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Effect of exogenously applied growth regulators on morpho-physiological and biochemical attributes of wheat (*Triticum aestivum* L.)

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Abstract
In the present investigation, a field experiment with nine treatments and three replications of each treatments was conducted with Randomised Block Design (RBD) to ascertain the effect of exogenously applied plant growth regulators on wheat morpho-physiological and biochemical attributes. Wheat plants were sprayed with growth regulators at tillering and before anthesis stage. The treatments were subjected to Indole 3 Acetic Acid (IAA) 25 ppm and 50 ppm, Gibberellins (GA_3) 20 ppm and 40 ppm, Cytokinin (Kinetin) 5 ppm and 10 ppm, Alar 1000 ppm and 2000 ppm. Results shows that exogenous application of plant growth regulator helpful in improving plants growth traits such as plants height (cm), number of tillers plant^{-1}, leaf area plant^{-1} (cm^2), total dry matter plant^{-1} (g), relative growth rate (mg g^{-1} day^{-1}) and Chlorophyll content (SPAD Value). All the parameters were significantly influenced by plant growth regulators in respect to control.

Keywords: Plant growth regulators, wheat, plant height, relative growth rate, chlorophyll content

Introduction
Wheat (*Triticum aestivum* L.) which is physiologically categorized as a C_3 plant, being second most important staple food crop. Wheat is widely cultivated as a cash crop because it produces a good yield per unit area, grows well in a temperate climate even with a moderately short growing season (Sureshkumar *et al.*, 2014) [17]. Its sustainable production is crucial for India and other south Asian countries and also for a large number of countries where demand of daily calorie intake from wheat. The demand of wheat is rising due to increasing population and food consumption patterns while the land will either remain same or may decline. Due to increasing population food security become a global challenge and its needs to enhanced the production of cereals crops (Ziska *et al.*, 2012) [18]. Among the wheat producing countries china rank 1st followed by India (FAOSTAT, 2016) [7]. In India, it is the second important stable food crop next to rice. The major wheat producing States of India are Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan etc (Annual Report 2016–2017, http://agricoop.nic.in/). Punjab is the most fertile region and ideal for growing wheat. The exogenous application of plant growth regulators (PGRs) are involved in promoting plant growth and development under normal and stressful conditions. Although plants are capable of producing PGRs endogenously, they respond well to the exogenous application (Giannakoula *et al.*, 2012; Asgher *et al.*, 2015) [9, 3]. Plants can store excessive amounts of exogenously supplied hormones in the form of reversible conjugates that are released in active forms when needed in any plant part during growth (Sembdner *et al.*, 1994) [16]. Nowadays, the utility of hormonal research in India has been progressively recognized. The effect of various growth regulators on morphological characters like plant height, number of tillers, days to flower initiation indicated that these parameters differed significantly due to growth regulators. In the present study, therefore, an attempt was made to find out how for certain growth regulating substances with their various concentrations influence physiology of growth and metabolism of wheat crop. These growth regulators are likely to play an important role in many aspects of crop production. They added a new dimension to the possibility of obtaining high yield because it is modifying plants growth, development and metabolism.

Material and Methods
Seeds of promising wheat cultivar (PBW-343) were used for present investigation. Seeds were obtained from the Economic Botanist (*Rabi* cereals) of this university. The field experiment was carried out at Student Instructional Farm, C.S. Azad University of Agriculture and Technology, Kanpur for the purpose to evaluate the effect of exogenous application of plant

growth regulators on plant growth and development. The treatments were: T1- Control**,** T2- IAA (25 ppm)**,** T3- IAA (50 ppm), T4- Gibberellic acid (20 ppm), T5- Gibberellic acid (40 ppm), T6- Kinetin (5 ppm), T7- Kinetin (10 ppm), T8- Alar (1000 ppm), T9- Alar (2000 ppm) and each treatment replicated three times. The desired quantity of each growth regulators e.g. IAA 25 mg & 50 mg, GA_3 20 mg & 40 mg, Kinetin 5 mg & 10 mg, Alar 1000 mg & 2000 mg were weighed on a chemical balance and dissolved in a few drops of alcohol and there after the alcoholic solution was added to 50 ml distilled water with constant stirring. This volume of solution was finally made up to 1 litre in volumetric flask and Stoppard well. The prepared solution was sprayed carefully on the plants with the help of 1 liter capacity hand sprayer (*i.e.* Hand Compressed Knumatic Sprayer) 750ml of the solution was sprayed on the foliage in each treatment, two times *viz.*, 30 days after sowing and 60 days after sowing respectively. After sowing the first spraying was done at 3-5 leaves stage. While second spraying was given at pre-anthesis stage. The estimation was made with following methods:

Plants height (cm): Plant height was measured in standing condition at different growth stages. Plant height of the main shoot was recorded from ground level of shoot to the tip of the leaf. But after ear emergence, plants height of the main shoot was recorded from ground level to the joint of ear (excluding ear) in cm and its fraction.

Number of Tillers Plant^{-1}: Simultaneously with height measurement number of tillers were also counted and recorded on tagged plants including the main shoot. All axillary tillers (including main shoot) was counted in number and recorded in each treatment.

Total Leaf area Plant^{-1} (cm^2): The length and width of the third leaf from the top of the main shoot tillers was measured at successive growth stages by the use of Portable Leaf Area Meter. The value so obtained gave the total leaf area per plant.

Total dry matter Plant^{-1} (g): Plant samples were taken, washed and dried at 105^0 C till weight was become constant. The weight of different plants parts was recorded separately.

Relative Growth Rate (RGR) (mg g^{-1} day^{-1}): Relative growth rate (RGR) is the ratio of increase in dry weight per unit dry weight already present and it's expressed in mg per gram dry weight per day. Relative growth rate at various stages was calculated as suggested by Radford (1967) [14] and also given by Hoffman and Poorter (2002) [12].

$$\mathrm{RGR} = \frac{\mathrm{Log_e}, \mathrm{W_2} - \mathrm{Log_e W_1}}{\mathrm{T_2} - \mathrm{T_1}} \mathrm{mg\, g^{-1} day^{-1}}$$

Where, $\mathrm{Log_e}$ = Exponential Logarithm value, W_2 = Dry weight (mg) (Final stage), W_1 = Dry weight (mg) (Primary or Initial stage), T_2 = Time in days (Final), T_1 = Time in days (Initial)

Chlorophyll Content: It was recorded by a device, Chlorophyll Meter Model: SPAD- 502 PLUS (Company MINOLTA).

Results

Plant height (cm): Plant height was influenced by plant growth regulators at various crop growth days of wheat is presented in table-1. Plant height increased continuously from 30 days till third interval and then ceased up to harvest. The increase was more between first to third observation as compared to third and fourth. At 30 DAS, the maximum plant height (31.4 cm) was achieved by the treatment of GA_3 40 ppm and it was significantly superior over control. GA_3 ppm was also found better than others. However, minimum plant height (25.5 cm) was recorded in the Alar 2000 ppm treated plant. All treatments found significantly superior against control. At 60 DAS, the maximum plant height (42.3 cm) was achieved in GA_3 (40 ppm) which was significantly superior over control. IAA 50 ppm and GA_3 20 ppm found better than others. However, decreased plant height (36.8 cm) was recorded in the Alar 2000 ppm. All the concentration of Kinetin and IAA were unable to appreciate plant height significantly. At 90 DAS, GA_3 40 ppm again proved better than others and enhanced the height to 56.3 cm which is higher in comparison to others. GA_3 20 ppm followed this and results a height of about 54.6 cm. The rest treatment was not significantly increasing the plant height. At final stage i.e. harvest, all the treatments had significant influence on height of wheat plant than untreated plant.GA_3 40 ppm treatment accelerated the wheat height followed by GA_3 20 ppm and IAA 50 ppm. Both the concentration of GA_3 20 and 40 ppm produced superior effect over control but was statistically at par with each other. Similar result was also observed in other growth regulators treatment except Alar. Both concentration of Alar showed significant response in comparison to control plant and also differed significantly from each other.

Number of tillers plant^{-1}: Table no 2 revealed that growth-regulators significantly appreciated the number of tillers plant^{-1} against control. Maximum number (3.6) was obtained in IAA 50 ppm while the lowest tiller was observed in control (2.0). All the concentration of GA_3 and kinetin failed to indicate significant influence on tiller number. At 60 DAS, higher dose of IAA registered its presence in hastened the tiller number up to 5.6 cm. Both the concentration of IAA i.e. 25 ppm and 50 ppm were at par with each other but significantly superior than control. All the treatments of GA_3 and Alar had significant effect on tiller number; however, kinetin 25 ppm and 50 ppm failed to indicate significant influence on tiller number. At 90 DAS, IAA 50 ppm again proved better than others and an enhanced in the tillers value up to 7.0 cm while the lowest tillers produced under the control (4.7). Alar 2000 ppm and IAA 25 ppm treatments also improved their effect and achieved significant more tiller number/plant, while all treatment of GA_3 and Kinetin resulted non-significant increment in tiller number of wheat plant than untreated plants. Finally, at the harvest stage, a slight reduction in tiller number was noticed and IAA, 50 ppm (6.3) retained their first position for tiller number followed by IAA 25 ppm, Alar 1000 ppm and Alar 2000 ppm had also shown better effect than control but statistically at par with each other. All treatment of GA_3 and kinetin resulted non-significant increment in tiller number of wheat plant than untreated plant.

Total Leaf area plant $^{-1}$(cm^2): Table No. 3 represent the leaf area plant^{-1}, showed that growth regulators significantly improved total leaf area of wheat crop. Leaf area value was continuously rise from 98.33 to 1830.0 between 30 DAS to 90 DAS. Individually, consideration of 30 DAS, all treatments produced significant superior values against control plants except kinetin which unable to show significant result.

Maximum leaf area (147.18 cm2) was recorded in IAA 50 ppm followed by Alar 2000 ppm (145.26 cm2). Both concentration of GA_3 showed significant response in comparison to control plant and also differed significantly from each other. At 60 DAS, overall similar trend was noted and significantly the highest value on leaf area (1062.08 cm2) was noticed IAA 50 ppm. All the growth regulators treatment significantly superior over control. At 90 DAS, leaf area value improved from 754.46 cm2 to 1830.0 cm^2. The highest leaf area was recorded in IAA 50 ppm (1830.0 cm^2). All treatment produced significantly more leaf area in comparison to untreated plant. The value on leaf area index was decremented from 90 DAS to harvest and thus maximum leaf area value was observed by the treatment of IAA 50 ppm (1830.00 cm^2) followed by IAA 25 ppm. All the treatments were significantly superior over untreated plant.

Total dry matter plant^{-1} (g): It is perceived from the table-4 that dry weight of the plant continuously increased. There was a sharp enhancement in the dry matter content from 30 to 90 DAS to at maturity. GA_3 20 and 40 ppm differed significantly with each other. Effect of all growth regulators significantly incremented the total dry matter content of wheat crop. Regarding, at 30 DAS, maximum dry matter was obtained by the application of IAA 50 ppm (2.80 g) followed by Alar 2000 ppm. The lowest dry matter was recorded in control (1.14 g). Two doses of GA_3 *i.e.*, 20 and 40 ppm had significantly influenced on the dry matter content of wheat in comparison to control but had statically at par with each other. All treatment of kinetin had also better and produced significant effect over control. At 60 DAS, there was sharp rise in dry weight of plant from 30 DAS to 60 DAS. At 60 DAS, IAA 50 ppm achieved maximum dry weight (6.30 g) in comparison to control (4.15 g). Furthermore, both the concentrations of GA_3 were significantly superior over control but statistically at par among themselves. IAA 25 and 50 ppm, Alar 1000 and 2000 ppm were showed similar influence on dry matter but significantly at par with each other. Kinetin 10 ppm show non-significant result. At 90 DAS, there was also sharp elevation in dry matter from 8.4 to 12.96 g. thus this was noted that foliar spray of these growth regulators significantly affected dry matter of wheat plant in comparison to untreated plants. Maximum dry matter was accumulated in IAA 50 ppm (12.96 g) as reported in earlier stage. All doses of GA_3 and Alar improved the dry matter against control simultaneously but had statistically at par with each other. As for as Kinetin is concerned, both doses, 5 and 10 ppm were produced significantly more dry matter than control, however, both showed statistically at par value. Finally, at harvest stage, the maximum dry matter value was noted 21.76g as previous stage and the similar trend was obtained. Maximum dry matter per plant was obtained by the treatment of IAA 50 ppm (21.76 g). IAA 25 ppm and IAA 50 ppm both has significantly superior value over control but non-significant with each other. Kinetin 10 ppm and 5 ppm were significantly responding better than untreated plant (18.51 g) and also differ with each other. GA_3 20 ppm and GA_3 40 ppm both had significantly superior value over control plant but was also had statistically similar value with each other. Both dose of Alar was also indicates significant effect over control.

Relative growth rate (mg g^{-1}day^{-1}): Table no. 5 represent the relative growth rate (RGR), three observations were noted i.e. between tillering to heading, between heading to dough and between dough to maturity, and all proved significant response against control. The upper most RGR value (0.058) was noted in GA_3, 40 ppm followed by IAA 50 ppm while IAA 25 ppm treated plant secured second position in RGR value (0.057). Alar 2000 and Kinetin 10 ppm treated plant responded significant influence than control plant. In between heading to dough stage, the relative growth rate varied. The data significantly affected by the foliar spray of Kinetin 5 ppm and IAA 50 ppm treatment (0.066 and 0.064). IAA 25 ppm and Alar 2000 had similar value, both concentration of GA_3 also had similar RGR value. Regarding the data between dough to maturity, a slight decrease in the RGR level was observed. The RGR was observed in IAA 50 ppm and Alar 2000 ppm which display significant superior value than control. All the treatment had non-significant effect on RGR.

Chlorophyll Content (SPAD value): Table no. 6 revealed that chlorophyll content of wheat plant increased up to 30 DAS and the observations which had been provided indicate significant response of growth regulators against control. Exclusively, at 30 DAS, maximum chlorophyll content (50.88) was observed by the treatment of IAA 50 ppm while lowest content was recorded in control (47.76). All treatment shown significant result compare to control. As days advanced *i.e.* 60 DAS of crop, the average value of chlorophyll content was 43.98 and similar response was recognized as previous data. IAA 50 ppm possessed highest chlorophyll content 44.86 followed by Alar 2000 ppm (44.5). Which show at par effect with Alar 1000 ppm, GA_3, 20 and 40, kinetin 5 and 10 ppm, IAA 25 and 50 ppm considering all these were failed to enhance the chlorophyll content.

Discussion

Spraying of bio-regulators brought about the significant changes in growth characters *viz.*, plant height, number of tillers, leaves area per plant, and dry matter per plant. It has been concluded that GA_3, 20 and 40 ppm, IAA, 25 and 50 ppm and Kinetin,5 and 10 ppm promoted the plant height (Table No. 1 & Fig. 1). While Alar, 1000 and 2000 ppm were reducing the plant height at initial stage of crop growth, while at later stage all the treatment had better performance. The increment in plant height might be due to stimulation of cell division and increase in plasticity of cells. This finding is supported by the result of Roitsch and Ehness (2000) [15], Gherroucha (2003) [8], Nitumoni *et al*., (2009) [13]. Number of tillers per plant increased with the advancement of the crop days in case of all growth-regulators against control. More number of tillers per plant (Table No. 2 & Fig. 2) was mainly extended by the foliar spray of IAA 50 ppm followed by Alar 2000 ppm and IAA 25 ppm. GA_3 40 ppm has also showed an elevating effect on tiller number as the days advanced. Other hormones were also supported this character. Untreated plant possessed a smaller number of tillers per plant in comparison to treated plants. Similarly, Nitumoni *et al.*, (2009) [13] also find an improvement in tillers number in rice crop. A morphological and major yield supporting character *i.e.* green leaf area per plant of wheat crop was accelerated significantly against control after the treatment of growth regulators. Extended green leaf area (Table No. 3 & Fig. 3) was found by the treatment of IAA 50 ppm followed by IAA 25 ppm and Alar 2000 ppm. Both the concentration of kinetin, and GA_3 and Alar 1000 ppm also yield an expanded form of green leaf area. The most probable reason behind this character might be cell division and its elongation. Atta (2005) [5] also observed extended leaf area in wheat crop that accelerated by growth regulators. Dry matter which is a yield of photosynthates, significantly appreciated as compared with control by the foliar application of bio-regulators on wheat crop. Among these growth regulators, total assimilates were generated by the higher concentration of IAA *i.e.* 50 ppm followed by Alar 2000 ppm, which also promoted dry matter content (Table No. 4 & Fig. 4) in comparison to control at initial stage. All the treatment had significant. But at the time of harvest, maximum dry matter was produced by IAA 50 ppm. The most important reason for higher dry weight at initial stage might be due to more assimilates production by the leaves and at harvest stage, higher dry matter production is mainly due to higher assimilate translocation toward by sink. Similar trend also observed by Hadole *et al.*, (2002) [11], Asli *et al.*, (2011) [4] and Aldesuquy *et al.*, (2001) [1]. Growth parameter trait *i.e.* RGR is increased between different days of crop growth namely between tillering to heading and heading to dough, after this it decline to maturity stage. The inclined value of RGR was found in case of GA_3 20 ppm followed by control. GA_3 is also successful in accelerating RGR (Table No. 5 & Fig. 5). Other treatment is also had an uplifting value on RGR. At harvest the highest value was produce in IAA 50 ppm application followed by 50 ppm level. The elevated value of RGR is due to the assimilation of higher biomass and economic yield (Baruah, 1990) [6]. Chlorophyll content, which is a biochemical character and play a key role in photosynthesis, is mainly induced by the use of IAA 50 ppm and 25 ppm followed by GA_3 40 ppm at initial stage (Table No. 6 & Fig. 6). Increment in chlorophyll content was due to its formation, maintenance and development of chloroplast by the treatment of these hormones. Our statement is also similar with the observation of Gupta *et al.*, (2000) [10].

Conclusion
According to above outcome of the experiment, it may be inferred that the foliar application of plant growth regulators such as IAA, GA_3, Kinetin and Alar improved the parameter related to morphological, growth and biochemical of wheat crop. Application of GA_3 40 ppm at pre tillering and anthesis stage appreciated the plant height and relative growth rate of wheat crop while tiller number, leaf area, total dry weight and chlorophyll content improved by IAA 50 treatment. Thus, application of growth regulators support to sustainable growth and improvement of crops.

Table 1: Influence of growth-regulators on plant height (cm) at different days after sowing in wheat

Treatments	Plant height (cm)			
	30 DAS	60 DAS	90 DAS	At Harvest
Control	25.5	38.3	51.9	56.6
IAA 25ppm	28.5	39.3	53.4	57.4
IAA 50 ppm	30.0	40.8	53.7	57.8
GA_3 20ppm	30.3	40.8	54.6	58.2
GA_3 40ppm	31.4	42.3	56.3	59.6
KN 5ppm	27.6	38.4	52.2	56.8
KN 10ppm	28.2	38.6	52.8	57.2
Alar 1000 ppm	24.2	37.9	50.6	56.2
Alar 2000 ppm	23.5	36.8	49.8	53.0
SEm±	0.57	1.45	1.27	0.887
CD at 5%	1.22	3.06	2.69	1.88

Table 2: Influence of growth- regulators on number of tillers at different days in wheat

Treatments	Number of tillers plant^{-1}			
	30 DAS	60 DAS	90 DAS	At Harvest
Control	2.0	3.1	4.6	4.3
IAA 25 ppm	3.3	5.3	6.3	5.7
IAA50ppm	3.6	5.6	7.0	6.3
GA_3 20 ppm	2.4	4.6	5.3	5.0
GA_3 40 ppm	2.6	4.7	5.7	5.3
KN 5 ppm	2.3	4.3	5.3	5.0
KN 10 ppm	2.4	4.0	5.0	4.6
Alar 1000 ppm	3.1	5.0	6.0	5.4
Alar 2000 ppm	3.4	5.2	6.7	5.5
SEm±	0.487	0.680	0.757	0.681
CD at 5%	1.023	1.460	1.606	1.440

Table 3: Influence of growth-regulators on leaf area plant^{-1} (cm^2) at different days in wheat

Treatments	Leaf area plant^{-1} (cm^2)			
	30 DAS	60 DAS	90 DAS	At harvest
Control	98.35	452.10	754.46	817.52
IAA25ppm	143.61	970.33	1410.10	1468.65
IAA50 ppm	147.18	1062.08	1830.00	1834.25
GA_3 20 ppm	107.88	503.40	786.67	795.93
GA_3 40 ppm	137.95	848.90	1256.20	1254.06
KN 5 ppm	103.30	553.00	853.34	897.61
KN 10 ppm	99.60	591.36	1148.23	1184.43
ALAR 1000 ppm	139.82	630.66	1020.3	1035.40
ALAR 2000 ppm	145.26	697.10	1253.41	1192.08
SEm±	3.013	16.975	7.752	23.443
CD at 5%	6.388	35.987	16.435	49.700

Table 4: Influence of growth-regulators on total dry matter plant^{-1} (g) at different days in wheat

Treatments	Dry matter plant^{-1} (g)			
	30 DAS	60 DAS	90 DAS	At Harvest
Control	1.19	4.15	8.40	18.51
IAA 25 ppm	1.99	6.05	11.95	20.90
IAA 50 ppm	2.80	6.30	12.96	21.76
GA_3 20 ppm	1.82	5.11	11.23	20.24
GA_3 40 ppm	1.84	5.23	11.5	20.6
KN 5 ppm	1.80	5.01	10.25	20.15
KN 10 ppm	1.71	4.68	10.16	19.03
ALAR 1000 ppm	1.85	5.51	11.81	20.65
ALAR 2000 ppm	2.32	6.08	12.41	21.30
SEm±	0.059	0.289	0.496	0.43
CD at 5%	0.125	0.612	1.052	0.92

Table 5: Influence of growth-regulators on Relative growth rate (mg g^{-1}day^{-1}) between different growth days in wheat.

Treatments	Relative Growth Rate		
	between tillering to heading	between heading to dough	between dough to maturity
Control	0.045	0.058	0.022
IAA 25 ppm	0.053	0.062	0.007
IAA 50 ppm	0.057	0.064	0.032
GA_3 20 ppm	0.050	0.061	0.019
GA_3 40 ppm	0.058	0.061	0.004
KN 5 ppm	0.052	0.067	0.008
KN 10 ppm	0.049	0.059	0.007
Alar 1000 ppm	0.048	0.060	0.033
Alar 2000 ppm	0.049	0.062	0.010
SEm±	0.0011	0.0010	0.0021
CD at 5%	0.0024	0.0037	0.0046

Table 6: Influence of growth-regulators on Chlorophyll content (SPAD Value) in leaves at different growth days in wheat.

Treatments	Chlorophyll content	
	30 DAS	60 DAS
Control	47.76	42.43
IAA 25 ppm	50.69	44.41
IAA 50 ppm	50.88	44.86
GA_3 20 ppm	50.40	43.93
GA_3 40 ppm	50.43	44.11
KN 5 ppm	49.85	43.81
KN 10 ppm	49.83	43.66
Alar 1000 ppm	50.48	44.11
Alar 2000 ppm	50.86	44.50
SEm±	0.61	0.24
CD at 5%	1.36	0.52

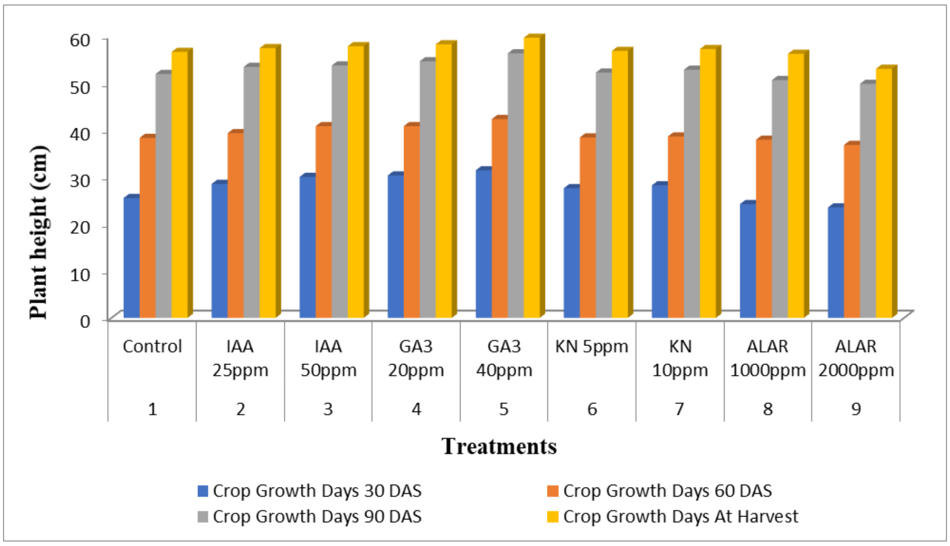

Fig 1: Influence of various hormonal treatments on plant height (cm) of wheat at different stages.

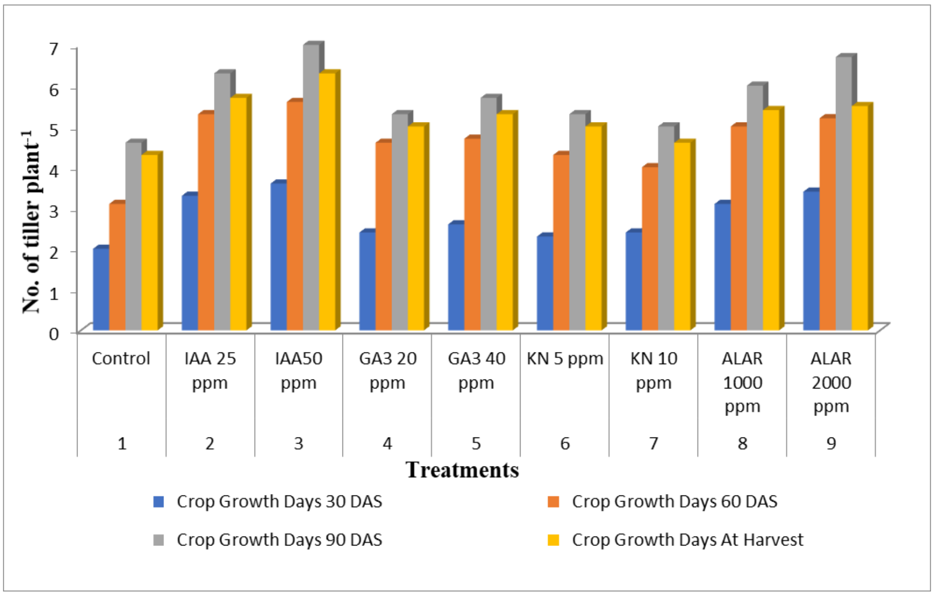

Fig 2: Number of tillers of wheat plant as influence by various hormonal treatments

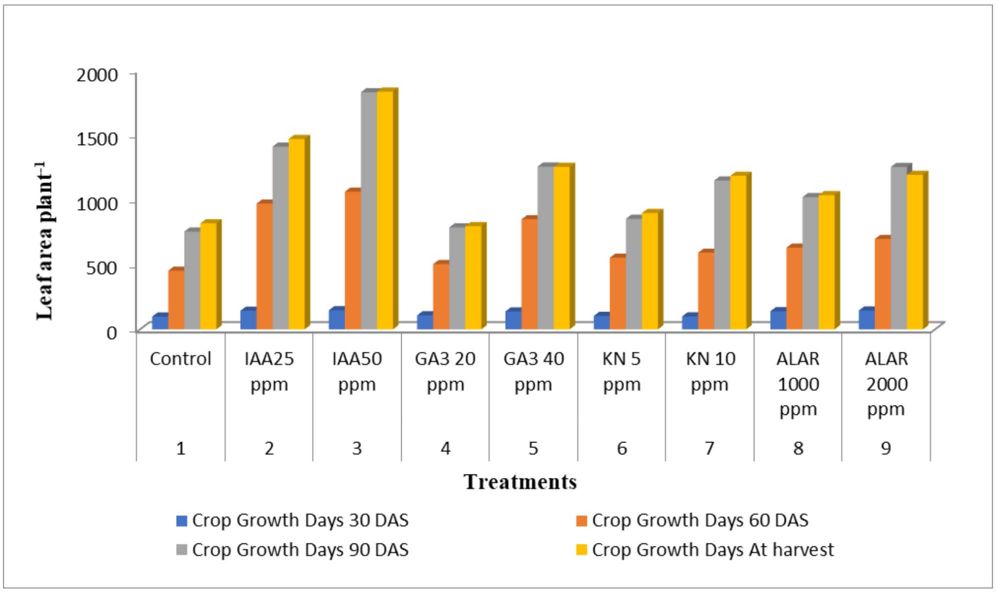

Fig 3: Influence of various hormonal treatments on leaf area of wheat crop.

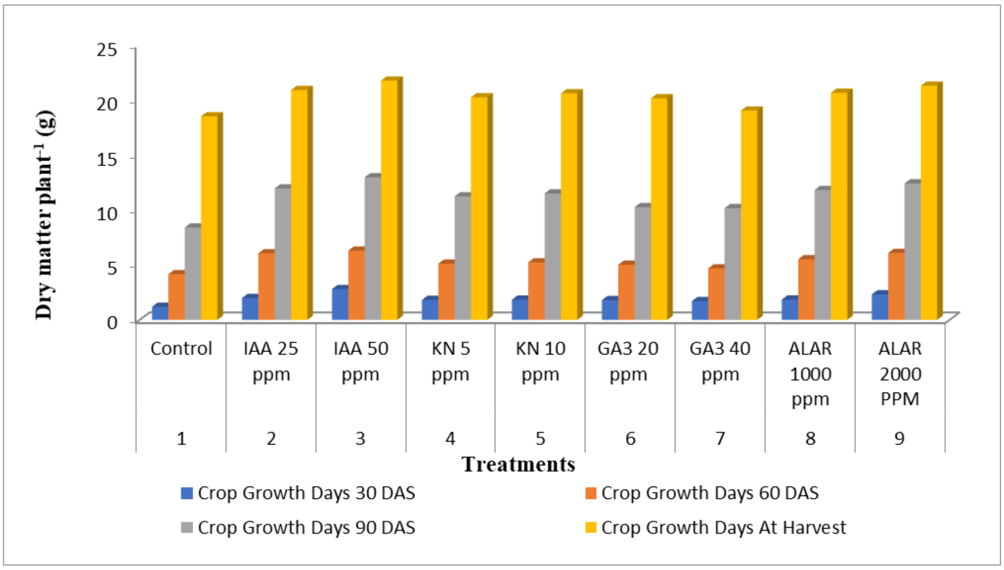

Fig 4: Influence of various hormonal treatments on dry matter production of wheat

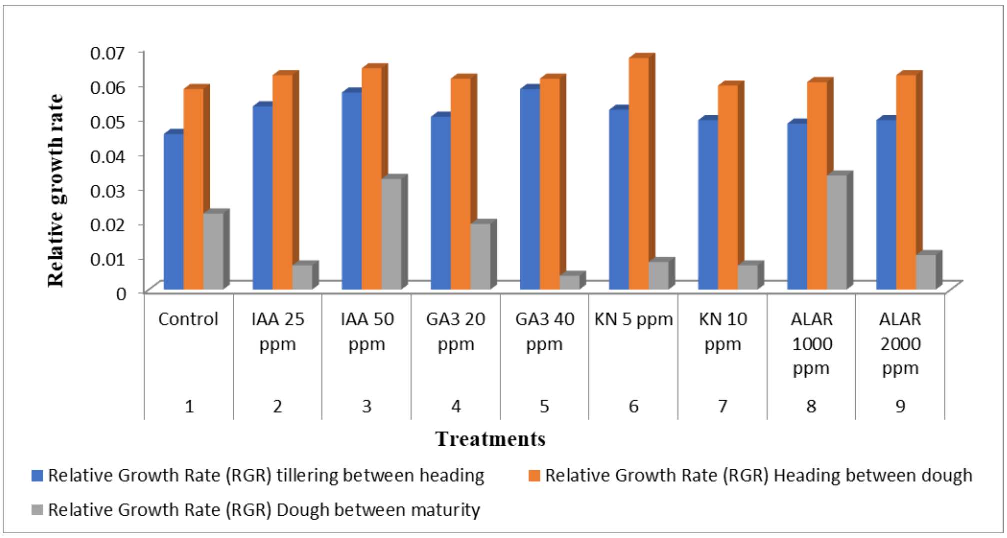

Fig 5: Influence of various hormonal treatments on relative growth rate of wheat crop

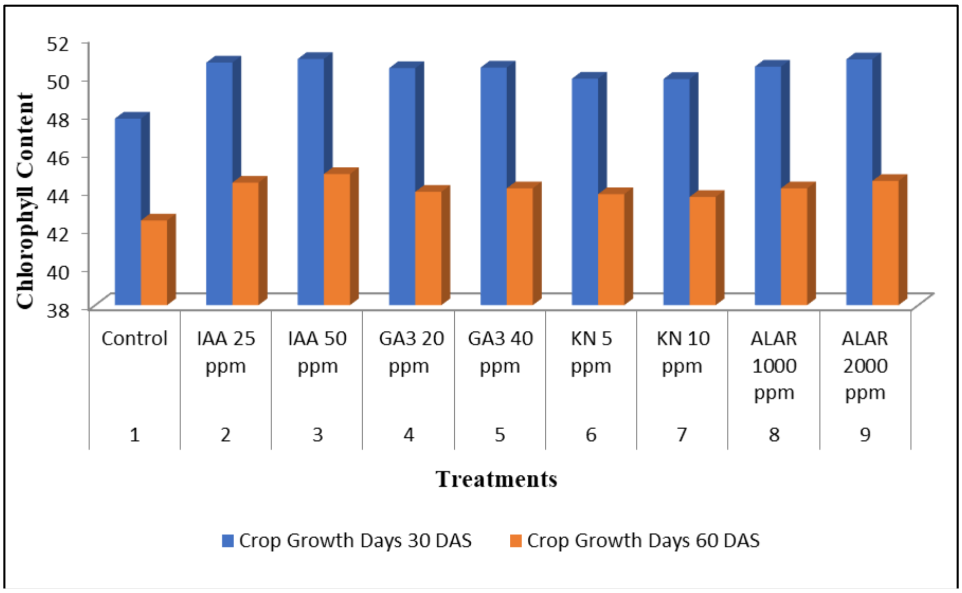

Fig 6: Influence of various hormonal treatments on chlorophyll content (SPAD value) at pre-anthesis and post anthesis stage of wheat crop.

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