EFFECT OF 3-INDOLE ACETIC ACID AND GIBBERELLIC ACID ON GROWTH AND YIELD OF ALFALFA BC_3 HYBRID

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Abstract. Alfalfa (*Medicago sativa* L.) is a globally important leguminous fodder crop because of its high production and nutritional value. The investigation was conducted to determine an effect of 3-Indole Acetic Acid (IAA) and Gibberellic acid (GA3) on the alfalfa BC_3 hybrid at the breeding room of the institute of grassland science, Yangzhou University, Jiangsu Province, China. Plant height, stem diameter, leaves number per plant, leaf length, leaf width, leaf area, fresh shoot weight, and dry shoot weight were measured. The study used four different IAA and GA3 concentrations (0, 50, 75, and 100 mg L^{-1}) with three replications in a completely randomized design. The results revealed that the use of IAA and GA3 was significantly effective in the growth of the alfalfa BC_3 hybrid at the two cutting times. The use of 25 mg L^{-1} IAA concentration was significantly improved the plant height, leaf width, and leaf area. Exogenous GA3 application significantly enhanced stem diameter, leaf length, leaf area, fresh shoot weight, and dry shoot weight, except for leaf width, which showed a non-significant difference but was higher than that in the control group. Thus, the application of GA3 (i.e., GA3 at 75 mg L^{-1}) can be suggested for a higher yield of the alfalfa BC_3 hybrid.
Keywords: *growth regulator, plant height, stem diameter, leaf area, shoot dry weight*

Introduction

Alfalfa (*Medicago sativa* L.) is one of the most commonly cultivated perennial feed crops globally due to its high yield, nutritional values, and adaptability. Alfalfa is produced primarily for feed production due to its high biomass and nutritional value; seed yield is considered secondary (Iannucci et al., 2002). Furthermore, due to its perennial nature and rapid biomass accumulation, alfalfa is an excellent source for biofuel production, which may help meet the energy requirements of the world's growing population in the future (Arshad et al., 2017). Increasing livestock production has also led to a considerable increase in the consumption of alfalfa forage in countries such as China over the last 50 years (Bai et al., 2018). Thus, alfalfa can increase global food security and be an essential crop (Khoury et al., 2014). Therefore, enhancing productivity of alfalfa through novel genetic approaches like alfalfa BC_3 hybrid was necessary.

The plant hormone is widely used in agriculture to enhance crop production (Gangwar et al., 2012). Development, differentiation, and evolution are the most critical processes. However, secondary processes, including stomatal movement, may be distributed (Petri et al., 2009). Growth regulators are used for various purposes at different crop growth stages, and several of these hormones have been seen to interact. Plant hormones are chemicals that, in small concentrations, can influence plant

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physiological processes, resulting in changes in production and quality (Sajid et al., 2016). The primary auxin present in plants is indole-3-acetic acid (IAA), which regulates essential physiochemical processes such as cell elongation and division, tissue differentiation, phototropism, and geotropism (Hussain et al., 2011). The most common auxin in the plants is (IAA) 3-Indole acetic acid (Ludwig-Müller, 2000). Auxin, which comprises IAA, is a stimulator driven by tryptophan, enhancing the plant's maximum characteristics (Singh et al., 2015). Indole acetic acid (IAA) is a type of auxin that regulates the growth and development of plants (Gangwar et al., 2011). Gibberellins (GAs) are essential plant hormones involved in different developmental processes, including germinating of seeds, stem elongation, expansion of trichomes and leaves, pollen maturity, and blooming stimulation (Davière and Achard, 2013). Gibberellins are essential in various physiological processes (Yang et al., 2012). GA3 promotes flower initiation, whereas, during short days, it encourages flower bolting (Mutasa-Göttgens et al., 2010). Auxin and gibberellins (GA3) played essential roles in plant development and were frequently found to respond in tandem. This relationship occurs when a single hormone influences the amount of a supplemental hormone in the target organ. During the growth of main stems, gibberellin and auxin have been shown to work together. Hormones likely make this synergy more effective (Husen et al., 2016). Gibberellins are cyclic diterpenes used as plant hormones because they are essential for stem, leaf, and root growth and can increase the number and color of flower buds. Even though 136 gibberellins have been identified, only a few of them exhibit biological activity in plants (Achard et al., 2009; Gupta and Chakrabarty, 2013). Gibberellins were discovered in different actively developing tissues after being extracted from the fungus Gibberella (Gupta and Chakrabarty, 2013). Gibberellic acid (GA3) is the most commonly generated and used gibberellin (Gupta and Chakrabarty, 2013). By promoting ribonucleic acid and protein synthesis directly, GA3 can regulate cell length, length of stem internodes, grass leaf length, and the division of cells in plant shoots (George et al., 2008).

In light of the above literature, (IAA) and (GA3) plant growth regulators were investigated to evaluate their effects on alfalfa BC_3 hybrid morphological growth and yield. The two cutting times were tested to determine the variability of plant height, stem diameter, number of leaves per plant, leaf length, leaf width, leaf area, shoot fresh weight and shoot dry weight on successive growths of alfalfa.

Material and methods

Plant material

A pot study was done in mid-February 2021 at the breeding room of the institute of grassland science, Yangzhou University, Jiangsu Province, China (*Fig. 1*). Huaiyin alfalfa trifoliate leaves as the non-recurrent male parent and BC_2 with high multifoliate leaves as the recurrent female parent were crossed to produce a BC_3 hybrid plant, then the seeds were harvested after maturity. The seeds were under 3 months old. Deionized water was used to soak the seeds (control) or various GA3 and IAA solution concentrations at 25°C for 12 h in the dark. Then, the same color, size, and shape of seeds were sown in pots (diameter 16 cm, height 17 cm) filled with 3 kg of clay soil. The soil of the experiment is clay having pH 7.2, available potassium 38.25 mg kg^{-1}, organic matter content 9.3%. Five seeds were sown at 1.5 cm depth in each pot after irrigating to field capacity. The study was designed as a completely randomized design

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(CRD) with three replicates for each treatment. The temperature in the breeding room was set at 25/20°C of light/darkness, 60% relative humidity, 200 mol m^{-2} s^{-1} of light intensity, and a 16/8 h photoperiod of light/darkness. Two different hormone solutions were chosen for this study: 3-Indole acetic acid (IAA) (0, 50, 75, and 100 mg L^{-1}) and Gibberellic acid (GA3) (0, 50, 75, and 100 mg L^{-1}) were applied during the shoot growth stage. Each pot was treated with 50 ml of the solution, whereas the control group was treated with water. The plants were treated twice each week. During the growth, weeding was done when desired.

Growth attributes

Throughout the experiment alfalfa was harvested two times at the early flowering stage. However, three plants were randomly harvested from each pot (9 plants from each treatment and control group). Plant height (cm) was measured using a diameter tape, and the average was calculated. The stem diameter was measured at 1 cm above ground using a vernier caliper (mm). The leaves number per plant was recorded directly. The leaf length and width were measured, and the average value was calculated. Furthermore, leaf area per plant was calculated by tracing the leaf on graph paper. Fresh shoot weight (g) was calculated using a digital balance. For shoot dry weight (g), we calculate the dry weight after oven-drying at 80°C for 48 h until the plant reaches a consistent weight.

Figure 1. *Experimental site: the breeding room of the Institute of Grassland Science, Yangzhou University, Jiangsu Province, China*

Statistical analysis

The experiment was designated in a completely random design and replicated three times. Each variable data was analyzed using MSTAT-C statistical software (Gomez and Gomez, 1984). When the F values were significant (P 0.05), the LSD test separated the means.

Results

In this experiment, we investigated the effects of IAA and GA3 on the alfalfa BC_3 hybrid at two successive cutting times. Most of the morphological studies of the alfalfa

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BC_3 hybrid show positive responses to different concentrations of IAA and GA3. For morphological studies on the alfalfa BC_3 hybrid, the following variables were determined using Analysis of Variance (ANOVA): plant height (cm), stem diameter (mm), number of leaves, leaf length (cm), leaf width (cm), leaf area (cm^2), fresh shoot weight (g), dry shoot weight (g) (*Tables 1–2*).

Table 1. *ANOVA represents mean squares for phenotypic traits in first cutting time*

Source of variance	DF	PH	SD	LN	LL	LW	LA	FSW	DSW
Hormone (H)	1	0.06*	1.359*	80.667ns	0.427*	0.004 ns	0.103ns	1.354 *	0.220*
Treatment (T)	3	335.19ns	0.122 ns	1279.22ns	0.238*	0.017 ns	0.441*	18.294ns	1.662 ns
H*T	3	257.37 ns	0.214 ns	1072.78ns	0.076ns	0.004 ns	0.029ns	4.822 ns	0.435 ns
Error	16	195.35	0.129	618.79	0.076	0.026	0.072	22.819	1.527
Total	23								
C.V%		18.06	14.02	23.07	11.29	14.56	17.00	40.53	41.60

PH: plant height (cm), SD: stem diameter (mm), LN: leaves number per plant, LL: leaf length (cm), LW: leaf width, LA: leaf area (cm^2), FSW: fresh shoot weight (g), DSW: dry shoot weight (g). Note: DF: Degree of freedom; ns: Non-significant; *Significant at $P < 0.05$

Table 2. *ANOVA represents mean squares for phenotypic traits in second cutting time*

Source of variance	DF	PH	SD	LN	LL	LW	LA	FSW	DSW
Hormone (H)	1	296.807ns	0.007*	1190.042ns	0.150ns	0.220*	1.972*	112.234ns	7.370*
Treatment (T)	3	343.219ns	0.088ns	2957.042ns	0.530*	0.070ns	1.412ns	12.314ns	1.730ns
H*T	3	87.900ns	0.054ns	6268.931*	0.050ns	0.052ns	0.424ns	17.234ns	1.216ns
Error	16	362.596	0.244	1249.0	0.138	0.068	0.587	27.120	1.745
Total	23								
C.V%		20.28	18.28	25.21	14.40	17.19	27.48	35.68	34.72

PH: plant height (cm), SD: stem diameter (mm), LN: leaves number per plant, LL: leaf length (cm), LW: leaf width, LA: leaf area (cm^2), FSW: fresh shoot weight (g), DSW: dry shoot weight (g). Note: DF: Degree of freedom; ns: Non-significant; *Significant at $P < 0.05$

The effect of IAA and GA3 on alfalfa BC_3 hybrid plant height (cm) in the first and second cutting times

The obtained result revealed that the concentration of 25 mg L^{-1} of growth regulator IAA significantly affected the plant height in the first cutting times (*Fig. 2*). The highest plant height (97.57 cm) was seen at a 25 mg L^{-1} IAA concentration, while the concentration of 100 mg L^{-1} IAA showed the least plant height (68.23 cm). However, the interaction was not significant. All concentrations of GA3 showed no significant difference in plant height of the alfalfa BC_3 hybrid in the first cutting times. The result of the second cut showed no significant difference in both growth regulators (*Fig. 2*). The concentration of 75 mg L^{-1} of IAA showed the highest plant height (105 cm), whereas the 100 mg L^{-1} concentration of GA3 showed the smallest plant height (76.37 cm). In addition, the interaction between hormones and treatment at the two cutting times was not statistically significant.

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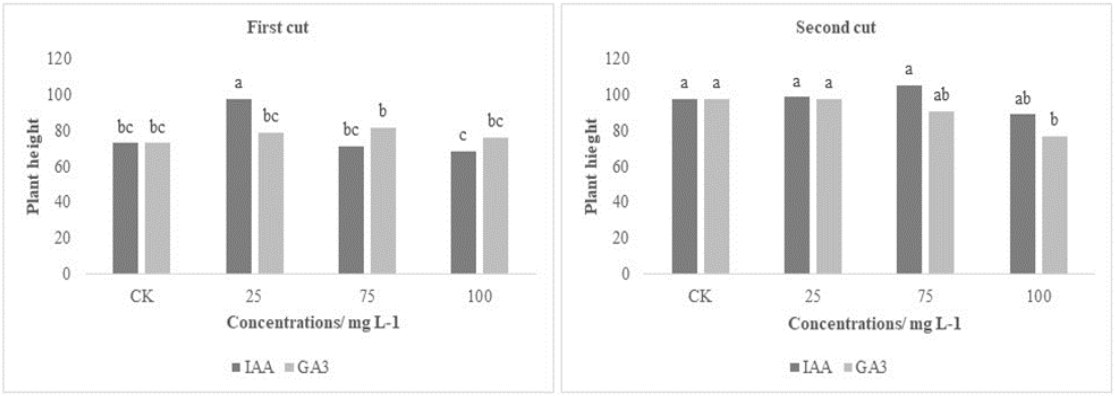

Figure 2. *The effect of IAA and GA3 on the plant height (cm) of the alfalfa BC_3 hybrid in the first and second cutting times. The same letters in columns means did not show any significance*

The effect of IAA and GA3 on alfalfa BC_3 hybrid stem diameter (mm) in the first and second cutting times

The study showed that using IAA and GA3 significantly increased alfalfa stem diameter at a concentration of 75 mg L^{-1} in the first and second cutting times (*Fig. 3*). Among the different hormones, the highest value of stem diameter was (3.2 mm) at 75 mg L^{-1} concentration of GA3 in the first cutting time, while the highest stem diameter recorded in the second cutting time was 2.96 mm. The lowest stem diameter was recorded at plants treated with 100 mg L^{-1} of the IAA (2.12 mm) and GA3 (2.49 mm). Furthermore, a non-significant difference was observed in the interaction between hormones and treatment at the two cutting times.

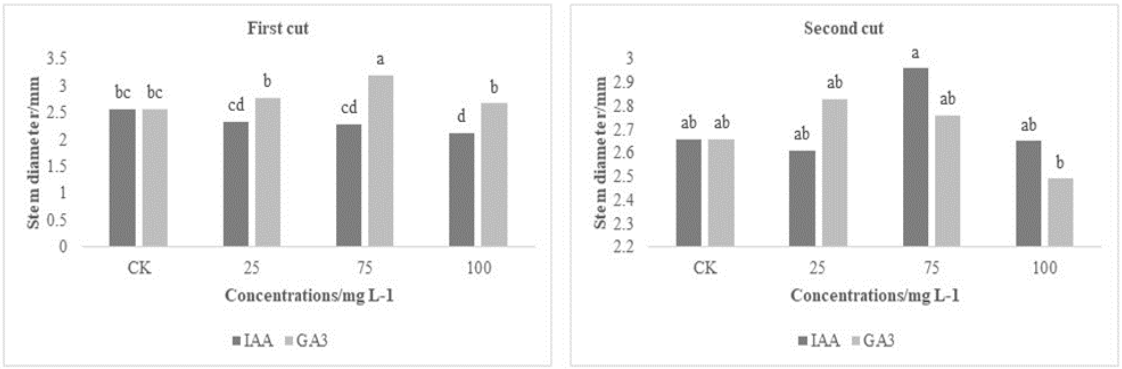

Figure 3. *The effect of IAA and GA3 on the stem diameter of the alfalfa BC_3 hybrid in the first and second cutting times. The same letters in columns means did not show any significance*

The effect of IAA and GA3 on alfalfa BC_3 hybrid leaves number per plant in the first and second cutting times

A significant effect of IAA at 75 mg L^{-1} concentration was observed on the leaves number per plant at the first cutting time. In contrast, the GA3 at 25 mg L^{-1} significantly affected the leaves number per plant at the second cutting time (*Fig. 4*). Maximum

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leaves number per plant (134.7) was recorded in plants sprayed with IAA at 75 mg L^{-1} and lowest leaves number plant (73.33) was recorded in plants sprayed with IAA at 100 mg L^{-1} at the first cutting times. The plants sprayed with GA3 at 25 mg L^{-1} recorded the highest leaves number per plant (209.7) at the second cutting time, while the lowest leaves number per plant (96.67) was recorded in plants sprayed with GA3 at 75 mg L^{-1}. In addition, the interaction between the two-growth regulator and treatment showed a significant difference at the second cutting time.

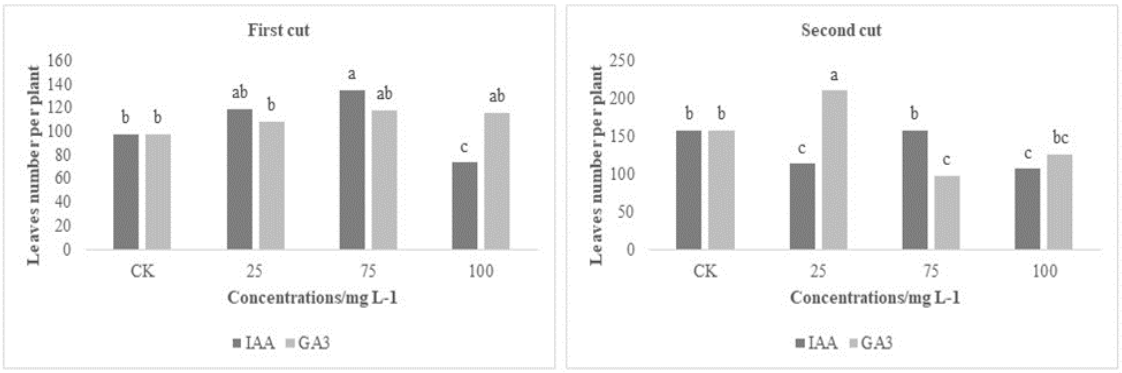

Figure 4. *The effect of IAA and GA3 on the leaves number per plant of the alfalfa BC_3 hybrid in the first and second cutting times. The same letters in columns means did not show any significance*

The effect of IAA and GA3 on alfalfa BC_3 hybrid leaf length (cm) in the first and second cutting times

At first cutting times, IAA at a concentration of 25 mg L^{-1} significantly affected leaf length, while GA3 had a significant impact at all concentrations (*Fig. 5).* Highest leaf length (2.8 cm) was observed in plants sprayed with GA3 at 25 mg L^{-1} and lowest leaf length (2.23 cm) was recorded in plants sprayed with IAA at 100 mg L^{-1}. At second cutting times, IAA and GA3 significantly increased leaf length at a concentration of 75 mg L^{-1} (*Fig. 5*). Highest leaf length (3.2 cm) was observed in plants sprayed with GA3 at 75 mg L^{-1} and lowest leaf length (2.4 cm) was recorded in plants sprayed with IAA at 25 mg L^{-1}. In addition, the interaction between the phytohormones and treatments at the two cutting times showed a non-significant different.

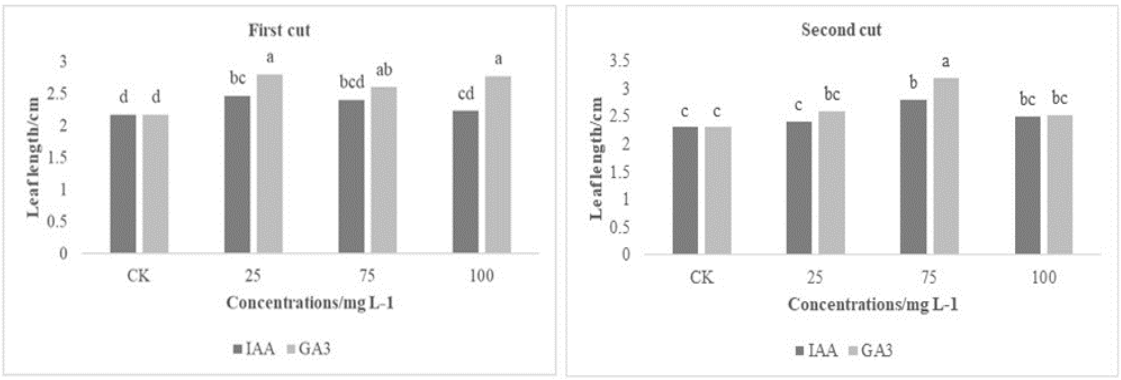

Figure 5. *The effect of IAA and GA3 on the leaf length of the alfalfa BC_3 hybrid in the first and second cutting times. The same letters in columns means did not show any significance*

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The effect of IAA and GA3 on alfalfa BC_3 hybrid leaf width (cm) in the first and second cutting times

The application of IAA and GA3 at different concentrations showed no significant difference in the first cutting time (*Fig. 6*). Highest leaf width (1.2 cm) was observed in plants sprayed with IAA at 25 mg L^{-1} and lowest leaf width (1.03 cm) was recorded in plants sprayed with GA3 at 75 mg L^{-1}. In the second cutting time, the GA3 at 75 mg L^{-1} showed a significant difference in alfalfa leaf width. Maximum leaf width (1.8 cm) was recorded in plants sprayed with GA3 at 75 mg L^{-1}, while the lowest leaf width (1.37 cm) was observed in plants sprayed with IAA at 100 mg L^{-1}. Additionally, the interaction between the phytohormones and treatments at the two cutting times was non-significant difference.

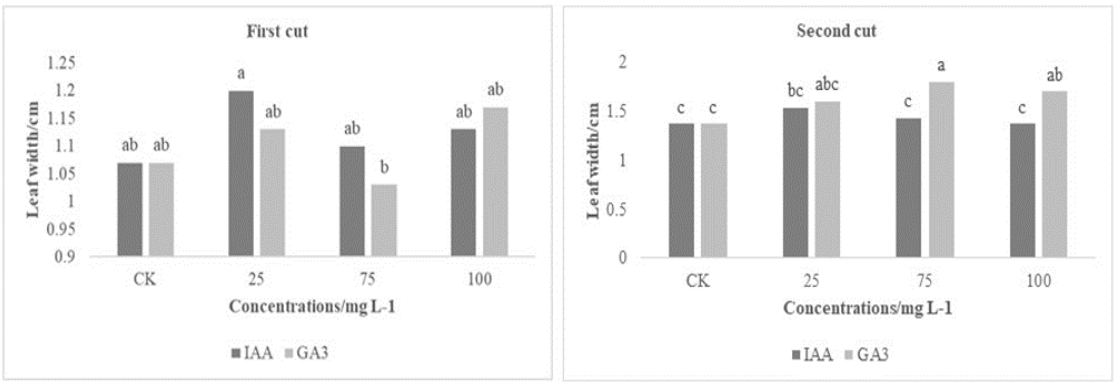

Figure 6. *The effect of IAA and GA3 on the leaf width of the alfalfa BC_3 hybrid in the first and second cutting times. The same letters in columns means did not show any significance*

The effect of IAA and GA3 on alfalfa BC_3 hybrid leaf area (cm^2) in the first and second cutting times

The use of IAA and GA3 at different concentrations showed significant differences in the first cutting time (*Fig. 7*). The maximum leaf area (1.86 cm^2) was recorded in plants sprayed with GA3 at of 100 mg L^{-1} concentration, while the lowest leaf area (1.54 cm^2) was observed in plants sprayed with IAA at 100 mg L^{-1}. In the second cutting time, the GA3 at a concentration of 75 mg L^{-1} significantly affected the alfalfa leaf area, while the IAA at different concentrations showed no significant difference. Among the different hormones, the highest value of alfalfa leaf area was (4.09 cm^2) at 75 mg L^{-1} concentration of GA3, while the lowest leaf area (2.29 cm^2) was observed in plants sprayed with IAA at a concentration of 100 mg L^{-1}. In addition, the interaction between the phytohormones and treatments at two cutting times showed a non-significant different.

The effect of IAA and GA3 on alfalfa BC_3 hybrid fresh shoot weight (g) in the first and second cutting times

The study showed that using GA3 significantly increased alfalfa fresh shoot weight at a concentration of 75 mg L^{-1} in the first cutting time. In contrast, the application of IAA at different concentrations showed no significant difference in the second cutting time (*Fig. 8*). In the first cutting times, GA3 at 75 mg L^{-1} recorded the maximum fresh

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shoot weight (15.53 g), higher than the control group by about 52.25%. The lowest fresh shoot weight (10.53 g) was observed in plants sprayed with IAA at 100 mg L^{-1}, it is higher than the control group, about 3.24%. In the second cutting time, GA3 at 25 mg L^{-1} recorded the highest fresh shoot weight (19.6 g), higher than the control group by about 31.28%. While IAA at 100 mg L^{-1} was observed to be the lowest (10.57 g), it was lower than the control group. Furthermore, there was no significant interaction between the phytohormones and treatment at the two cutting times.

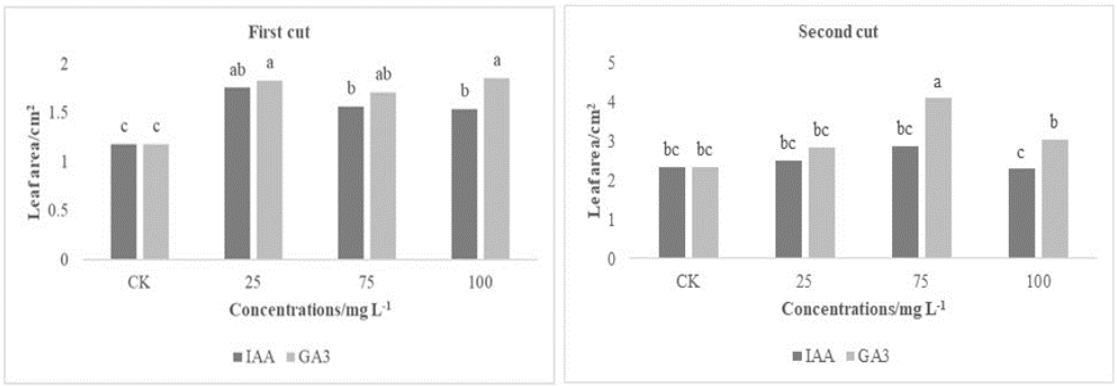

Figure 7. *The effect of IAA and GA3 on the leaf area of the alfalfa BC_3 hybrid in the first and second cutting times. The same letters in columns means did not show any significance*

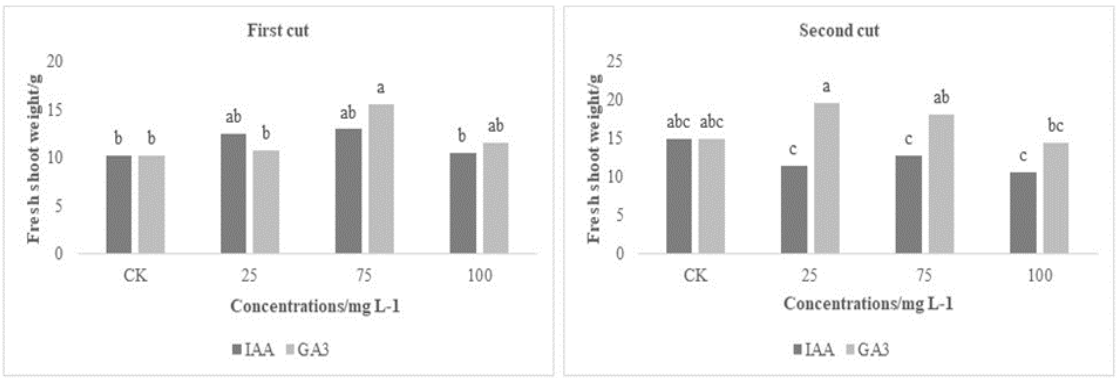

Figure 8. *The effect of IAA and GA3 on the fresh shoot weight (g) of the alfalfa BC_3 hybrid in the first and second cutting times. The same letters in columns means did not show any significance*

The effect of IAA and GA3 on alfalfa BC_3 hybrid dry shoot weight (g) in the first and second cutting times

The study revealed that applying GA3 at a concentration of 75 mg L^{-1} significantly increased alfalfa fresh shoot weight in the first and second cutting times. In contrast, there were no significant differences in the second cutting time when IAA was applied at different concentrations (*Fig. 9*). In the first cutting time, the GA3 at 75 mg L^{-1} recorded the highest dry shoot weight (4.1 g) per plant. It was higher than the control group, about 62.06%. In contrast, the concentration of IAA at 100 mg L^{-1} showed the lowest dry shoot weight (2.43 g) per plant. In the second cutting time, the GA3 at 75 mg L^{-1} recorded the

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highest alfalfa dry shoot weight (5.47 g) per plant, while IAA at 100 mg L^{-1} was observed to be the lowest alfalfa dry shoot weight (2.8 g). Finally, the interaction between the hormones and treatments at the two cutting times was non-significant.

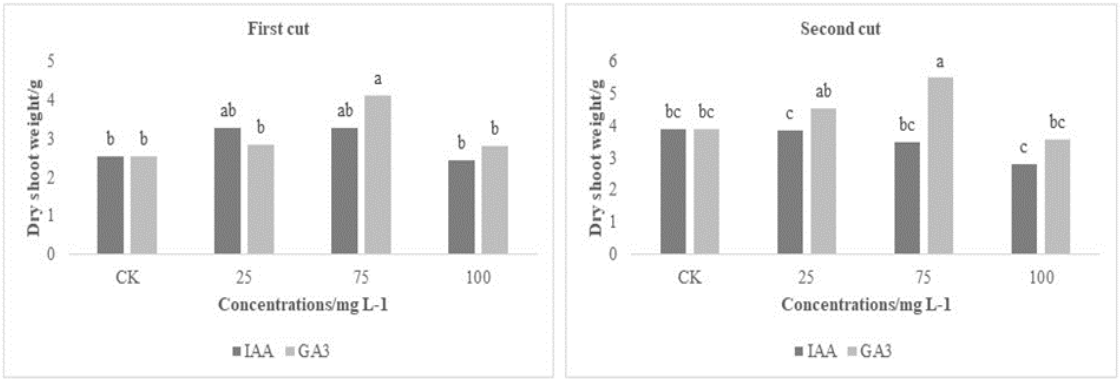

Figure 9. *The effect of IAA and GA3 on the dry shoot weight (g) of the alfalfa BC_3 hybrid in the first and second cutting times. The same letters in columns means did not show any significance*

Discussion

The results mentioned above noted that the maximum plant height of the alfalfa BC_3 hybrid was 97.57 cm when plants were sprayed with 25 mg L^{-1} IAA at the first cutting time. The application of IAA and GA3 increased the height of the alfalfa BC_3 hybrid plant. This increase in plant height was highly significant when the IAA growth regulator was used at 25 mg L^{-1}. The findings of pea plants treated with IAA foliar support our study (Hussain et al., 2020; Khalid et al., 2017). Even though there was no significant difference in the plant treated with GA3, all treatments enhanced its height; it was higher than the control group. The same result was observed by (Torres-Pio et al., 2021), who reported that foliar application of gibberellic acid increased the stem height of Lilium cv., and by (Mirheidari et al., 2021), who reported that treatment with 400 and 800 mg L^{-1} IAA and GA3 alone resulted in increased plant height. A similar result by (Muniandi et al., 2018), reported that exogenous spraying of gibberellic acid at a low concentration (1.25 mL L) increased the internode length and plant height in kenaf (*Hibiscus cannabinus* L.) plants. It has been proposed that GA3 may stimulate cell elongation, division, and internodal elongation, which may increase plant growth (Xu et al., 2016), and the role in phytohormone regulation (Hamayun et al., 2010). GA3 stimulates growth by encouraging cell division and elongation by increasing mechanical extensibility and cell wall flexibility. This is followed by hydrolysis of starch to sugar, which reduces water potential and allows water to enter the cell.

The application of IAA growth hormone increased plant height in field and pot environments (Rastogi et al., 2013). Different concentrations of GA3 have shown an increase in the stem diameter of the alfalfa BC_3 hybrid at the first cutting time. This increase in stem diameter was highly significant in the concentration of 75 mg L^{-1}. (Çavuşoğlu et al., 2008) observed the same result. Similar results were observed that the stem diameter was increased when castor (*Ricinus communis* L.) plants were treated with GA3 at a concentration of 250 µM (Jiao et al., 2019). While no significant difference was observed when all concentrations of IAA were applied to the stem diameter, it was smaller than the control group.

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The number of leaves on the treated plants was more significant than in the control group. Due to the application of GA3, a significant increase in the number of leaves was observed at the concentration of 25 mg L^{-1} on the second cutting time. On the other hand, the IAA treatment significantly increased the leaves on the alfalfa BC_3 hybrid at the first cutting time. The IAA growth regulator found the highest number of leaves per plant at 75 mg L^{-1}. An earlier study on Lentil (Naeem et al., 2004) found a similar result.

In the first cutting time, the alfalfa BC3 hybrid leaf area increased in all IAA and GA3 concentrations. The maximum values of leaf area (1.83 and 1.86 cm2) were observed by GA3 at 25 mg L^{-1} and 100 mg L^{-1}, respectively, when the compound concentrations were increased. In the second cutting time, applying GA3 at 75 mg L^{-1} showed a significant increase in leaf area, while IAA at the different concentrations showed no significance. In general, it could be seen that GA3 was more effective than IAA in increasing the leaf area of the alfalfa BC_3 hybrid when used as a growth regulator in this study. Applying GA3 significantly increased alfalfa fresh shoot weight at a concentration of 75 mg L^{-1} in the first cutting time, while the application of IAA at different concentrations showed no significant difference but was higher than the control group. The application of IAA and GA3 at a 75 mg L^{-1} improved the fresh and dry shoot weight of the alfalfa BC_3 hybrid in this study. A similar finding was seen in pea plants (Hussain et al., 2020), where foliar applications of IAA and GA3 substantially increased shoot fresh and dry weights during germination and mature stages. Higher IAA concentrations (100 mg L^{-1}) were less effective for most growth parameters, i.e., plant height, stem diameter, leaves number per plant, leaf length, leaf area, fresh shoot weight, and dry shoot weight in the two cutting times.

Conclusions

It was concluded that the foliar application of 3-indole acetic acid (IAA) and Gibberellic acid (GA3) improved the morphological and yield parameters such as plant height, stem diameter, leaf length, leaf width, leaf area, fresh shoot weight, and dry shoot weight. Applying 25 mg L^{-1} of IAA improved the plant height, leaf width, and leaf area at the first cutting time, whereas the other concentrations did not affect the plant height. Furthermore, applying GA3 at 75 mg L^{-1} showed significant and improved stem diameter, leaf length, leaf width, leaf area, fresh shoot weight, and dry shoot weight. The experiment showed that the 3-Indole acetic acid and gibberellic acid applications affected the phenotypic traits of the alfalfa BC_3 hybrid depending on the different concentrations. Further research is needed to determine which concentration produced the best result.

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