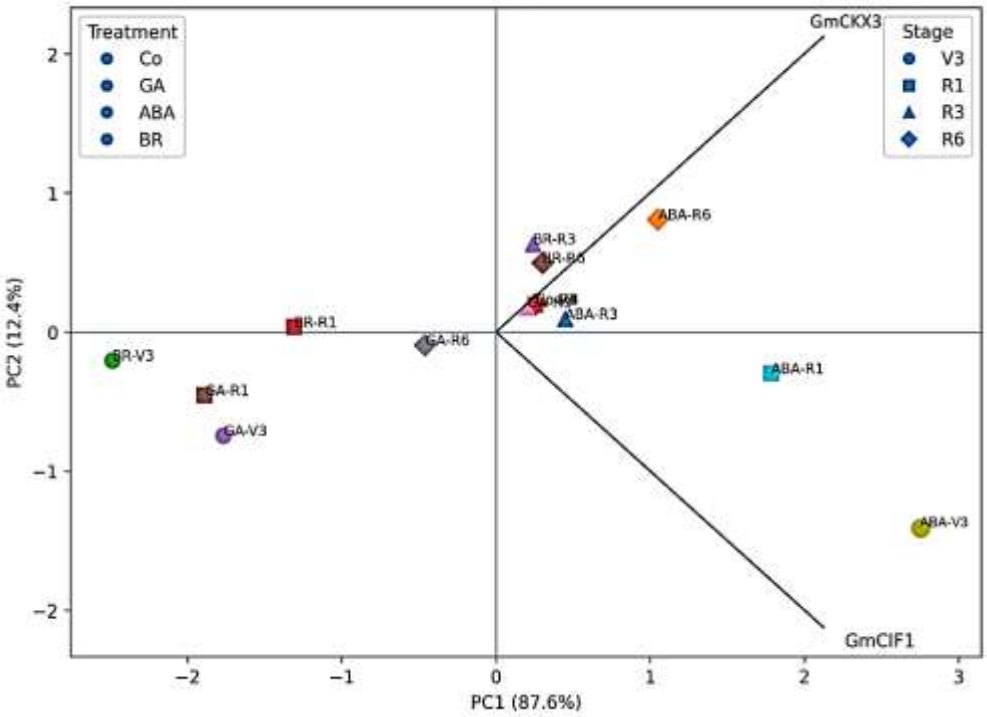


Figure 3: Principal Component Analysis (PCA) biplot of the relative expression levels of GmCKX3 and GmCIF1 as influenced by plant growth regulators (GA, ABA, BR) and application stages (V3, R1, R3, R6).

Conclusions and Recommendations

Conclusion

This study demonstrates that foliar application of plant growth regulators (PGRs)—particularly Brassinolide (BR) at the late vegetative stage (V3)—represents an effective strategy for improving soybean seed traits and increasing total productivity.

At the Molecular Level: BR application at the V3 stage led to a significant down-regulation in the expression levels of the *GmCKX3* and *GmCIF1* genes. This suppression limits cytokinin degradation and enhances cell wall invertase activity, thereby increasing sink strength and accelerating the translocation of sugars and nutrients into the developing seeds.

At the Phenotypic Level: This molecular impact translated into a reduction in the percentage of wrinkled seeds, an increase in 100-seed weight, and the achievement

of the highest total seed yield (reaching 5.726 t ha^{-1} for the BR and V3 treatment.

The study recommends adopting foliar spraying of the growth regulator BR at the vegetative stage (V3) to mitigate the deterioration of seed traits, address the

ISSN 2072-3857

issue of seed wrinkling, and enhance both crop quality and overall yield.

References

1. Afzal, M., Alghamdi, S. S., Migdadi, H. H., Khan, M. A., Nurmansyah, Mirza, S. B., and. (2020). Legume genomics transcriptomics: From classic breeding to modern technologies. *Saudi. J. Biol. Sci.* 27, 543–555. doi: 10.1016/j.sjbs.2019.11.018
2. Alghazi, S., Abdelhakam, S., and Abogadallah, G. M. (2025). Impact of resource limitation during seed development on yield and seed composition in soybean. *Scientific Journal for Damietta Faculty of Science*, *15*(3), 156-168.
3. Delic, I., Kocar, M., Jukic, G., Varnica, I., and Spanic, V. (2026). Biological activities of soybean (Glycine max L.) oil and its bioactive constituents: a focus on volatile fractions and functional potential. International Journal of Experimental Botany, 95: 3.
4. Dwivedi, S. L., Spillane, C., Lopez, F., Ayele, B. T., Ortiz, R. (2021). First the seed: Genomic advances in seed science for improved crop productivity food security. *Crop Sci.* 61, 1501–1526. doi: 10.1002/csc2.20402CrossRef Full Text | Google Scholar
5. Egli, D. B. (2010). The relationship between the duration of seed filling and seed yield in soybean. Crop Science, 50(5), 1618–1625.
6. Gao, H., Wang, Y., Li, W., Gu, Y., Lai, Y., Bi, Y., and. (2018). Transcriptomic comparison reveals genetic variation potentially underlying seed developmental evolution of soybeans. *J. Exp. Bot.* 69, 5089–5104. doi: 10.1093/jxb/ery291 PubMed Abstract | CrossRef Full Text | Google Scholar
7. Hu Y, Liu Y, Wei JJ, Zhang WK, Chen SY, Zhang JS (2023). Regulation of seed traits in soybean. aBIOTECH. 27;4(4):372-385
8. Hudson, K. (2022). Soybean protein oil variants identified through a forward genetic screen for seed composition.
9. Le, D. T., Nishiyama, R., Watanabe, Y., Tanaka, M., Seki, M., Ham, L. H., & Tran, L. S. P. (2012). Genome-wide survey and expression analysis of the cytokinin oxidase/dehydrogenase (CKX) gene family in soybean (Glycine max L.). Journal of Experimental Botany, 63(3), 1271–1282.
10. Le, D. T., Nishiyama, R., Watanabe, Y., Tanaka, M., Seki, M., Ham, L. H., & Tran, L. S. P. (2012). Genome-wide survey and expression analysis of the cytokinin oxidase/dehydrogenase (CKX) gene family in soybean. Journal of Experimental Botany, 63(3), 1271–1282.
11. Liu, D.; Yu, Z.; Zhang, G.; Yin, W.; Li, L.; Niu, M.; Meng, W.; Zhang, X.; Dong, N.; Liu, J.; and. Diversification of plant agronomic traits by genome editing of BRassinolide signaling family genes in rice. *Plant physiol.* 2022, *187*, 2563–2576. [Google Scholar] [CrossRef] [PubMed]
12. Liu, M Zhang, F Feng, Z Tian Toward a “green revolution” for soybean Mol Plant, 13 (5) (2020),

ISSN 2072-3857

pp. 688-697 (2020), pp. 688-697 View PDF View articleView in ScopusGoogle Scholar

13. Magray, M.M. (2021). Plant growth regulators their role in horticultural crop production development. *Researc h Management in Horticultural Crops*.

14. Mominova, S.S., Tastanbekova, G.R., Kashkarov, A.A., Azhimetova, G.N. Balgabaev, A.M. (2022). Effect of foliar mineral fertilizer plant growth regulator application on seed yield yield components of soybean (*Glycine max*) cultivars. *Eurasian Journal of Soil Science*. 11(4): 322-328.

15. Müller, M. Foes or friends: ABA ethylene interaction under abiotic stress. Plants 2021, 10, 448. [Google Scholar] [CrossRef [PubMed].

16. Nawaz, M. A., Chung, G., Pamirsky, I. E., and Golokhvast, K. S. (2026). Breeding Climate-Resilient Soybeans for 2050 and Beyond: Leveraging Novel Technologies to Mitigate Yield Stagnation and Climate Change Impacts. *Plants*, *15*(8), 1201.

17. Oliveira, A. J. 2021. Processamento pós colheita de sementes. Lavras: UFLA, 168p.

18. Rao, P. J. M., Pallavi, M., Bharathi, Y., Priya, P. B., Sujatha, P., and Prabhavathi, K. (2023). Insights into mechanisms of seed longevity in soybean: a review. *Frontiers in Plant Science*, *14*, 1206318.

19. Rodrigues, D. DA S.; Schuch, L. O. B.; Meneghello, G. E.; Peske, S. T. 2018. Desempenho de plantas de soja em função do vigor das sementes e do estresse hídrico. Revista Científica Rural 20: 144-158. https://doi.org/10.30945/rcr-v20i2.260

20. SchmüllingT.WernerT.RieflerM.K rupkováE.Bartrina y MannsI. (2003). Structure and function of cytokinin oxidase/dehydrogenase genes of maize, rice, arabidopsis and other species. *J. Plant Res.*116, 241–252. doi: 10.1007/s10265-003-0096-4 CrossRef Google Scholar View reference in article

21. Smýkal, P., Vernoud, V., Blair, M. W., Soukup, A., & Thompson, R. D. (2014). The role of the seed coat in seed development and germination. Frontiers in Plant Science, 5, 357.

22. SoyBase (2023). Available at: https://www.soybase.org/ (Acc essed FeBRuary 2023).Google Scholar

23. Staniak, M., Stępień-Warda, A., Czopek, K., Kocira, A., and Baca, E. (2021). Seeds quality and quantity of soybean [Glycine max (L.) Merr.] cultivars in response to cold stress. *Agronomy*, *11*(3), 520.

24. *Tang X, Su T, Han M, Wei L, Wang W, Yu Z, Xue Y, Wei H, Du Y, Greiner S, and. Suppression of extracellular invertase inhibitor gene expression improves seed weight in soybean (Glycine max) J Exp Bot. 2017;68:469–482. doi: 10.1093/jxb/erw425.* [DOI] [PMC free article] [PubMed] [Google Scholar

25. Tang, X. L., Su, T. H., Cao, Y. Y., Wang, L. G., & Zhang, Z. W. (2017). Cell wall invertase and its inhibitor genes regulate sucrose metabolism and seed weight in grain legumes. Frontiers in Plant Science, 8, 1284.

26. Van Niekerk, G.L. (2020). Evaluation of the effect of selected plant growth regulators on soybean yield parameters in South

Africa (Doctoral dissertation, North-West University (South Africa).

27. Wang, L., & Ruan, Y. L. (2012). Critical roles of vacuolar invertase and cell wall invertase in floral organ development and seed vigor. Plant Physiology, 160(3), 1152–1166.
28. Wang, X., Sun, J., Yi, Z. *and*. Effects of seed size on soybean performance: germination, growth, stress resistance, photosynthesis, and yield. *BMC Plant Biol* 25, 219 (2025). https://doi.org/10.1186/s12870-025-06224-3
29. Wang, J., Liu, Y., & Zhang, H. (2020). Brassinosteroid signaling and gene networks regulating seed size and assimilate transport in leguminous crops. Frontiers in Genetics, 11, 892.

ISSN 2072-3857