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Santanu Maity
M.Sc. Scholar, Department of Vegetable Science, Post Graduate Institute, Dr. PDKV, Akola, Maharashtra, India

DH Paithankar
Associate Professor, Officer in Charge ACRIP Citrus, Dr. PDKV, Akola, Maharashtra, India

AM Sonkamble
Professor and Head, Department of Vegetable Science, Dr. PDKV, Akola, Maharashtra, India

AP Wagh
Professor, Department of Vegetable Science, Dr. PDKV, Akola, Maharashtra, India

YV Ingle
Professor, Department of Plant Pathology, Dr. PDKV, Akola, (Maharashtra, India

Corresponding Author:
Santanu Maity
M.Sc. Scholar, Department of Vegetable Science, Post Graduate Institute, Dr. PDKV, Akola, Maharashtra, India

Screening of plant growth substances on cowpea (*Vigna unguiculata*) for waterlogging stress tolerance

Santanu Maity, DH Paithankar, AM Sonkamble, AP Wagh and YV Ingle

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Abstract
In the India, cowpea (*Vigna unguiculata*) is primarily cultivated after the summer rains, depending on rainfall or supplemental irrigation for its water supply. However, when there is excessive rainfall or inadequate soil drainage, the plants may experience waterlogging, which reduces the oxygen available to their submerged roots. Although cowpea is vulnerable to waterlogged conditions, too much moisture early in its growth can trigger several detrimental physiological and structural changes. These changes often hinder plant growth and lead to a decrease in yield potential. Use of plant growth substances is a very effective and cost benefit method to reduce the detrimental effect of the waterlogging condition. The presence study aimed to find out the effect of different plant growth substances at different concentration NAA (10 & 20 ppm), GA (15 & 25 ppm), Brassinolide (2 & 4 ppm) and 4CPPU (10 &20 ppm) on cowpea (*Vigna unguiculata*) in waterlogging condition. Two foliar sprays of these plant growth substances are given at 30 and 45 days after sowing. Plant height, number of leaves, number of branch per plant, leaf area, days to 50% flowering, number of flower per cluster, days required to first harvest, days required to last harvest, number of green pods per plant, average weight of green pod, green pod yield per plant, green pod yield per plot, green pod yield per hector, green pod length, green pod width, chlorophyll content of leaves were observed. The results showed that plant height and leaf area increased significantly with GAA 25 ppm, while branch and leaf number were higher with Brassinolide 2 ppm. GA 15 ppm led to early 50% flowering, and the highest flower clusters were found with NAA 10 ppm. NAA 20 ppm required the lowest days to harvest, followed by NAA 10 ppm. Brassinolide 2 ppm produced the highest yield for green pods per cluster, plant, plot, and hectare. GAA 15 ppm improved pod length and width, while 4CPPU resulted in higher chlorophyll content. The identified waterlogging tolerant plant growth substances Brassilonoides can serve as potential candidate for production of cowpea in waterlogging area and it can be used in climate resilient cultivation practices.

Keywords: Cowpea, waterlogging stress, plant growth substances naphthalene acetic acid (NAA), gibberellic acid (GA), forchlorfenuron (4CPPU), brassinolide, climate resilient cultivation

Introduction
Cowpea (*Vigna unguiculata* L. Walp.), a member of the Leguminaceae family and sub-family Fabaceae, has a chromosome number of 2n=22 and is a vital component of farming systems across arid and humid tropical regions. Originating from Africa, *V. unguiculata* consists of three primary subspecies: *ssp. unguiculata*, *ssp. cylindrica*, and *ssp. sesquipedalis*. These varieties are cultivated for multiple uses, including as leafy vegetables, fresh pods, and mature dry seeds. In its dry grain form, cowpea is commonly referred to as black-eyed pea, southern bean, China pea, or marble pea, whereas its fresh pod form is known as yard-long bean, asparagus bean, body bean, or snake bean.
Cowpea is a rich source of plant-based protein, containing essential amino acids, except for cysteine and methionine. Due to its high biological value, it is often termed "vegetable meat." In addition, it provides essential micronutrients such as zinc and iron, which are critical for human health. The grain consists of approximately 53% carbohydrates and about 2% fat (FAO, 2012). Its green leaves contain between 23% and 32% protein, while immature pods provide 4% to 5% protein content. Apart from its nutritional benefits, cowpea plays a crucial role in sustainable agriculture by enhancing soil fertility through biological nitrogen fixation (BNF) and contributing to nutrient cycling.

Furthermore, its relatively short growing season allows it to adapt to changing climatic conditions and tolerate various environmental stresses.

Despite its adaptability, cowpea production faces significant challenges due to abiotic stresses, particularly waterlogging. Waterlogged conditions reduce oxygen availability in the root zone, leading to hypoxia-induced physiological disruptions such as inhibited root function, impaired nutrient uptake, and oxidative stress. To counteract these adverse effects, plant growth regulators (PGRs) have been explored for their potential in enhancing stress tolerance. Phytohormones such as Naphthalene Acetic Acid (NAA), Gibberellic Acid (GA), 4-Chlorophenyl N-Phenylurea (4CPPU), and Brassinolides (BRs) play crucial roles in modulating plant responses under stress. NAA promotes root elongation and adventitious root formation, aiding in oxygen acquisition. GA stimulates cell elongation and aerenchyma development, improving internal aeration. 4CPPU, a cytokinin analog, delays senescence, maintains chlorophyll stability, and enhances antioxidant activity. Meanwhile, BRs activate stress-responsive genes and enzymatic antioxidants, improving waterlogging tolerance. Given the importance of these growth regulators in alleviating waterlogging stress, this study aims to evaluate the effectiveness of NAA, GA, 4CPPU, and BRs in improving cowpea growth and physiological responses under waterlogged conditions. By analyzing their impact on stress mitigation, this research seeks to provide insights into optimizing plant hormone treatments to enhance cowpea resilience in challenging environments.

Materials and Methods

This study was conducted at the instructional farm of the Department of Vegetable Science, Dr. PDKV Akola, during the kharif season of 2023-24. The experiment site was chosen in a low-lying area where water stagnation commonly occurs during the rainy season. The cowpea variety used for the study was PDKV Rutuja, sown at a spacing of 60 cm × 45 cm. The experiment followed a randomized block design (RBD) with nine treatment combinations of plant growth regulators. The treatments included Naphthalene Acetic Acid (NAA) at 10 ppm (T_1) and 20 ppm (T_2), Gibberellic Acid (GA) at 15 ppm (T_3) and 25 ppm (T_4), Brassinolide at 2 ppm (T_5) and 4 ppm (T_6), Forchlorfenuron at 10 ppm (T_7) and 20 ppm (T_8), and an untreated control (T_9). Each treatment was replicated three times.

Growth regulator solutions were prepared and applied as foliar sprays at two stages 30 and 45 days after sowing (DAS). Growth, yield, and quality parameters were assessed by randomly selecting five plants from each treatment plot. The recorded parameters included plant height, leaf count, number of branches per plant, leaf area, days to 50% flowering, number of flowers per cluster, duration from sowing to the first and last harvest, number of green pods per plant, average green pod weight, green pod yield per plant, per plot, and per hectare, as well as green pod length, width, and chlorophyll content of leaves.

The collected data were statistically analyzed following the methods outlined by Panse and Sukhatme (1954) [5]. The standard error and critical difference (CD) at a 5% significance level were computed to evaluate the differences between treatment means.

Results and Discussion

Plant height (cm): The application of various plant growth regulators had a significant impact on cowpea (*Vigna unguiculata*) plant height. Among the treatments, the application of GA at 25 ppm (T_4) resulted in the tallest plants, recording a height of (90.40) at 45 DAS and (99.90) at 60 DAS. This significant increase in plant height can be attributed to the elongation of internodes, leading to an overall enhancement in plant stature. Similar findings were reported by Nabi *et al.* (2014) [4] In contrast, the control treatment (T_9) exhibited the shortest plant height, with a recorded value of 47.97 cm. The increase in plant height observed in response to GA_3 application is likely due to its role in promoting internodal elongation.

Number of branches plant^{-1}.

The data presented in Table 1 indicates that the application of plant growth regulators significantly influenced the number of branches per cowpea plant. Among the treatments, the highest number of branches (7.27) was recorded in plants treated with Brassinolide at 2 ppm (T_5), whereas the control treatment (T_9) exhibited the lowest branch count (4.40). The increase in branching can be attributed to the role of Brassinolide, a steroidal hormone known to suppress apical dominance, thereby promoting lateral shoot development.

Number of leaves plant^{-1}

The application of plant growth regulators had a significant effect on the number of leaves per plant in cowpea. Among the treatments, the highest number of leaves (64.13) was recorded in plants treated with Brassinolide at 2 ppm (T_5), while the lowest number of leaves (45.73) was observed in the control (T_9). The increase in leaf production under Brassinolide treatment can be attributed to its role in promoting cell division, expansion, and overall vegetative growth.

Leaf Area (cm^2) per plant

The application of plant growth regulators had a significant impact on the leaf area of cowpea plants. The maximum leaf area (146 cm^2) was recorded in plants treated with GA_3 at 25 ppm (T_4), while the minimum leaf area (101 cm^2) was observed in the control (T_9). Gibberellic acid (GA_3) is known to promote cell elongation and expansion, leading to an overall increase in leaf surface area, which enhances the plant's photosynthetic capacity.Several studies have reported that GA_3 application positively influences leaf area development in various crops. Bhat (2011) [1] observed that GA_3 treatment in cowpea significantly increased leaf expansion due to its role in stimulating cell division and elongation.

Flowering paramers

Days to 50% flowering

The application of plant growth regulators significantly influenced the time required for 50% flowering in cowpea. The earliest (54.33) 50% flowering was observed in plants treated with GA_3 at 15 ppm (T_3), whereas the maximum number of days (62.33) to reach 50% flowering was recorded in plants in control (T_9). The role of GA_3 in promoting early flowering can be attributed to its influence on breaking dormancy, enhancing enzymatic activities, and accelerating floral initiation. Several studies have confirmed the impact of GA_3 on early flowering in legumes and other crops. Sravya (2024) [7] reported that GA_3 application significantly reduced the time required for flowering by stimulating the synthesis of flowering hormones and improving nutrient mobilization.

Flower cluster plant^{-1}

The application of plant growth regulators significantly influenced the number of flower clusters per plant in cowpea. The maximum number of flower clusters (4.60) was observed in plants treated with Naphthalene Acetic Acid (NAA) at 10 ppm (T_1), whereas the minimum number of flower clusters (2.61) was recorded in the control (T_9). The increased flower cluster formation in NAA-treated plants can be attributed to its role in enhancing floral bud initiation, improving nutrient mobilization, and regulating auxin transport, which collectively stimulate reproductive growth.

Table 1: Effect of plant growth substances on Plant height (cm), Number of leaves per plant, Number of branches per plant, leaf area(cm^2), Days to 50% flowering, Number of flower cluster, Pod length (cm), Pod width(mm), and Chlorophyll index (SPAD unit)

Treatments	Plant height (cm)	Number of leaves per plant	Number of branches per plant	leaf area (cm2),	Days to 50% flowering	Number of flower cluster	Pod length (cm)	Pod width (mm)	Chlorophyll index (SPAD unit)
T_1 (NAA 10ppm)	63.89	56.64	6.03	131.96	57.66	4.60	25.75	0.44	53.28
T_2 (NAA 20ppm)	62.40	52.50	5.77	126.43	57	4.21	25.43	0.52	58.30
T_3 (GA 15ppm)	84.84	55.53	5.53	135.10	54.33	3.21	26.40	0.56	48.71
T_4 (GA 20ppm)	99.90	53.27	5.07	146.36	55.66	3.23	25.25	0.55	45.23
T_5 (Brassinolide 2 ppm)	61.32	64.13	7.27	109.50	60	4.10	25.55	0.39	52.82
T_6 (Brassinolide 4 ppm)	57.40	55.47	6.29	111.60	60	3.40	25.15	0.40	57.09
T_7 (Forchlorfenuron 10 ppm)	54.78	59.13	6.27	105.73	61.33	3.41	24.63	0.38	62.33
T_8 (Forchlorfenuron 2 0ppm)	54.50	55.47	6.07	111.53	61.67	3.35	25.53	0.43	56.92
Control	47.97	45.73	4.40	101.06	62.33	2.61	21.83	0.36	53.60
F test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE(m)±	0.81	1.95	0.18	1.69	0.61	0.13	0.11	0.03	1.34
CD at 5%	2.38	5.71	0.54	4.96	1.79	0.39	0.35	0.09	3.93

Table 2: Effect of plant growth substances on days required for first harvest, days required for last harvest number of green pods per plant, average weight of green pod (g), green pod yield per plant (g), green pod yield per plot (kg), green pod yield per hectare

Treatments	Days required for first harvest	Days required for last harvest	Number of green pods per cluster	Green yield per plant	Average weight of green pod (g),	Green pod yield per plant (g)	Green pod yield per plot (Kg)	Green pod yield per hectare
T_1 (NAA 10 ppm)	58.66	102.66	4.15	27.78	7.50	208.31	8.33	77.13
T_2 (NAA 20 ppm)	58.33	101	4.05	26.71	7.65	210.65	8.42	77.96
T_3 (GA 15 ppm)	59.33	99.66	3.20	26.30	7.88	201.21	8.04	74.47
T_4 (GA 20 ppm)	60	100.33	3.18	25.25	7.01	177.18	7.08	65.58
T_5 (Brassinolide 2 ppm)	60.66	104.33	3.97	33.46	7.16	242.16	9.68	89.62
T_6 (Brassinolide 4 ppm)	61.66	103.33	3.16	32.10	7.25	232.57	9.30	86.10
T_7 (Forchlorfenuron 10 ppm)	64	102.66	3.25	28.10	7.26	195.88	8.17	75.64
T_8 (Forchlorfenuron 20 ppm)	64.33	103	3.06	27.46	7.06	181.93	7.73	71.57
Control	65.66	96.66	2.36	24.46	6.90	168.87	6.75	62.49
F test	Sig	Sig	Sig	Sig	Sig	Sig	Sig	Sig
SE(m)±	0.38	0.62	0.13	0.90	0.10	5.26	0.23	2.04
CD at 5%	1.12	1.84	0.39	2.46	0.30	15.45	0.68	6.00

Yield Parameters

Days required for first harvest

The number of days required for the first harvest was significantly influenced by plant growth regulators. The earliest harvest was (58.33) observed in plants treated with NAA at 20 ppm (T_2), while the control (T_9) took the maximum number of days (65.66) for the first harvest. NAA plays a crucial role in accelerating the reproductive phase by promoting early flowering and fruit set, ultimately leading to an earlier harvest.

Days required for last harvest

The maximum number of days (104.33) required for the last harvest was recorded in plants treated with Brassinolide at 2 ppm (T_5), whereas the lowest duration (96.66) was observed in the control (T_9). Brassinolide is known for extending the productive lifespan of crops by delaying senescence and maintaining plant vigor.

Number of green pods per cluster

The highest number of green pods per cluster (4.15) was recorded in plants treated with Brassinolide at 2 ppm (T_5), while the lowest (2.36) was observed in the control (T_9). Brassinolide enhances flower retention and pod formation by improving hormonal signaling and nutrient uptake.

Number of green pods per plant

The highest number of green pods (33.46) per plant was recorded in Brassinolide 2 ppm (T_5), whereas the lowest (24.46) was observed in the control (T_9). The increase in pod number with Brassinolide application can be attributed to its role in promoting cell elongation, enhancing nutrient uptake, and improving stress tolerance.

Average weight of green pod (g)

The highest average pod weight (7.88) was observed in plants treated with NAA at 20 ppm (T_2), while the lowest (6.90) was recorded in the control (T_9). NAA enhances cell division, improves nutrient mobilization, and promotes pod enlargement. Similar result is find by Desai and Deore (1985) [2].

Green Pod Yield per Plant (g)

The highest green pod yield per plant (242.16) was recorded in Brassinolide 2 ppm (T_5), while the lowest yield (168.87) was observed in the control (T_9). Brassinolide enhances overall plant growth and increases reproductive success by improving carbohydrate metabolism and nutrient absorption.

Green Pod Yield per Plot (kg)

The highest green pod yield per plot (9.68) was recorded in Brassinolide 2 ppm (T_5), whereas the lowest (6.75) was observed in the control (T_9). This increase can be attributed to the hormone's ability to enhance flower retention, improve pod set, and increase nutrient uptake.

Green Pod Yield per Hectare (q)

The highest green pod yield per hectare (89.62) was recorded in Brassinolide 2 ppm (T_5), while the lowest (62.49) was observed in the control (T_9). The increased yield is due to improved pod set, enhanced biomass accumulation, and prolonged reproductive activity.

Quality Parameter

Green pod length (cm)

The maximum green pod length (26.40) was recorded in GA 15 ppm (T_3), while the lowest length (21.83) was observed in the control treatment (T_9). Gibberellic acid plays a crucial role in cell elongation and division, leading to increased pod length. Almost similar result was found by Emongor and Ndambole (2011) [3].

Green pod width (cm)

The highest green pod diameter (0.56 cm) was observed in GA 15 ppm (T_3) and GA 15 ppm (T_4), while the lowest (0.36 cm) was recorded in the control treatment (T_9). Gibberellic acid promotes cell expansion and improves vascular differentiation, which contributes to an increase in pod girth.

Chlorophyll content of leaves (SPAD unit)

The highest chlorophyll content in SPAD units (62.33) was recorded in Forchlorfenuron 20 ppm (T_7), whereas the lowest (45.23) was observed in GA 25 ppm (T_4). Cytokinins such as forchlorfenuron enhance chlorophyll biosynthesis and delay leaf senescence, thereby improving photosynthetic efficiency.

Conclusions

Based on the findings of the present investigation, it can be concluded that application of different plant growth regulators significantly influences the growth, yield and quality of cowpea. Among the various treatments, the foliar spray of Brassinolide 2ppm at 30 days and 45days after sowing demonstrated notable advantages, resulting in enhanced growth, increased green pod yield. Foliar application GA at lower concentration improved quality of pods of cowpea.

Future Scope

This study investigates the role of plant growth regulators (NAA, GA, 4CPPU, and Brassinolide) in enhancing waterlogging stress tolerance in cowpea (*Vigna unguiculata*). It examines their effects on physiological, biochemical, and agronomic traits, including seedling vigor, root development, antioxidant enzyme activity, and yield performance. The research aims to identify the most effective PGR for mitigating waterlogging stress, thereby improving cowpea resilience in flood-prone areas. Findings from this study could offer valuable insights for sustainable agricultural practices and crop management strategies.

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