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Evaluating the impact of phyto-hormones on the morpho-biochemical traits of soybean through seed treatment and foliar application

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ABSTRACT

Global climate variations, mainly extreme temperatures reduce crop production and contributed to global food insecurity. Plant growth regulators (PGRs) improve physiological efficiency, photosynthetic capacity, and assimilate partitioning in field crops. However, their influence on soybean (Glycine max L.) yield and seed quality is under-researched. This study investigates the synergistic effects of auxin (2,4-D) and gibberellin (GA₃) at 60 and 90 mg L^{-1} concentrations. This study investigates the impact of phytohormones, specifically gibberellic acid (GA3) and 2,4-dichlorophenoxyacetic acid (2,4-D), on the morphological and biochemical traits of soybean (Glycine max L.) through seed treatment and foliar application. These Growth regulators were applied through seed treatment at the time of sowing and foliar application at the time of flowering initiation stage. The experiment was designed using a RCBD arrangement and each block replicated thrice time. Seed treatment with 2,4-D at 90 mg/L showed that maximum plant height (116.80 cm), first node height (8.04 cm), No. of branches per plant (18.67), root length (18.12 cm) stem diameter (0.05 cm), no. of pods per plant (32.60), seed yield (1532.30 kg ha⁻¹), biological yield (4109.30 ha⁻¹), and harvest index (39.36 %). The GA₃ (seed treatment) at conc. of 90 and 60 mg L^{-1} resulted in a significantly increased no. of nodule fresh weight, nodules per plant, and dry weight respectively. But the no. of seeds per pod did not showed significant results throughout the study. However, the foliar applied of 2,4-D at 90 mg L^{-1} resulted in significantly increased leaf area index (LAI) compared to the seed treatment. PGRs had a significant influence on quality traits. Thus, the application notably improved the potential of soybean and successful oilseed crop. Therefore, it is showed that using PGRs are extremely helpful for attaining higher growth, yield production, and improved quality of soybean.

1. Introduction

Soybean (*Glycine* max L.) is an imperative and critical legume besd oil-seed-crop. It originated in East Asia and It may thrive in temperate,

tropical, and subtropical climates worldwide. About 90 % of the world's soybean production is produced by the United States, India, China, Brazil and Argentina (Amoanimaa-Dede et al., 2022). Soybean is an imperative source of minerals, proteins, fats, vitamins, and energy for

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livestock, and humans. Its seeds contain 20-22 % oil, 40-42 % protein, 20-26 % carbohydrates, and a high amount of P, Ca, and vitamins (Rahman et al., 2011). It is a multi-purpose crop as it is extensively grown for its edible beans. It is sometimes considered as "the meat grows on plant" because of the good quality protein. Soymeal is used as a supplementary diet for human food and poultry and livestock feed. Other uses include the provision of the raw material for industries e.g., soy-oil is used in the manufacturing of lubricants, paints, varnishes, antibiotics, and adhesives (Khanzada et al., 2013). Pakistan is one of the biggest importers of soybean oil; majorly Pakistan imports soybeans from USA and Argentina. Originally, this crop was introduced in Pakistan as an oilseed crop in the 1960 s, and it gained much attention from research and industry for a decade but latter farmers shifted back to conventional crops. Nevertheless, it has been grown in the Northern areas of Pakistan: Hazara, Swat, Dir, and Azad Jammu and Kashmir. The crop has shown better results and higher yields under mild climatic conditions in northern regions than in the severe climate of Sindh and Punjab. The area under cultivation of soybean is negligible in Pakistan. Efforts have been made by Pakistan Agricultural Research Council (PARC) to promote innovative ideas for sovbean growth as an oilseed crop in Pakistan. Other academic and research institutes of Pakistan are now actively participating in research and farmer-reach projects to identify suitable varieties for different regions and dissemination of its production technology. Efforts are underway to identify and breed biotic and abiotic stress-tolerant soybean varieties and improve the nutritive potential of currently available soybean cultivars. To increase soybean production, one of many strategies is the use of plant growth regulators since they promote the normal growth and development of soybean plants and guarantee higher yield. There are five major phytohormone groups including auxin, abscisic acid, cytokinins, gibberellins, and ethylene. Application of these growth regulators is a valuable practice, which has shown better yield of beans, corn, and soybean (Vieira and Castro, 2004). Exogenous application of these growth regulators increases the crop yield in field thereby enhances the dry mass (Cho et al., 2008). In numerous vegetable crops, pre-soaking, Seed priming, and other pre-sowing treatments with PGRs have boosted seed performance (Ashraf, 2010). Typically, priming responses are found much faster, quicker and more reliable to seedling emergence in the seedbed environment and versatile temperature ranges. It results in a better crop stand and ultimately improves harvest quality and yield responses especially for suboptimal, stressed conditions, prevailing in the field (Halmer, 2004). Comparably, foliar application of phytohormones e.g., GA₃ promoted plant height, increased first node height, stem diameter, leaf area, and dry matter in soybean (Leite et al., 2003). Gibberellins (GAs) enhance seed germination, seedling establishment, flower establishment, fruit and seed development, and increase dry mass and yield responses (Yamaguch, 2008). Furthermore, GAs increases the photosynthetic competence of plants by influencing the photosynthetic enzymes through light interception, leaf-area index, and increased nutrient use efficiency (Khan et al., 2007). Contrastingly, when soybean plants were exposed to higher concentrations of 2,4-D, extreme leaf burning, plant stunting, and necrosis occurred (Johnson et al., 2012). Considering the prevailing climatic conditions i.e., intermittent rainfall, weed infestations, insect and disease attack, it is essential to understand how the application of different concentrations of GAs (specifically GA₃) and 2,4-D can be helpful for farmers to improve overall soybean plant performance. Thus, the purpose of this experiment was to evaluate the effects of PGRs on Soybean growth, productivity, and quality under various application scenarios

2. Materials and methods

2.1. Experimental site

The study was conducted in the Agronomic Field Research Area at the University of Poonch Rawalakot, Azad Kashmir with (33°51′32.18″N, 73° 45′34.93″E) at an altitude of 1638 m reported by Zafar et al. (2013) during the spring session of 2017. Soil analysis of this region, containing samples collected from several locations, has classified it as "*Thermic Lithic Eutrudepts*" with a temperate climate region. This classification orders with the Köppen climate classification scheme. The annual precipitation at the research experimental site is approx. 700–800 mm, with Avg. mean temperatures fluctuating from 5-28 °C (Khaliq and Abbasi, 2015). A pre-planting physico-chemical analysis were performed on base of soil samples and Samples collected from the randomly different place of each block to a depth of 15 cm, revealing silt-loam soil. The soil characteristics are presented in Table 1.

Crop husbandry detail: The experiment was planned and arrange randomized complete block design with three time replicated. Two plant growth regulators (PGRs), auxin (2,4-D) and gibberellins (GA3), were applied through seed treatments and foliar applications at different concentrations of 60 and 90 mg L^{-1} . In this experiment different treatments were used and details as follows: T0 = Control (unsoaked seed); $T1 = GA3 60 \text{ mg L}^{-1}$ seed treatment; $T2 = GA3 90 \text{ mg L}^{-1}$ seed treatment; T3 = GA3 60 mg L^{-1} foliar application; T4 = GA3 90 mg L⁻¹ foliar application; T5 = 2,4-D 60 mg L⁻¹ seed treatment; T6 = 2,4-D 90 mg L⁻¹ seed treatment; T7 = 2.4-D 60 mg L⁻¹ foliar application; and T8 = 2.4-D 90 mg L^{-1} foliar application. The seeds were used in this study, obtained from the gene-bank at the National Agriculture Research Institute (NARC) in Islamabad, Pakistan. Seed treatments were carried out before to sowing, and also foliar applications were applied at the crop flowering initiation stage. The sowing area tilled by three times, followed by planking to make an adequate seedbed. Each experimental plot was dignified 4 m², with plant-to-plant spacing arranged of 20 cm and rowto-row spacing of 50 cm, covering four rows per plot. The approved soybean genotypes, Rawal-1, was cultivated using a seed dibbler and seed rate was used 100 kg ha⁻¹. The recommended doses of different fertilizer were applied such as nitrogen (urea), phosphorus (SSP), and potassium (SOP) at rates of 25, 60, and 50 kg ha⁻¹, respectively. For foliar application stock solutions were prepared of GA3 and 2,4-D (1000 mg L^{-1}) and diluted to applications of 60 and 90 mg L^{-1} . For seed treatment, measured quantities of total seeds and seeds were soaked separately in each dilution of the phyto-hormones for 6 h and then sun dried in the shade. Foliar applications were performed at the flowering initiation stage with a sprayer. All the treatments were applied separately as assigned.

2.2. Data collected

Ten plants were randomly obtained from each treatment for measurements record. Data were noted for plant height, root length, first node height, No. of branches per plant, stem diameter, leaf area, No. of nodules per plant, fresh and dry weight of nodules, No. of pods per plant, No. of seeds per pod, crude protein (CP), oil content, and neutral detergent fiber (NDF). Means of three replicates were calculated for further analyses. A sample of 100 grains was randomly carefully chosen and weighed via digital electric balance to record the 100-grain weight. The chlorophyll content of leaves was imposed at the vegetative stage from mature three palnts via a portable chlorophyll meter (SPAD) per

Table	1
Table	1

Soil Physio-chemical attributes of the experimental site.

Soil Characteristics	Observations	
рН	6.63	
Soil Organic matter (g kg ⁻¹)	7.33	(Walkley and Black, 1934)
Sand (g kg ⁻¹)	237	
Silt (g kg ⁻¹)	536	
Clay (g kg ⁻¹)	208	
Textural Class	Silt loam	(Bouyoucos, 1962)
Total Nitrogen (mg kg ⁻¹)	0.33	(Bremner and Mulvaney, 1982)
Available Phosphorus (g kg ⁻¹)	6.90	(Olsen and Sommers. 1982)
Extractable Potassium (g kg $^{-1}$)	78.9	(Simard, 1993)

plot, and the average value was recorded. At maturity, harvesting and threshing were through manually performed. Grain yield from 1 m^2 was measured with an electronic scale and then changed to kg ha⁻¹ using the following formula:

Grain Yield =
$$\frac{\text{Yield}(\text{Kg})}{\text{Area}(m^2)} \times 10000m^2$$

After harvesting, the biological yield was measured by weighing the complete plot. The mean yield was then changed to kg ha^{-1} using the appropriate formula:

$$Biological Yield = \frac{Biological Yield(Kg)}{Area (m^2)} \times 10000m^2$$

Harvest index was considered by the given formula:

$$Harvest Index = \frac{Seed Yield}{Biological Yield} \times 100$$

Before quality analysis value, soybean seeds were crushed and conceded through a 2 mm sieve. Crude protein contents were determined with the Kjeldahl method (AOAC, 2012). For this, 1 g of oven-dried sample was processed in a Kjeldahl flask with 30 ml of determined H₂SO₄ and 5 g of a digestion mixture (K₂SO₄; CuSO₄; FeSO₄ in a 100:10:5 ratio). A 10 ml aliquot was reserved for distillation. Ammonia was composed in a boric acid (2 %) solution with mixed displays (Bromocresol green and methyl red) and titrated alongside standard 0.1 N H₂SO₄. The crude protein measurement in (% percentage) was calculated by increasing the H₂SO₄ titration reading by 6.25. Neutral detergent fiber was assessed with the Van Soest et al., (1991) method. Oil contents were determined using the Soxhlet extraction method. Place the grinded seed sample in a thimble and insert it into a Soxhlet extractor. Add a suitable solvent (commonly hexane) to the extraction flask. Heat the flask so that the solvent vaporizes, rises through the column, condenses, and then drips over the seed sample. This process is repeated over several hours or even overnight to ensure complete extraction. After extraction, remove the solvent from the collected oil by evaporating it. Weigh the extracted oil to determine its mass. Calculate the oil content as a percentage of the initial seed weight (Smith, 2010)

Statistical analysis; The Composed data were analyzed using Fisher's analysis of variance technique, and treatments mean was compared using Tukey's (HSD) test at a 5 % probability level (Steel et al., 1997).

3. Results and discussion

Conferring to the results, most of the treatments were non-significant from each other for all growth attributes except control. The maximum plant height, first node height, root length, number of branches per plant and stem diameter was observed when seed was primed with 2,4-D at 90 mg/L while minimum value of all mentioned parameters were noted in control. Likewise, higher leaf area was measured in foliar application with 2,4-D 90 mg/L and lowest value was observed in control (Table 2). It might be due to early and synchronized germination and improved photosynthetic ability which leads to effective partitioning of accumulates. These results also corroborate with the findings of Amoanimaa-Dede *et al.* (2002), who reported the highest plant height, stem diameter, and the number of leaves when soybean seed was primed in a solution.

Similar findings were also noted by Patel et al. (2016) in chilli. Regarding GA₃, an earlier study by Agawane and Parhe (2015) reported that seed priming with GA3 at 100 mg/L for 12 h caused significant variation in plant height, stem girth, No. of pods, No. of branches and leaf area of soybean. These results are consistent with the findings of Leite et al. (2003), Dhakne et al. (2015) and Khatun et al. (2016), who reported that maximum plant height, first node height, root length, number of branches per plant, stem diameter and leaf area of soybean in response to the foliar application of GA₃. In this field investigation, seed soaking in growth regulators had also varying impact on other yield attributes of soybean. Seed treatment with 2,4-D 90 mg/L remained at par with T2 and T5 for recording the highest number of nodules per plant, while minimum was recorded in control treatment. The nodules fresh weight and nodules dry weight was found non-significant (Table 3). The preferente performance of seed treatments with 2,4-D at 90 mg L^{-1} may be attributed to its significante physiological effects on plants, including seed germination, growth, stem elongation, leaf expansion, photosynthesis, flowering, and cell expansión (Taiz and Zeiger, 2010). These results are in toeing the line with those of Basuchaudhuri (2016), Bawa et al. (2020), Agawane and Parhe (2015) and Azizi et al. (2012) who also reported that plant growth regulators can improve the physiological efficiency in field crops. Likewise, No. of pods per plant could not reach a level of significance from T6 > T8 > T4 > T2 > T1 except control that which was statistically at par with T3 and T7. This might be due to the reduced pod abortion which resulted in an increased No. of pods (Passos et al., 2011; Nonokawa et al., 2012). No. of seeds per pod showed no response of the PGRs used. The increase in 100 grain weight was ordered as T6 > T8 > T3 > T4 > T2 > T5 > T1 > T0. As seed treatment and foliar application was effective in early emergence and good stand establishment of soybean crop which ultimately led to better nutrient uptake and more 100 grain weight. These results differ with the conclusion of Kalyankar et al. (2007), who reported that Naphthalene Acetic Acid at 50 mg/L increased the No. of pods per plant, seeds per pod and 100 seed weight. Similar results were also reported by Azizi et al. (2012). It concluded that different levels of GA₃ and the soybean genotypes had significant effect on No. pod per plant, No. seed per pod, 1000-seed weight, and economic as well as biological yield (p < 0.01). Seed priming with PGR is an unusually used priming method to increase the crop growth and yield under hectic environment (Jisha et al. 2013; Hu et al. 2013). Soybean seed imbibed in solution of 2,4-D at 90 mg/L showed greenish leaves owing to more chlorophyll contents (Table 4). The enzyme chlorophyllase was responsible for the growth retarder of chlorophyll, which improved the chlorophyll content. Application of growth regulators at different stages of plant exhibited a

Table	2
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Influence of seed treatment and foliar application of GA₃ and 2,4-D on growth attributes of soybean.

Treatments	Plantheight (cm)	First nodeheight (cm)	Rootlength (cm)	No. of branches per plant	Stemdiameter (cm)	Leafarea (cm²)
T0 = Control	88.20 d	2.28c	12.08 d	10.80 e	0.03	1078.11 d
T1 = GA3 60 mg/L seed treatment	105.20 abc	6.00 ab	14.22b	15.21 bc	0.04	1421.00c
T2 = GA3 90 mg/L seed treatment	113.07 ab	7.93 a	17.10 a	17.33 ab	0.05	1421.33c
T3 = GA3 60 mg/L foliar application	100.40c	3.02c	12.10 cd	11.56 de	0.04	1550.44 abc
T4 = GA3 90 mg/L foliar application	103.33 bc	4.01 bc	13.52 bc	13.78 cde	0.05	1626.00 abc
T5 = 2,4-D 60 mg/L seed treatment	107.31 abc	6.51 a	16.51 a	16.10 abc	0.04	1494.14 bc
T6 = 2,4-D 90 mg/L seed treatment	116.80 a	8.04 a	18.12 a	18.67 a	0.05	1515.30 bc
T7 = 2,4-D 60 mg/L foliar application	104.03 bc	3.90c	13.70 bc	14.02 bcde	0.05	1745.73 ab
T8 = 2,4-D 90 mg/L foliar application	104.67 bc	4.13 bc	14.11 bc	14.80 bcd	0.05	1799.82 a
Tukey's critical value	12.10	2.05	2.11	3.44	NS	283

Influence of seed treatment and foliar application of GA3 and 2,4-D on yield attributes of soybean.

Treatments	No. of nodules $plant^{-1}$	Nodule Fresh weight (g)	Nodule Dry weight (g)	No. of pods $plant^{-1}$	No. of seeds per pod	100-grain weight (g)
T0 = Control	11.67f	0.52	0.29	27.40 d	2.26	13.60 e
$T1 = GA_3 60 \text{ mg/L seed treatment}$	17.33 bc	0.67	0.38	34.03 abc	2.60	17.40 abcd
$T2 = GA_3 90 \text{ mg/L seed treatment}$	18.67 ab	0.70	0.39	36.00 ab	2.80	18.56 ab
$T3 = GA_3 60 \text{ mg/L}$ foliar application	14.33 e	0.66	0.38	30.56 cd	2.60	19.26 ab
$T4 = GA_3 90 \text{ mg/L}$ foliar application	15.00 de	0.68	0.37	36.33 ab	2.70	19.03 ab
T5 = 2,4-D 60 mg/L seed treatment	19.33 ab	0.65	0.35	32.60 bc	2.63	18.26 abc
T6 = 2,4-D 90 mg/L seed treatment	20.33 a	0.69	0.38	37.53 a	2.73	20.10 a
T7 = 2,4-D 60 mg/L foliar application	16.00 cd	0.62	0.36	30.46 cd	2.60	17.06 bcd
T8 = 2,4-D 90 mg/L foliar application	17.67 bc	0.67	0.36	36.76 ab	2.70	19.46 ab
Tukey's critical value	2.18	NS	NS	4.91	NS	2.99

Means with different letter indicate statistical difference (Tukey; $p \le 0.05$).

Table 4

Influence of seed treatment and foliar applied of GA3 and 2,4-D on chlorophyll contents, seed yield, biological yield and harvest index of soybean.

Treatments	Chlorophyll contents	Seed yield (kg ha ⁻¹)	Biological yield (kg ha ⁻¹)	Harvest index (%)
T0 = Control	20.95 e	1191.00 e	3255.30 e	32.76c
$T1 = GA_3 60 \text{ mg/L}$ seed treatment	22.32 de	1258.70 d	3686.70 d	34.10 bc
$T2 = GA_3 90 \text{ mg/L}$ seed treatment	24.63 bc	1496.30b	4011.00b	37.26 ab
$T3 = GA_3 60 mg/L$ foliar application	23.51 bcd	1442.30c	3953.70 bc	36.43 abc
$T4 = GA_3 90 \text{ mg/L}$ foliar application	23.82 bcd	1492.00b	3893.70c	38.23 a
$T5 = 2,4-D \ 60 \ mg/L$ seed treatment	24.11 bcd	1519.70 ab	4066.30 ab	36.93 abc
T6 = 2,4-D 90 mg/L seed treatment	27.23 a	1532.30 a	4109.30 a	39.36 a
T7 = 2,4-D 60 mg/L foliar application	23.21 cd	1426.30c	3756.30 d	38 a
T8 = 2,4-D 90 mg/L foliar application	25.48 ab	1489.00b	3985.00 bc	37.36 ab
Tukey's critical value	2.23	35.49	97.83	3.29

Means with different letter indicate statistical difference (Tukey; $p \le 0.05$).

significant effect on chlorophyll contents (Khatoon et al., 2016). Similarly, Devi et al. (2011) concluded that cycocel at the rate of 500 mg/L applied as foliar spray increased the chlorophyll content and carotenoids in leaves.

Basuchaudhuri (2016) found increased in chlorophyll contents with the use of auxins, cytokinins, gibberellins and abscisic acid in soybean. In contrast to our findings, El-Shraiy and Hegazi (2009) revealed that a decreasing in chlorophyll contents with the use of PGRs such as GA3. Yield production, obviously, is the final goal of cultivated crop. Therefore, the economic significance of PGRs basically depends on their capability to increasing crop yield. The different PGRs had a significant influence on soybean yield. More seed yield was noted in 2,4-D at 90 mg/L as seed treatment that was at statistical parity with T5 followed by T2 > T3 > T8. The minimum was recorded in control treatment. Dhakne et al. (2015) illustrious that foliar applied of 40 mg/L of NAA increased the growth productivity of soybean. Similarly, Khatun et al. (2016) concluded that, Foliar sprayed of salicylic acid at reproductive stage of soybean increased the biomass and production. The weight of the total biomass determines the growth potential of the crop in general under the foliar applied and seed treatments. The seed primed with 2, 4-D at 90 mg/L of seed treatment exhibited improved biological characteristics as compared with other treatments excepted T5. The lowest was observed in control. The results corroborate with the conclusion of Basuchaudhuri (2016) who explained that growth hormones when applied at suitable concentration influenced the yield components and yield of soybean especially seed yield, biological yield and harvest

index. Seed priming significantly influenced seed yield and traits that contribute to soybean yield, showing the corresponding favorable improvement in number of pods per plant, number of seeds per pod, seed yield, and biological yield (Agawane and Parhe, 2015). The physiological aptitude and efficacy of any crop for converting the dry mass yield into the economic yield is called as its harvest index. The higher value was found by 2,4-D at 90 mg/L seed treatment that could not reach to a level of significance from T2, T3, T5, T7 and T8 except control which is at par with T1 and T3. These results are supported by the evaluations of Basuchaudhuri (2016) who concluded that no doubt exogenous application and stage of application of plant growth hormones have shown some impacts in index of yield attributes. Upadhyay and Ranjan (2015) observed that application of GA3 (20 mg/L) increased the biological yield and seed yield along with harvest index.

Quality traits among these treatments are significantly varied (Table 5). As for as the crude protein is concerned, the maximum value of crude protein (42.23 %) was noted T6 treatment where soybean seed was soaked in 2,4-D 90 mg/L solution which is followed by GA₃ 90 mg/L as seed treatment ($p \le 0.05$). Minimum seed protein contents (37.56) were accounted for control Highest crude protein content in the seeds might be due to more translocation of photosynthates to the seeds. Khatoon et al. (2016) reported maximum crude protein contents in soybean with the application of Salicylic acid. Application of higher dose of NAA resulted higher protein content and lower oil content is of soybean with the use of GA₃ 60 mg/L foliar application that was at par with T1 and T7. Travaglia et al (2009) concluded that GA₃ as foliar spray increased oil contents but reduced protein contents in soybean. Application of higher dose of NAA resulted higher protein contents and lower

Table	5
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Influence of seed treatment and foliar applied of GA3 and 2,4-D of crude protein contents, oil contents and Neutral detergent fibre (NDF) of soybean.

Treatments	Crude Protein	Oil Contents	NDF
T0 = Control	37.56 e	14.44 d	14.32 e
$T1 = GA_3 60 \text{ mg/L seed treatment}$	38.54 d	18.81 ab	17.67 bcd
$T2 = GA_3 90 \text{ mg/L seed treatment}$	41.32b	15.10 cd	17.89 bc
$T3 = GA_3 60 \text{ mg/L}$ foliar application	38.24 de	19.10 a	16.17 d
$T4 = GA_3 90 \text{ mg/L}$ foliar application	39.89c	16.78 bc	17.10 cd
T5 = 2,4-D 60 mg/L seed treatment	40.10c	16.43 cd	18.88 ab
T6 = 2,4-D 90 mg/L seed treatment	42.23 a	14.50 d	19.76 a
T7 = 2,4-D 60 mg/L foliar application	39.90c	18.99 a	18.55 abc
T8 = 2,4-D 90 mg/L foliar application	40.33c	16.32 cd	18.64 ab
Tukey's critical value	0.82	2.20	1.52

Means with different letter indicate statistical difference (Tukey; $p \le 0.05$). NDF were noted maximum in T6 where 2,4-D 90 mg/L used as seed treatment while lowest in control. The increase in NDF might be due to the response of cell wall constituents. In Alfalfa forage NDF contents were significantly reduced by the applied of various type of PGRs (Buck et al., 1988).

oil content in soybean (Basuchaudhuri, 2016).

4. Conclusions

Significant differences among plant growth regulators (PGRs) and their mode of application were observed which indicated that use of PGRs should be stimulated. However, Auxin; (2,4-D) at 90 mg/L as seed priming presented the higher values for overall studied variables. Hence, it is recommended that the use of PGRs must be exhilarated for soybean crop in rain-fed and hilly area.

CRediT authorship contribution statement

Muhammad Shehzad: Writing - review & editing, Writing - original draft, Visualization, Supervision, Software, Resources, Project administration, Investigation, Formal analysis, Data curation. Muhammad Sajid Munir: Writing - review & editing, Writing - original draft, Visualization, Supervision, Software, Resources, Project administration, Investigation, Formal analysis, Data curation. Muhammad Nazim: Writing - review & editing, Writing - original draft, Visualization, Supervision, Software, Resources, Project administration, Investigation, Formal analysis, Data curation. Majid Mahmood Tahir: Writing - review & editing, Visualization, Validation, Supervision, Investigation, Conceptualization. Mehdi Maqbool: Writing - review & editing, Visualization, Validation, Supervision, Investigation, Conceptualization. Muhammad Amjad Nawaz: Writing - review & editing, Validation, Software, Conceptualization. Hafiz Muhammad Rashad Javeed: Writing - review & editing, Supervision, Methodology, Formal analysis. Abdullah Ahmed Al-Ghamdi: Writing - review & editing, Supervision, Software, Resources, Project administration, Funding acquisition, Formal analysis.

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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References

- Agawane, R.B., Parhe, S.D., 2015. Effect of seed priming on crop growth and seed yield of soybean (*Glycine max* (L) Merill). The Bioscan 10, 265–270.
- Amoanimaa-Dede, H., Su, C., Yeboah, A., Zhou, H., Zheng, D., Zhu, H., 2022. Growth regulators promote soybean productivity: a review. Peer J. 10:e12556. 10.7717/ peerj.12556.
- Aoac, 2012. Official methods of analysis, 19th, rev. ed. Association of Official Analytical Chemists, Washington, DC, USA.
- Ashraf, M., 2010. Inducing drought tolerance in plants: recent advances. Biotechnol. Adv. 28 (1), 169–183. https://doi.org/10.1016/j.biotechadv.2009.11.005.
- Azizi, K., Moradii, J., Heidari, S., Khalili, A., Feizian, M., 2012. Effect of different concentrations of gibberellic acid on seed yield and yield components of soybean genotypes in summer intercropping. International Journal of Agricultural Sciences 2, 291–301. http://www.inacj.com/attachments/sect.
- Basuchaudhuri, P., 2016. Influences of Plant Growth Regulators on yield of Soybean. Indian Journal of Plant Science 5, 238–255.
- Bawa, G., Feng, L., Chen, G., Chen, H., Hu, Y., Pu, T., Cheng, Y., Shi, J., Xiao, T., Zhou, W., et al., 2020. Gibberellins and auxin regulate soybean hypocotyl elongation under low light and high-temperature interaction. Physiol. Plant. 170 (3), 345–356. https://doi.org/10.1111/ppl.13158.

Bouyoucos, G.J., 1962. Hydrometer method improved for making particle size analysis of soils. Agron. J. 54 (5), 464–465.

Bremner JM and Mulvaney CS. 1982. "Nitrogen-Total," in Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties, Ed. by A. L. Page, et al. (Soil Science Society of America, American Society of Agronomy, Madison, WI) pp. 383-411.

- Buck, D.C., Cosen, R.D.H., Christensen, D.A., 1988. Effects of various plant growth regulators on the nutritive value and yield of alfalfa. Can. J. Plant Sci. 68, 95–101. https://doi.org/10.4141/cjps88-010.
- Cho, M.H., No, H.K., Prinyawiwatkul, W., 2008. Chitosan treatments affect growth and selected quality of sunflower sprouts. J. Food Sci. 73, 570–577. https://doi.org/ 10.1111/j.1750-3841.2007.00607.x.
- Devi, K.N., Vyas, A.K., Singh, M.S., Singh, N.G., 2011. Effect of bioregulators on growth, yield and chemical constituents of soybean (*Glycine max*). J. Agric. Sci. 3, 151–157. https://doi.org/10.5539/jas.v3n4p151.
- Dhakne, A.S., Mirza, I.A.B., Pawar, S.V., Awasarmal, V.B., 2015. Yield and economics of soybean (*Glycine max* (L) Merill) as influenced by different levels of sulphur and plant growth regulator. Int. J. Trop. Agric. 33, 2645–2648.
- El-Shraiy AM, Hegazi AM. 2009. Effect of acetylsalicylic acid, indole-3-butyric acid and gibberellic acid on plant growth and yield of pea (*Pisum sativum* L.). Australian Journal of Basic and applied Sciences 3 (4): 3514-3523.
- Halmer, P., 2004. Methods to improve seed performance in the field. Food Products Press, The Harworth Press Inc, Handbook of seed physiology. New York, pp. 125–166.
- Hu YF, Zhou G, Na XF, Yang L, Nan WB, Liu X, Zhang YQ, Li JL, Bi YR. 2013. Cadmium interferes with maintenance of auxin homeostasis in Arabidopsis seedlings. Journal of Plant Physiology Jul 15: 170(11): 965-75.
- Jisha, K.C., Vijayakumari, K., Puthur, J.T., 2013. Seed priming for abiotic stress tolerance: an overview. Acta Physiol. Plant. 35, 1381–1396.
- Johnson, V.A., Fisher, L.R., Jordan, D.L., Edmisten, K.E., Stewart, A.M., York, A.C., 2012. Cotton, peanut, and soybean response to sublethal rates of dicamba, glufosinate, and 2, 4-D. Weed Technol. 26, 195–206.
- Kalyankar, S.V., Kadam, G.R., Borgaonkar, S.B., Deshmukh, D.D., Kodam, B.P., 2007. Effect of foliar application of growth regulators on seed yield and yield components of soybean (Glycine max (L.) Merill). Asian Journal of Bioscience 3, 229–230.
- Khaliq, A., Abbasi, M.K., 2015. Improvements in the physical and chemical characteristics of degraded soils supplemented with organic–inorganic amendments in the Himalayan region of Kashmir, Pakistan. Catena 126, 209–219.
- Khan, N.A., Singh, S., Nazar, R., Lone, P.M., 2007. The source-sink relationship in mustard. The Asian and Australasian Journal of Plant Science and Biotechnology 1 (1), 10–18.
- Khanzada, S.R., Khanzada, M.S., Abro, G.H., Syed, T.S., Soomro, K., Khanzada, A.M., Anwar, S., Shakeel, N., 2013. Relative resistance of soybean cultivars against sucking insect pests. Pak. J. Sci. 65 (2), 197–201.
- Khatun S, Roy TS, Haque MN, Alamgir B. 2016. Role of plant growth regulators on growth and yield of soybean at different stages of application. Scientia Agriculturae 15 (3): 380-386. 10.15192/PSCP.SA.2016.15.3.380386.
- Leite M, Rosolem CA, Rodrigues JD. 2003. Gibberellin and cytokinin effect on soybean growth. Scientia Agricola 60 (3): 537-541. htts://dpoi.org/10.1590/S0103-90162003000300019.
- Nonokawa, K., Nakajima, T., Nakamura, T., Kokubun, M., 2012. Effect of synthetic cytokinin application on pod setting of individual florest with in raceme in soybean. Plant Prod. Sci. 15 (2), 79–81. https://doi.org/10.1626/pps.15.79.
- Olsen SR and Sommers LE. 1982. Phosphorus. In: Methods of Soil Analysis, Chemical and Microbiological Properties, Part 2. (Eds.): A.L. Page, R.H. Miller & D.R. Keeney. ASASSSA, Madison, WI, USA, pp. 403-430.
- Passos, A.M.A.D., Rezende, P.M.D., Alvarenga, A.A.D., Baliza, D.P., Carvalho, E.R., Alcantara, H.P.D., 2011. Yield per Plant and Other Characteristics of Soybean Plants Treated with Kinetin and Potassium Nitrate Ciencia e Agrotechnologia 35 (5), 965–972. https://doi.org/10.1590/S1413-70542011000500014.
- Patel, V.P., Lal, E.P., John, S., 2016. Comparative study of the effect of plant growth regulators on growth, yield and physiological attributes of chilli, *Capsicum annuum* L cv Kashi Anmol. International Journal of Farm Sciences 6 (1), 199–204.
- Rahman, M.M., Hossain, M.M., Anwar, M.P., Uraimi, J.A.S., 2011. Plant density influence on yield and nutritional quality of soybean seed. Asian J. Plant Sci. 10 (2), 125–132.
- Simard, R.R., 1993. Ammonium acetate-extractable elements. In: Carter, M.R. (Ed.), Soil Sampling and Methods of Analysis. Lewis Publishers, Boca Raton, FL, pp. 39–42.
- Smith, J., 2010. Analysis of Oil Content in Plant Seeds. Journal of Agricultural Chemistry 20 (3), 345–358.
- Steel RGD, Torrie JH, Dickey DA. 1997. Principles and Procedures of Statistics. A biometrical approach 3rd Edition. McGraw Hill Book Co., Incorporated New York, 400-428 pp.
- Taiz, L., Zeiger, R., 2010. Plant Physiology, 5th edition. Sinauer Associates Inc Sunderland, MA, USA.
- Travaglia, C., Reinoso, H., Boltini, R., 2009. Application of abscisic acid promotes yield in field cultured soybean by enhancing production of carbohydrates and their allocation in seed. Crop Pasture Sci. 60, 1131–1135. https://doi.org/10.1071/ CP08396.
- Upadhyay RG, Ranjan R. 2015. Effect of growth hormones on morphological parameters, yield and quality of soybean (*Glycine max* L.) during changing scenario of climate under midhill conditions of Uttarakhand. International Journal of Tropical Agriculture 33 (2):1899-1904. http://serialsjournals.com/archives.p.
- Van Soest, P.J., Robertson, J.B., Lewis, B.A., 1991. Methods for dietary fiber, neutral detergent fiber, and nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 74 (10), 3584–3597. https://doi.org/10.3168/jds.S0022-0302(91) 78551-2.
- Vieira EL, Castro PRC. 2004. Acao de biosetimulante na cultura da soja (Glycine max L. Merrill). Cosmopolis: Stoller do Brasil Ltda., 74 pp.

M. Shehzad et al.

- Walkley, A., Black, I.A., 1934. An examination of Degtjareff method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Soil Sci. 37, 29–38.
- Yamaguch, S., 2008. Gibberellin metabolism and its regulation. Annu. Rev. Plant Biol. 59, 225–251. https://doi.org/10.1146/annurev.arplant.59.032607.092804.
- Zafar, M., Abbasi, M.K., Khaliq, A., 2013. Effect of different phosphorus sources on the growth, yield, energy content and phosphorus utilization efficiency in maize at Rawalakot Azad Jammu and Kashmir. Pakistan. J. Plant Nutrition 36 (12), 1915–1934.