PAKISTAN JOURNAL OF AGRICULTURE
ISSN: 3079 – 3866 (ONLINE), 3079 – 3858 (PRINT)
https://isciencepress.com/journals/index.php/pja

Research Article

Gibberellic Acid (GA_3) Enhances biomass production, nitrogen accumulation and crude protein content of *Sesbania Aculeata* Pers. at Seedling Stage

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Received: 03.01.2025, **Accepted:** 22.02.2025, **Published Online:** 03.03.2025 **DOI:** https://doi.org/10.38211/PJA.2025.02.85

Abstract | The use of plant growth regulators has been highlighted as a very essential practice in agriculture. A pot experiment was designed and conducted in a green-house of the Department of Soil Science, Faculty of Crop Production, SAU, Tandojam. The aim of the experiment was to investigate the influence of different concentrations of gibberellic acid (GA_3) on the vegetative growth, development and biomass yield of a commercially important plant species locally called *Manjhandri* (*Sesbania aculeata* Pers.). The experiment was laid out in a complete randomized design with three replications. The seed was purchased from the market and sown in the pots by placing ½ inch deep in soil. After one week the seedlings were thinned to allow single plant per pot. The crop was irrigated with canal water and fertilized with chemical macronutrient fertilizer (ENGRO-NPK) based on their specific growth requirement. Thirty-days after planting the seedlings were sprayed with GA_3 solutions: The GA_3 concentrations included in the study for spray were: G_0 = Control (without GA_3 application), G_1 = 40 mg L^{-1}, G_2 = 60 mg L^{-1}, G_3 = 80 mg L^{-1}, G_4 = 100 mg L^{-1} and G_5 = 120 mg L^{-1} (in G_5 treatment, seedlings could not survive, hence this treatment was excluded from the study). The data were recorded for plant height, number of composite leaves (plant^{-1}), shoot and root fresh and dry weights and stem girth (mm). The results obtained from the study revealed that almost all the recorded growth and development traits of Sesbania aculeata Pers. were significantly affected by GA_3. The seedlings sprayed with 40, 60, 80 and 100 mg L^{-1} GA_3 showed 10, 31, 44 and 63% increase in height; 22, 35, 58 and 81% increase in number of composite leaves (plant^{-1}); 33, 59, 83 and 148% increase in shoot fresh weight; 15, 27, 49 and 90% increase in root fresh weight; 45, 73, 98 and 169% increase in shoot dry weight; 18, 34, 52 and 75% increase in root dry weight; 14, 28, 38 and 62% increase in stem girth and 32, 38, 70 and 81% increase in nitrogen and crude protein over unsprayed (control) seedlings, respectively. The results advocate that the application of GA_3 (100 mg L^{-1}) at seedlings stage improves growth and development of Sesbania aculeata. Hence, GA_3 can be used to spray this industrially important plant species.

Keywords: *Sesbania aculeata* Pers., GA_3, Plant growth regulators, Foliar spray

Introduction

The *Sesbania aculeata* Pers. is an annual, fast growing, medium density fiber-wood plant species belonging to the family *Leguminosae*, which grows mostly in tropical and sub-tropical areas of the world. Sesbania is locally known as *Manjhandri* and *Savery*. It is mostly grown in some parts of Sindh province; particularly district Naushahro Feroze, Shaheed Benazirabad, Khairpur Mir's, etc. The plant is leguminous in nature and fixes atmospheric nitrogen into the soil. The soil and weather conditions of Sindh seem to be in favor of the growth and development of this valuable plant species. Very little research has been done on its production technology, including cultivation time, soil requirement, pest management, varieties, organic and inorganic fertilizer/nutrients management, irrigation requirement, pruning, marketing, etc.

Plant growth regulating substances are bio-stimulants or bio-inhibitors. They are generally used to improve crop production as they regulate a number of physiological processes inside the plants. Particularly PGRs are organic compounds, other than nutrients used to regulate internal physiological processes of plants. They are active in nature hence very low concentration of them can regulate the nutritionally important physiological processes (Gupta et al., 2023). Plant growth regulators are commercially valuable compounds, especially in intensive agriculture (Wu et al., 2024).

Compared to other growth regulators, GA_3 is cost-effective. Its lower concentration seems to be non-toxic to plants. It is widely used in the crop fields. During early 1970s China initiated the industrial production of GAs, since then it is being used in horticultural crops for enhancing fruit yield. Exogenous application of gibberellic acid results in bigger shoots, more leaves and healthy stem in several plants (Monteiro et al., 2024).

The exogenous application of GA_3 during the flowering and fruiting stage enhance the quality and yield (Pahi et al., 2020). Foliar application of GA_3 improve growth and development, increasing root and shoot length, shoot fresh weight and shoot dry weight (Fatima et al., 2024). Especially, it is essential for enhance yield, fruit edible quality and shelf life of plant (Uddin et al., 2024) and enhancing anti-oxidative activities (Saeed et al., 2014).

In addition to the above effects, it has been found by several researchers that PGRs are used for rapid stem elongation; mainly they encourage the rate of cell division, potential source and sink, etc. Keeping in view the above beneficial effects of GAs, this study was designed to investigate the influence of range of GA_3 concentrations on the growth and development of *Sesbania aculeata*. Seedlings under greenhouse condition. The study was planned with two specific objectives, viz. (i) to determine the effect of different concentrations of GA_3 on shoot growth and root development of *sesbania aculeata* Pers. and (ii) to evaluate the influence of GA_3 on nitrogen and protein contents of leaves of *sesbania aculeata* Pers.

Materials and Methods

Growth conditions: This pot study was designed to investigate the effect of a (GA_3) on the growth and biomass yield of *Sesbania aculeata*. The experiment was conducted, following a completely randomized arrangement, in a tropical greenhouse with no control of light, temperature, humidity, etc. at the Department of Soil Science, Faculty of Crop Production, SAU, Tandojam.

Experimental soil: Soil was collected from Latif Experimental farm of SAU, Tandojam. The soil was air-dried and sieved through a 4 mm sieve and placed in 5 kg black colored plastic pots, having drainage holes in their bottom. Before initiation of the experiment, the soil was sampled for detailed analyses. Soil nitrogen was determined by Kjeldahl's Method (Estefan et al., 2013), available P and extractable K were analyzed by AB-DTPA extraction method of Soltanpour and Schwab (1977). The EC (dS m^{-1}) and pH (1:2, H_2O) were determined. Soil texture was determined through Bouyoucos Hydrometer method of Chopra and Kanwar (1959). Soil organic matter (SOM) was determined by Walkley-Black Method (Rowell, 1994) and the lime content ($CaCO_3$) was determined by Acid-neutralization method of Jackson, (1958).

Seed sowing: The seed of sesbania was initially soaked in water for 12 hours, and then it was sown in the pots by placing 1 inch deep in the soil. Thinning was done to achieve single plant to grow in each pot.

Fertilizer application: The recommended rates of P and K fertilizers were applied to plants in each pot. Phosphorus was applied through Single Super Phosphate (SSP) @ 50 kg P_2O_5 ha^{-1} and K was applied through Sulphate of potassium (SOP) @ 50 kg K_2O ha^{-1}.

Irrigation: Canal water was regularly applied to the plants in the pot as per requirement of the plants.

Application of gibberellic acid: Following five concentrations of GA_3 were prepared by diluting GA_3 salt initially in alcohol and then final volume was adjusted with distilled water. These solutions were used to spray the 30-day-old seedlings with knapsack sprayer: G_0 = Control (without GA_3 application), G_1 = 40 mg L^{-1}, G_2 = 60 mg L^{-1}, G_3 = 80 mg L^{-1}, G_4 = 100 mg L^{-1} and G_5 = 120 mg L^{-1} (due to toxic effect of over application, this treatment was excluded from the plan immediately).

Plant data recorded: Plant data were generated from the experiment: plant height (cm), number of composite leaves ($plant^{-1}$), stem girth (mm), root fresh and dry weight, shoot fresh and dry weight, nitrogen and crude protein content.

Plants were harvested from each pot (90 days after planting) by cutting with sharp sickle at soil surface level. Roots were carefully washed with distilled water and separated from shoots with sharp scissors. Plant samples were washed with distilled water. Fresh weight of both shoot and roots was recorded. Nodules were detached from the roots for counting and photography. The samples were placed in an oven for drying at 70 ^{0}C for 48 hours. Finally, data on dry shoot and root weights was obtained.

Leaf nitrogen content: Leaf dry matter of each plant was processed and analyzed for nitrogen (N). Nitrogen was determined by Kjeldahl's method (Estefan et al., 2013). Crude protein content was calculated through the formula (Preston, 2018), Crude Proteins = Nitrogen × 6.25.

Statistical analyses: The collected plant data were subjected to analysis of variance using Statistix ver. 8.1

computer software (Statistix, 2006). Treatment means were compared using the least significant difference (LSD) test at alpha 0.05.

Results

Soil properties: The results related to soil properties used in the experiment are given in Table 1. The soil filled in pots was sandy clay loam in texture, it was alkaline in reaction (pH 7.80) and non-saline (EC 3.90 dS m^{-1}). The soil was low in organic matter (0.80%) and phosphorus (2.00 mg kg^{-1}) contents. The lime content was also determined, that was high (12.80%). It seems that the soil was deficient in total nitrogen (0.03%).

Table 1. Selected properties of soil under experiment

Soil Property	Value
Texture class	Sandy clay Loam
Electrical conductivity 1:2 (EC dS m^{-1})	3.90
pH (1:2)	7.80
Lime ($CaCO_3$) content (%)	12.80
Soil organic matter (%)	0.80
Total nitrogen content (%)	0.03
Phosphorus (mg kg^{-1})	2.00

Plant height: The data on the height of *Sesbania aculeata* plants sprayed with different concentrations of gibberellic acid are depicted in the Table-2. There was positive and significant ($P <0.05$) effect of GA_3 on the height of sesbania plants. The sesbania plants sprayed with 40, 60, 80 and 100 mg L^{-1} GA_3 were 10, 31, 44 and 63% taller in height over unsprayed plants, respectively. The over concentration of GA_3 (120 mg L^{-1}) showed negative impact on the seedlings of sesbania, plants died (treatment excluded from the study). The application of GA_3 (100 mg L^{-1}) at seedlings stage improves growth and development of *Sesbania aculeata.*

Shoot fresh weight: The data on shoot fresh weight of *Sesbania aculeata* as affected by different concentrations of GA_3 are given in the Table 2. The fresh shoot weight of *Sesbania aculeata* was positively and significantly (P <0.05) affected by GA_3 concentrations. The results obtained from the study indicated that the plants sprayed with 40, 60, 80 and 100 mg L^{-1} GA_3 produced 33, 59, 83 and 148% more shoot fresh weight over control, respectively.

Shoot dry weight: Like other traits, the effect of various GA_3 concentrations on shoot dry weight (Table 2.) also remained positively effective and significant (P <0.05) as well. The sesbania seedlings sprayed with 40, 60, 80 and 100 mg L^{-1} GA_3 solution displayed 45, 73, 98 and 169% more shoot dry weight, over control respectively.

Root fresh weight: The results related to the fresh root weigt of *Sesbania aculeata* as affected by gibberellic acid concentrations are given in Table 2 The data indicated that the application of 40, 60, 80 and 100 mg L^{-1} GA_3 remained positively effective and significant (P <0.05) for fresh root weight of *Sesbania aculeata*. As compared to unsprayed treatment, the plants receiving 40, 60, 80 and 100 mg L^{-1} GA_3 gave 15, 27, 49, and 90% more fresh root weight.

Root dry weight: Enhancement in root dry weight was also noticed with the application of four GA_3 concentrations (Table 2). The *sesbania aculeata* plants receiving 40, 60, 80 and 100 mg L^{-1} GA_3 solution showed 18, 34, 52 and 75% increase in their shoot dry weight over control plants, respectively.

Number of composite leaves: The effect of different concentrations of GA_3 on *Sesbania aculeata* leaves was also found positive and significant (P < 0.05). Generally, the plants grown in the treatments where plants were sprayed with 40, 60, 80 and 100 mg L^{-1} had 22, 35, 58 and 81% more leaves over control, respectively.

Stem girth: Like other traits, the effect of GA_3 for this important trait also remained significant (P <0.05) and beneficial. The seedling treated with 40, 60, 80 and 100 mg L^{-1} GA_3 solution displayed 14, 28, 38 and 62% thick stem over control, respectively. Over concentration of GA_3 for this trait was also found adverse and the plants treated with 100 mg L^{-1} GA_3 showed only increase in stem girth, over control.

Leaf nitrogen and crude protein: *Sesbania aculeata* leaves are mostly used as fodder for livestock in the countryside, hence, to check the nitrogen and crude protein status in leaf tissue was determined and the results are given in the Table 3. The data indicated that raising the concentration of GA_3 showed positive impact on nitrogen content. The seedlings sprayed with 40, 60, 80 and 100 mg L^{-1} GA_3 solution contained 32, 38, 70 and 81% more nitrogen in these leaf tissues, over control, respectively. Due to the increase in nitrogen content, the values calculated for crude protein content were also

Table 2. Effect of different concentrations of GA_3 on growth attributes of *sesbania aculeata*

GA_3 concentrations	Plant height (cm)	Shoot fresh weight (g $plant^{-1}$)	Shoot dry weight (g $plant^{-1}$)	Root fresh weight (g $plant^{-1}$)	Root dry weight (g $plant^{-1}$)
G_0 = Control	73.6	14.5	4.4	14	4.2
G_1 = 40 mg L^{-1}	81.3	19.3	6.4	16.3	4.9
G_2 = 60 mg L^{-1}	96.6	23.1	7.7	17.8	5.6
G_3 = 80 mg L^{-1}	106.3	26.6	8.8	20.8	6.4
G_4 = 100 mg L^{-1}	120.3	36.0	12	26.6	7.3
SED	0.7746	2.2440	0.7467	0.6791	0.1358
LSD (0.05)	1.7862	5.1746	1.7218	1.5659	0.3132

Table 3. Effect of different concentrations of GA_3 on growth, nitrogen, and crude protein content of *sesbania aculeata*

GA_3 concentrations	Composite leaves	Stem girth (mm)	Nitrogen (%)	Crude protein (%)
G_0 = Control	26.6	6.2	5.16	32.29
G_1 = 40 mg L^{-1}	32.6	7.1	6.83	42.7
G_2 = 60 mg L^{-1}	36.0	8.0	7.13	44.33
G_3 = 80 mg L^{-1}	42.0	8.6	8.76	54.71
G_4 = 100 mg L^{-1}	48.3	10.1	9.36	58.54
SED	1.7385	0.3565	0.0416	2.3351
LSD (0.05)	4.0089	0.8222	0.23208	4.115

found higher with the application of GA_3. The seedlings receiving 40, 60, 80 and 100 mg L^{-1} GA_3 had 32, 38, 70 and 81% more crude proteins.

Discussion

Growth regulators or hormones are used in agriculture and biology for regulating the growth and development of high commercial value plants (Kandil et al., 2014). Particularly, these growth hormones are used for depressing and/or enhancing potential morphological traits like height, number and size of leaves, bud and flower formation, ripening of fruits, hastening maturity, etc. (Sumanasiri et al., 2013). The naturally occurring plant growth regulators or plant hormones such as; cytokinins, auxins, abscisic acid, gibberellin and ethylene having their own specific effect on plant growth and development (Thapa et al., 2024). Among the growth hormones, gibberellins are found very useful to improve growth and development of high commercial value field and pot grown plants (Chang & Sung, 2000).

In this study, gibberellic acid (GA_3) was selected and used to improve growth and development of *Sesbania aculeata* plant species at an early/seedling stage under greenhouse environment. This plant species has got commercial value in different parts of the world; including Egypt, India, Malaysia, Pakistan, and other countries, etc.

In Sindh province of Pakistan, the cultivation of *Sesbania aculeata* has been initiated by some growers with the collaboration of some industries, particularly involved in the production of medium density fiber wood (MDF) board sheets. Particularly, the Al-Noor MDF Board has been found very active in this practice. Currently the MDF has encouraged the growers in some parts of Sindh; like Noushehro Feroze, Shaheed Benazirabad and may be Dadu districts. However, a planned scientific study so far has not been initiated on this plant. Keeping in view the commercial value of *Sesbania aculeata* it was hypothesized that the growth hormones can significantly enlarge and develop this plant. Therefore, the study reported in this manuscript was planned to investigate the influence of different concentrations of gibberellic acid (GA_3) i.e. 40, 60, 80, 100 and 120 mg L^{-1}. Although the crop requires 18 months to attain maturity but in this pot study the experiment was terminated after 6 months, just to observe the its response to GA_3 at early/seedling stage.

It is interesting to report that the *Sesbania aculeata* plants treated with various concentrations of GA_3 in this study showed positive response in all respects including plant height, fresh and dry weight of roots and shoot (Table 2), number of composite leaves, stem girth (Table 3). It seems that gibberellic acid can be used to stimulate/promote the growth and development of roots, leaves and stem of *Sesbania aculeata* plants. Such numerous positive effects of gibberellins can be the results of various biochemical mechanisms. Faster mobilization of foods and mineral elements in plants has also been reported by (Thapa et al., 2024); as gibberellins stimulate the mobilization of food and nutrient elements, especially at early and young seedling stage. Similarly reserve mineral elements also become more readily available as a result of gibberellins action. GA_3 causes elongation of more than 15-times as great as in the untreated. It increases cell-wall plasticity by promoting cell elongation and organ growth (Shah et al., 2023) that promotes roots and leaves on large scale. Gibberellins also loosen cell-walls and increase formation of hydrolytic enzymes leading to stem elongation (Thapa et al., 2024). Cell-wall polysaccharides might be synthesized faster or become more active in gibberellins-treated plant cells.

Much significant improvement in almost all traits was found by spraying seedlings with 40, 60, 80, and 100 mg L^{-1} GA_3 solution; whereas the plants sprayed with 120 mg L^{-1} GA_3 were found negatively effective for seedlings. This logically indicates that these plants should not be sprayed with over concentration of GA_3. Several other studies and reports have also indicated the similar response of various crop species to over application of GA_3 concentrations. That was possibly due to the excess accumulation of gibberellins in plants; because plants also synthesize gibberellins, but not in enough quantity. Hence, the exogenous application of gibberellins can meet the demand, however, it may not be in over quantity.

Conclusion

It can be concluded from the study that the *Sesbania aculeata* seedlings responded well to the GA_3 concentrations up to 100 mg L^{-1}. Beyond 100 mg L^{-1} application of GA_3 on this species showed negative impact on its growth and development. Hence, it is recommended that GA_3 (100 mg L^{-1}) can be applied to this plant, for stimulating its growth and development.

Acknowledgements

We sincerely thank Prof. Dr. Inayatullah Rajpar for providing technical guidance and logistic support during this study. We also thank our Biosaline Agriculture Greenhouse and Laboratory staff for their assistance during this research study.

Author Contribution

ZL: Conceiving the idea and conducting pot study, collected data, writing initial draft of MS; ND: Data analysis, editing all drafts of MS; AL: Literature review; KJ: Data presentation; FNM: Chemical analysis; RO: Updating citations and references section, proof reading, format and style. All authors approved and assumed the responsibility of the content of MS.

Conflict of Interest

No competing interests are disclosed by the authors.

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