Journal of King Saud University – Science 35 (2023) 102385

Contents lists available at ScienceDirect

Journal of King Saud University – Science

journal homepage: www.sciencedirect.com

Original article

Effect of presoaking various growth regulators on the yield and biochemical characteristics of cowpea plants (*Vigna sinensis L.*)

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ARTICLE INFO

Article history:
Received 8 May 2022
Revised 25 September 2022
Accepted 15 October 2022
Available online 4 November 2022

Keywords:
Cowpea plants
Honnone bioregulators
Yield components
IAA

ABSTRACT

Objectives: Cowpea *(Vigna sinensis L.) is* one of the world's most popular and widely consumed vegetable crops, and its economic success in Saudi Arabia is dependent on high-quality yield. Cowpea is the most important indigenous legume crop in terms of economic value. Cowpea seed has been employed as both a nutritious cow feed and a human nutritional component. Cowpea seed has more amino acids, tryptophan, and lysine than other cereal grains, but fewer methionine and cystine than animal proteins. The purpose of this study was to look at the role of growth bioregulators in cowpea plant yield and yield characteristics, as well as complete protein and soluble sugar levels in seed production.

Methods: This study used plant and growth condition analysis, yield and yield component analysis, total soluble sugars and proteins in yielding seeds computation, and endogenous hormone determination.

Results: In this work, three growth regulators were utilized to increase the physiological activity and production of cowpea plants. When compared to untreated control plants, seed presoaking in various concentrations of IAA, GA3, and kinetin appeared to improve various yield parameters such as 100-seed weight and relative growth yield, pod weight, pod length number, and total amount of seeds/pots of cowpea plants.

Conclusion: This study concludes that IAA, GA3, and kinetin appeared to improve different yield parameters in cowpea plants.

1. Introduction

Vegetables that are often grown in the tropics of Latin America, Africa, and Asia under dry agroecological conditions are known as cowpeas (*Vigna unguiculata* (L.) Walpers) or black eye peas. This legume is a member of the Fabaceae family, tribe Phaseoleae, genus Vigna, and section Catiang of the genus *Vigna. Unguiculata* is a subspecies of cowpea that people grow for food. There are five groups of cultivars: Unguiculata, Sesquipedalis, Textilis, Biflora, and Melanophthalmus (Boukar et al., 2019). In terms of micronutrients and amino acids, cowpeas outperform or complement cereals, making them ideal candidates for solving the global shortage of micronutrients. Legumes include a variety of micronutrients, including carotenoids, which have been shown to enhance human health. Consumption of these foods has been shown to increase cellular differentiation and reproduction, reduce the risk of vision impairment, cancer, cardiovascular disease, and newborn mortality, and contribute to the body's antioxidant defense system. Carotenoids present in legume crops include α- and β-carotenes, as well as hydroxylated versions of these compounds (lutein, zeaxanthin, violaxanthin, etc.). Even though all carotenoids have isoprene units in common, their structural and functional characteristics vary (Sodedji et al., 2022). Therefore, cowpea seed is regarded as an important source of additional protein in cereals and as a means of extending the shelf life of animal protein sources (Rangel-Montoya et al., 2022). It's one of eight grain legume crops that the Consultative Group for International Agricultural Research (CGIAR) wants to improve. Cowpea species is one of them (Salinas-Gamboa et al., 2016). Most of the research on cowpea is devoted to promoting active breeding in underdeveloped nations in order to alleviate poverty. In sustainable farming, this plant is

Abbreviations: IAA, indole acetic acid; GA_3, Gibberellic acid; ppm, part per million; NaOCl, Sodium hypochlorite.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.jksus.2022.102385
1018-3647/

used because of its ability to withstand drought and water deficiency, as well as high temperatures and high salinity. In many areas, it serves as a stress-tolerant, low-cost, and environmentally friendly source of protein, making it especially important for vegetarians (Jayawardhane et al., 2022).

Studies explored the use of plant growth regulators to boost plant growth and production. Because it reduces soil oxygen levels, waterlogging stress can cause hypoxia and anoxia in plants. In waterlogging-sensitive crops, such as cowpeas, lower diffusion rates of gases that can induce cell and tissue death are signs of a hypoxic environment, as is plant anaerobic respiration (Olorunwa et al., 2022). Gibberellic acid at 50 and 75 ppm was observed to improve G. arborum's photosynthetic activity and, as a result, the total amount of photosynthetic metabolites, soluble sugars, and proteins. Foliar spraying of 50 or 100 ppm gibberellic acid to *Vicia faba* plants improved seed yield, pod yield, and harvest index (Choudhury et al., 2013). The utilization of Indole acetic acid (IAA) and gibberellic acid (GA_3) can be used to manage a variety of agricultural growth and development phenomena. IAA has been proven to increase plant height, leaf number per plant, fruit size, and seed yield in a variety of species, including groundnuts, cotton, cowpeas, and rice. Flowers and fruit set increase, as well as the amount of dry matter in a crop. A similar effect was seen with GA_3, which promoted stem lengthening, dried matter accumulation, and an increase in overall yield (Sarkar et al., 2002). Previous research found that soaking carrot roots in IAA at 100 ppm increased seed output and harvest index. The effects of soaking soybean seeds in IAA at various concentrations were studied using soybean and Seseamum plants (50, 75, and 100 ppm). Another study found that IAA treatment boosted blooming stage endogenous auxin and gibberellin activity (Khandaker et al., 2018; Prajapati et al., 2015).

Kinetin foliar applications at 100 and 200 ppm were sprayed on wheat leaves, and the plants' shoot fresh weight increased considerably. *Helioanthus annus* grew taller, had more chlorophyll, and had a larger leaf area when it was given Kinetin. According to previous studies, wheat plants' fresh and dry weights were dramatically boosted when kinetin was applied. Soybean seeds that had been soaked in various growth hormones exhibited a considerable increase in the amount of protein and oil in the final product compared to untreated plants. It was hoped that this study will examine theeffect of growth bioregulators on cowpea plant yield and yield characteristics. Seed yields were also tested for total protein and total soluble sugar content.

2. Materials and methods

2.1. Plant growth conditions

Surface sterilization with 5 % NaOCl for 10 min followed by thorough washing with deionized H_2O was performed on cow pea seeds. A total of 10 groups of uniformed, sterilized seeds were created. To act as a control, the seeds from the first set were immersed in deionized water for three hours, while the seeds from the 2–4 sets were soaked in GA_3 at concentrations of 25, 50, or 75 ppm for the same amount of time. Sets 5–7 were immersed for three hours in IAA at concentrations of 50, 100, and 150 ppm. Kinetin concentrations (25, 50, or 75 ppm) were applied to the 8–10 sets and then washed with distilled H_2O. On a wet filter paper at 250^{o} F, these seeds germinated for five days. Sown in 30 cm diameter plastic pots with equal soil (Sand and Clay 2:1) and grown under regulated conditions in the greenhouse of the Facility of Science, King Saud University, Riyadh, Saudi Arabia These variables were studied: phosphorus (pH), conductivity of electric current, total organic matter, total nitrogen, total phosphorus, magnesium kg-1, potassium kg^{-1}, and calcium mg kg^{-1}. With the goal of keeping the soil moisture level in the field at 75–80 %, all plants were watered as needed and fed once per week with a solution of 35 g of potassium and 35 g of nitrogen. A total of 6 plants from each treatment were harvested 12 weeks after the seeds were sown.

2.2. Yield and yield component analysis

Harvest index = grain yield/above ground dry matter × 100 (Abdel-Fattah et al., 2002).

Relative grain yield = yield in treated plants/yield in untreated plants × 100, (Abdel-Fattah et al., 2002);

The pod length, number of pods, seed count, and 100-seed weight of each cowpea plant treatment were measured. Harvest index was measured according the references of Abdel-Fattah et al. (2002).

2.3. Determination of total soluble sugars and protein in the yielded seeds

To quantitatively evaluate the total soluble sugars (TSS), dry seeds from each treatment were extracted for 12 h at room temperature with occasional shaking in 10 ml of 80 % (v/v) ethanol. Both TSS and total protein were estimated based on modifications of previous studies (Abdel-Fattah et al., 2002; Bradford, 1976).

2.4. Tests for endogenous hormones

Each treatment yielded a certain amount of fresh seeds, which were frozen in liquid nitrogen. HPLC was used to assess the levels of endogenous hormones (IAA, GA_3, ABA, and cytokinin). Extraction and purification were carried out based on the previous study (Terry et al., 1982).

2.5. Analysis of statistical data

Duncan's multiple range tests were used to guide the 1-way ANOVA analysis. The Costat software uses the p value less than 0.05 ($p < 0.05$) technique to separate the means in this investigation.

3. Results

3.1. Yield and its constituents

When compared to control plants, seed presoaking in varied concentrations of IAA (50, 100. and 150 ppm) resulted in significant increases in plant length, pods, and seed count, as well as relative seed yield and harvest index (Table 1). Furthermore, the results revealed that, with the exception of relative seed yield, there were no significant differences in the increase in different yield components between IAA concentrations. Table 2 demonstrates that applying GA3 at 25 ppm, 50 ppm, and 75 ppm resulted in a significant increase in all yield and yield component parameters when compared to untreated control cowpea plants. This is consistent with prior research findings. The bulk of yield components did not differ substantially between plants treated with 50 ppm and 75 ppm of GA3. Presoaking the seed in kinetin resulted in a significant increase in yield and yield characteristics of cowpea plants at all concentrations (25, 50, and 75 ppm) (Table 3). As kinetin concentrations rise, the value of the various yield components of cowpea plants rises. When compared to other growth regulator treatments, kinetin at the various dosages used here is the most powerful growth regulator for enhancing cowpea plant output

Table 1
Effect of different concentrations of indole acetic acid (IAA) on yield and yield components of cowpea plants.

Parameters	Indole acetic acid (PPm)			
	control	50	100	150
Pod length (cm)	8.2 c	10.5 b	12.0 a	12.8 a
Weight of pod (g)	0.95 c	1.10 bc	1.89 b	2.94 a
Number of pods/plant	2.93 c	3.55 b	3.87 b	4.98 a
Number of seeds/pod	4.51 c	6.11 ab	7.34 a	8.01 a
Harvest index (%)	200 c	410 c	450 a	455 a
100 seeds weight(g)	9.21 b	10.5 ab	11.25 ab	12.50 a
Relative seed yield(%)	1.14 b	2.90 a	3.11 a	3.20 a

Values in each column followed by the same letter do not differ significantly P = 0.05 (Duncan multiple range test).

Table 2
Effect of different concentrations of Gibberellic acidGA$_3$) on yield and yield components of cowpea plants.

Parameters	Gibberellic acid (PPm)			
	control	25	50	75
Pod length (cm)	8.97 b	11.22 ab	12.0 a	12.50 a
Weight of pod (g)	0.73 b	1.11 ab	1.54 a	1.74 a
Number of pods/plant	1.93 bc	2.55 b	3.01 b	3.98 a
Number of seeds/pod	3.51 c	6.33 b	8.24 a	9.11 a
Harvest index (%)	190 c	310 b	350 b	477 a
100 seeds weight(g)	9.31 b	11.80 ab	12.26 ab	13.88 a
Relative seed yield(%)	1.25 c	3.44 b	3.91 ab	4.20 a

Values in each column followed by the same letter do not differ significantly P = 0.05 (Duncan multiple range test).

Table 3
Effect of different concentrations of kinetin on yield and yield components of cowpea plants.

Parameters	kinetin (PPm)			
	control	25	50	75
Pod length (cm)	8.25 c	12.56 b	13.00 a	14.18 a
Weight of pod (g)	0.90 c	1.66 bc	1.99 b	2.98 a
Number of pods/plant	1.93 c	4.05 b	5.88 b	6.77 a
Number of seeds/pod	4.11 c	6.81 b	7.99 ab	10.50 a
Harvest index (%)	199 d	520 c	640 b	785 a
100 seeds weight(g)	9.11 c	11.54 bc	12.28 b	14.90 a
Relative seed yield(%)	1.11 c	3.55 b	4.98 b	5.55 a

Values in each column followed by the same letter do not differ significantly P = 0.05 (Duncan multiple range test).

(IAA or GA3). Thus, plants treated with varied kinetin concentrations produced higher yield components.

3.2. Proteins and soluble sugars

When compared to the control group, treatment of cowpea plants with growth regulators (IAA and kinetin) significantly increased total soluble sugars in the seeds (Table 4). There were no significant differences in total soluble sugars in seeds produced by cowpea plants treated with different dosages of GA3. When the growth regulators were used at the highest concentrations, the total soluble sugars increased the most. Seeds from cowpea plants treated with growth regulators had more protein than those from control plants (Table 4). Kinetin produced a significant increase in protein content when compared to the other growth regulator hormones used in this investigation.

3.3. Regulators of endogenous growth

Cowpea seeds grown after the administration of IAA, GA$_3$, or kinetin had significantly altered amounts of endogenous auxins, cytokinins, gibberellins, and abscisic acid (Table 5). IAA, GA$_3$, and kinetin significantly enhanced total auxin, gibberellin, and cytoki-

Table 4
Effect of seed presoaking in different concentrations of IAA, GA3 and kinetin (ppm) on protein content (μg. g^{-}dwt) and total soluble sugars (μg. g^{-}dwt) in yielded seeds of cowpea plants.

Treatments	Parameters	
	Protein	Total soluble sugars
control	950 e	480 d
LAA		
50	1050 e	610 cd
100	1410 cd	835 bc
150	1580 c	892 b
GA 3		
25		
50	1320 d	720 c
75	1560 c	725 c
	1808 ab	740 c
Kinetin		
25	1530 c	922 b
50	1710 b	1038 b
75	1985 a	1316 a

Values in each column followed by the same letter do not differ significantly P = 0.05 (Duncan multiple range test).

Table 5
Effect of different concentrations of IAA, GA3 and kinetin (ppm) on endogenous hormones content (μg. g^{-}fwt) in yielded seeds of cowpea plants.

Parameters	Parameters			
	IAA	GA_3	Cytokintin	
control	220 d	130 c	90 d	180 a
LAA				
50	410 b	140 b	105 c	160 b
100	557 a	147 b	125 b	155 b
150	584 a	156 b	130 b	140 c
GA_3				
25	250 c	135 b	98 c	155 b
50	278 c	142 b	110 c	130 d
75	310 bc	150 b	125 b	114 ab
Kinetin				
25	505 ab	150 b	105 c	160 b
50	566 a	188 a	135 b	143 c
75	586 a	210 a	150 a	129 d

Values in each column followed by the same letter do not differ significantly P = 0.05 (Duncan multiple range test).

nin levels when compared to control treatments. The endogenous levels of the found growth regulators increased as the concentration of each growth regulator applied was increased (total auxins, gibberellins and cytokinins). For control plants, there were more seeds with lower amounts of abscisic acid than cowpea plants. Cowpea plants that received kinetin had the highest amounts of cytokines and total auxins as well as the lowest levels of abscisic acid in their seeds when compared to other growth regulators.

4. Discussion

The goal of this study was to explore at the involvement of growth bioregulators in yield and yield qualities of cowpea plants, as well as the levels of complete protein and soluble sugars in yielding seeds. This study employed methodologies such as plant and growth conditions analysis, yield and yield component analysis, calculation of total soluble sugars and proteins in yielding seeds, and endogenous hormone determination. In this experiment, three growth regulators were utilized to increase the physiological activity and production of cowpea plants. When compared to untreated control plants, seed presoaking in various concentrations of IAA, GA_3, and kinetin appeared to enhance various yield parameters such as 100-seed weight and relative growth yield, pod weight, pod length number, and total amount of seeds/pots of cowpea plants. One of the causes again for increase in production of cowpea is the increased rate of transpiration and photosynthetic transfer from leaves to developing seeds produced by hormone treatments (Khandaker et al., 2018; Ayyub et al., 2013). This study found that growth regulators utilized in this study had a positive influence on the growth of cowpea plants, which in turn led to an increase in the quality of the plant's output as a result of increased leaf area, pigment production, and photosynthetic activity (Prajapati et al., 2015; Weiming and Aiwu, 1999; Vamil et al., 2010).

To treat persistent boils, ground up cowpea seeds mixed with oil is utilized. To satisfy thirst, a jelly prepared from cowpea starch is utilized. According to some medical professionals, spiced seeds and their boiling liquid may be a therapeutic cure for the common cold. Poultices made from the leaves and seeds can be used to treat swellings and infections, the leaves are chewed to treat tooth ailments, powdered carbonized seeds can be applied to insect stings, and the root is used as an antidote for snakebites as well as to treat epilepsy,$chest pain, constipation, $and dysmenorrhea. Many other biological actions are also demonstrated, such as effects on cellular signaling and animal infertility prevention. They are also expected to serve a preventative function in oxidative stress-related disorders such as cancer, cardiovascular disease, cataracts, age-related macular degeneration, central neurodegenerative diseases, and diabetes mellitus. A seed infusion can be used to cure amenorrhea, and powdered roots can be mixed into porridge to treat menstrual cramps, epilepsy, and chest pain.$Traditional healers use it to treat urinary shistomiasis$ (bilharzias). On relieve pain, the leaves can be put to burns and snuffed. Emetics derived from the plant are used to cure fevers. Tocopherols are the most important lipophilic antioxidants. Even though cowpea seed does not contain oil, it can be crushed and utilized to manufacture edible oils. Lipoproteins and membrane polyunsaturated fatty acids are protected from oxidation (Zia-Ul-Haq et al., 2010).

In this study, kinetin application was determined to be the most effective growth regulator for raising yield and yield components in cowpea plants when compared to other growth regulators. El-Saeid et al found that IAA boosted cowpea plant growth metrics, which is consistent with our findings (El-Saeid et al., 2010). IAA and GA_3 have been shown to increase tomato yield through changes in carbohydrate metabolism enzyme activity, (Choudhury et al., 2013). Cowpea seeds produced from this experiment had significantly higher levels of total soluble sugars than those produced without the growth regulators. Khandaker et al also found that floral$injection of IAA at 30 and 90 mg/L increased$ Abelmoschusesculentus' total soluble sugars (Khandaker et al., 2018). Furthermore. Soluble carbohydrate content and activity of the acid invertase enzyme were considerably boosted by the use of Kinetin (8, 19). Furthermore. The kinetin therapy, according to Prajapati et al, caused an excessive buildup of lemna minor starch (Prajapati et al., 2015).

The results of this study show that the protein content of growing cowpea seeds was greatly boosted by the use of growth regulators. Several researchers found that IAA elevated levels of protein and other nitrogenous substances in a variety of plant types when tested (Sarkar et al., 2002; Prajapati et al., 2015; Vamil et al., 2010). Before, it was reported that spraying 50 ppm IAA on the leaves of maize, bean, and cowpea plants increased their total nitrogen content, primarily protein. In addition, it was discovered that the protein content of the harvested cowpea seeds increased considerably when GA_3 was applied. In addition, the findings of Ayyub et al. and Choudhury et al. support these findings

(Choudhury et al., 2013; Ayyub et al., 2013). Furthermore, kinetin was found to be the most effective hormone for protein modification in growing seeds of cowpea plants (Prajapati et al., 2015; Weiming and Aiwu, 1999; El-Saeid et al., 2010; Al-Humaid, 2002). Because kinetin increases chlorophyll content, stomatal conductance, and the accumulation of dry matter within developing seed$, the apparent increase in seed protein content after kinetin application may be explained by the fact that kinetin enhances mobilization centers, which in turn directs the migration and accumulation of compounds into developing seeds (Khandaker et al., 2018; El-Saeid et al., 2010). In addition, Exogenous applications of some phytohormones (e.g., GA_3, IAA, ABA, and kinetin), have the potential to offset the adversities of salinity stress up to a certain limit in rice plants (Prajapati et al., 2015; Ayyub et al., 2013). Cytokinins indirectly affect tuberization by enhancing the activation of starch-synthesizing enzymes to support continuing starch deposition (Shah, 2011). Applications of exogenous kinetin in vitro stimulated accumulation of starch, possibly by activation of ADP glucose phosphorylase and pyrophosphorylase during tuberization (El-Saeid et al., 2010). Cytokinin activity can create a nutrient sink and that both ^{14}C-labelled sucrose and amino acids are mobilized to localized sites of high cytokinin accumulation. Local synthesis of cytokinins in axillary buds of transgenic tobacco resulted in an increase in starch accumulation in the lateral shoots that formed (Ayyub et al., 2013).

An increase in endogenous auxin and gibberellin activity in cowpea seeds was clearly observed in this study. An increase in gibberellin biosynthesis gene expression and polysaccharide production was detected (Prajapati et al., 2015); who discovered that foliar treatment of IAA caused similar results. The generation of secondary messengers by IAA activates preexisting H^+-ATPases and increases the activation of various growth and development related genes. IAA also starts a signal transduction pathway (Sarkar et al., 2002; Khandaker et al., 2018; Shah, 2011). When seeds were presoaked in GA_3 at varying concentrations, auxins, gibberellins, and cytokinin levels rose significantly. The concentration of abscisic acid was found to be decreasing. GA_3 was shown to stimulate proteolytic enzymes that may be predicted to release tryptophan, a precursor to IAA (El-Saeid et al., 2010). After kinetin treatment, there was a significant rise in auxins, gibberellins, and cytokinins in cowpea seeds. The induction of some metabolites, particularly sugars, into seeds may have led to this increase (Shah, 2011). An increase in osmotic water uptake ensued, leading to a rapid rise in fresh weight for the seed and, ultimately, to an increase in the seed's extractable gibberellin activity. Furthermore, kinetin treatment increased the cytokinin content of the cowpea seeds that were harvested. Because of an increase in seed division, the amount of cytokinin in the seeds produced may be higher than normal (Vamil et al., 2010; Al-Humaid, 2002).

5. Conclusion

The data reported here show that seed presoaking with varied doses of IAA, GA3, or kinetin increased yield quality of cowpea plants by generating a significant rise in yield components as well as increases in protein content and total soluble sugars in produced seeds. Kinetin treatment, according to the findings of this study, is the most effective hormone for raising productivity and endogenous auxins, gibberellin, and cytokinin in cowpea plants. Furthermore, more research is needed to better understand how growth bio-regulators alter cowpea plants' metabolic pathways, molecular analyses, and enzymes.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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