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Effects of Plant Growth Regulators on Biomass, Number of Nodes, Internodal Length and Leaf Water Relative Content of *Fagopyrum esculentum* Moench of Himalayan Region

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Abstract

An experiment was carried out to access the effect of plant growth regulators (PGRs) on biomass, number of nodes, internodal length and leaf water relative content of *Fagopyrum esculentum*. Four major hormones: Indole Acetic Acid (IAA), Benzylaminopurine (BAP), Abscisic Acid (ABA) and Gibberellic Acid (GA) were used in combinations i.e. IAA+BAP, IAA+ABA, IAA+GA, ABA+BAP, BAP+GA and ABA+GA in concentration of 25, 50 and 100 mg l^{-1} through foliar spray. Results revealed that higher concentration of IAA+BAP (50 and 100 mg l^{-1}) enhanced number of nodes and shoot fresh weight. Combination of BAP+GA at 100 mg l^{-1} concentration enhanced shoot fresh and dry weight. Root fresh and dry weight was improved in IAA+GA 100 mg l^{-1} treatment. Maximum relative water content was observed at 50 mg l^{-1} of ABA+GA concentration. No. of nodes and internodal length decreased in plants treated with ABA+GA (25 mg l^{-1}). Combination of ABA+BAP at 25 mg l^{-1} concentration reduced internodal length and 100 mg l^{-1} affected root fresh as well dry weight. 25 mg l^{-1} concentration of BAP+GA lowered leaf relative water content, root fresh and dry weight. ABA in combination with IAA (ABA+IAA) at 25 mg l^{-1} negatively affected shoot fresh as well as dry weight.

Keywords: Antioxidant activity, *Fagopyrum esculentum*, phenolic compounds

1. Introduction

Fagopyrum esculentum (Common Buckwheat) is regarded as medicinal plant belonging to family Polygonaceae. It is a dicotyledonous, summer-growing, annual plant with shallow root system and knotted stem of 30-60 height (Jamwal et al., 2020). It originated in the southwestern region of China and later became a well-known crop in the northern hemisphere. It is used in many traditional dishes, like memilguksu (Korean noodles), soba (Japanese noodles), galettes (French pancakes), pizzoccheri (Italian pasta) and pizzoccheri (Italian pasta) (Mizuno and Yasui, 2019). To achieve greater yields without affecting health and environment integrity, it's very important to use techniques which can contribute to global food demand. The use of PGRs is of considerable interest in diverse fields of agriculture. These are effective even in low concentrations and have definite effects on plant growth (Jamwal et al., 2018). Application of foliar spray in agriculture has been a popular practice with farmers since the 1950s when it was erudite that a foliar fertilizer was effective (Rekha et al., 2018). Number of studies is reported where PGRs enhanced fresh and dry weight of shoot **(**Khatun et al., 2016), fresh and dry weight of root (Latif, 2014; Naeem et al., 2017), number of nodes (Ajayi et al., 2017), internodal length (Meenakshi and Lingakumar, 2011; and relative water content. Till date, there are no reports concerning the effects of PGRs on shoot biomass, root biomass, number of nodes, internodal length and relative water content of common buckwheat. Keeping these facts in mind, present study has been undertaken to see the relative effect of *F. esculentum* to plant growth regulators.

2. Materials and Methods

The seeds were obtained from Himachal Pradesh Agricultural University, Research Station, Sangla, Kinnaur (HP).

2.1. Seedling growth assays and growing conditions

Surface sterilization of seeds was done with 0.1% $HgCl_2$, after which the seeds were rinsed three times with distilled water. Seeds were sown in the nursery beds, in the Herbal Garden of Shoolini University, Solan (Latitude 30°51'N, longitude 77°07'E and altitude 1195 m). The average maximum and minimum temperatures were 32°C and 2°C, respectively. When the first leaf appeared the seedlings were transferred to pots filled with 3 kg uniform soil mixture containing soil: sand: farm yard manure (FYM) in 1:1:1 ratio. Three seedlings per pot in replicates of three were used for each treatment.

2.2. Treatments with plant growth regulators

Four major hormones: indole acetic acid (IAA), benzylaminopurine

(BAP), abscisic Acid (ABA) and gibberellic Acid (GA), were used in combinations i.e. IAA+BAP, IAA+ABA, IAA+GA, ABA+BAP, BAP+GA and ABA+GA in concentration of 25, 50 and 100 mg l^{-1} through foliar spray. The foliar spray with plant growth regulator was carried out after every 10 days and quantity of spray was increased with life span of plant i.e. 1.5 ml up to 30 days, 3 ml from 31 to 60 days and 4.5 ml from 61 to 90 days per plant.

2.3. Fresh and dry weight of shoot and root

Fresh weight (g $plant^{-1}$) of shoot and root were recorded separately and were oven dried for 72 h at 70°C. Dry weight of shoot and root were recorded to determine the effect of different hormones on biomass at different growth stages i.e., at 30, 60 and 90 days after sowing.

2.4. Number of nodes and internodal length (cm)

Number of nodes and internodal length were measured after 30, 60 and 90 days of plant growth.

2.5. Relative water content (RWC)

The fresh weight of leaves from each treatment was recorded. The leaves were then immersed in distilled water in beakers and left for 24 h. Thereafter, fully turgid leaves were weighed again. The leaves were dried in oven for 72 h at 70°C, until constant weight of leaves was obtained. Relative water content (RWC) of leaves was calculated according to Wheatherley (1950):

RWC=[(fresh mass-dry mass)]/(saturated mass-dry mass) ×100

The data was analyzed statistically using Graph Pad Prism® 5.2. Mean values were calculated from measurements of three replicates and the standard error of means were determined. Two-way analysis of variance (ANOVA) was applied to determine the significance of results between different treatments and Bonferroni's post tests were performed at the significance level of $p<0.05$.

3. Results and Discussion

3.1. Shoot fresh weight

The fresh weight in *F. esculentum* shoots treated with different plant growth regulators increased and it was maximum in BAP+GA 100 mg l^{-1} at 30 days (2.884 g, 23.5% increase from control), IAA+BAP 100 mg l^{-1} at 60 days (5.905 g, 15.8% increase from control) and 90 days (8.547 g, 13.3% increase from control) was most effective in enhancing shoot fresh weight. Whereas, IAA+ABA 25 mg l^{-1} at 30 days (2.112 g, 9.6% decrease from control), ABA+GA 25 mg l^{-1} at 60 days (4.382g, 14.1% decrease from control) and 90 days (6.752 g, 10.5% decrease from control) reduced shoot fresh weight (Table 1). The two-way analysis of variance showed significant interactions for shoot fresh weight. IAA+BAP, IAA+GA and BAP+GA had promoting effect on shoot fresh weight whereas; ABA+GA and IAA+ABA had inhibitory effect.

3.2. Shoot dry weight

Shoot dry weight in *F. esculentum* showed an increase in dry weight with the growth of plant in treated and untreated plants. BAP+GA 100 mg l^{-1} treatment was most effective among all combinations in enhancing shoot dry weight at 30 days (1.153 g, 14% increase from control) and at 60 days (2.866 g, 39.9% increase from control). However, ABA+BAP 100 mg l^{-1} treatment was effective in increasing dry weight at 90 days (3.750 g, 27.8% increase from control). Lowest shoot dry weight at 30 days (0.829 g, 18.1% decrease from control) and 60 days (1.117 g, 45.5% decrease from control) was in IAA+ABA 25 mg l^{-1} treated plants and at 90 days (2.368 g, 19.3% decrease from control) in ABA+GA 25 mg l^{-1} treated plants (Table 1). In general, IAA+BAP, IAA+GA, ABA+BAP and BAP+GA didn't show any variation from control at 30 days but there after enhanced shoot dry weight with time; rest of the treatments retarded shoots dry weight.

Enhancement in the shoot fresh and dry weight was seen at higher concentration of growth regulators but at lower concentration it didn't show a significant effect. The improvement in plant biomass in the present study could be as a result of growth, improved photosynthesis and better translocation of photosynthates (Miniraj and Shanmugavelu, 1987). IAA+BAP and BAP+GA promoted shoot fresh weight whereas; BAP+GA and BAP+ABA promoted shoot dry weight. Similar effect of BAP was reported in *Scutellaria alpina* (Grzegorczyk-Karolak et al., 2015) and spinach (Di Mateo et al., 2015). As cytokinin stimulates xylem differentiation, therefore more absorption of water as well as nutrients from the soil takes place, which results in growth (Sadak et al., 2013) and increase in the photosynthetic efficiency of leaves (Della Gaspera et al., 2016). It is well known that IAA stimulates cell division and elongation that might have caused increased plant biomass. Increased biomass with IAA has been reported in *Vicia faba* (Sadak et al., 2013). The increased biomass due to GA_3 may be due to a rapid increase in cell division, cell enlargement and gathering of building units that is accompanied by higher saccharide content (Mostafa and Abou-Alhamd, 2011). Similar results from application of GA have been reported in *Trigonella foenum-graecum* (Dar et al., 2015). Shoot fresh and dry weight decreased in IAA+ABA and ABA+GA (25 mg l^{-1}). Foliar spray of IAA+Kinetin decreased dry matter of salt-stressed plants (Kaya et al., 2010). GA (150 ppm) decreased dry weight in the first season of *Balanites aegyptiaca* (Mostafa and Abou-Alhamd, 2011). Abscisic acid (ABA) enlarged the root/shoot proportion of hydroponically-grown plants of cauliflower by decreasing the dry weight of the shoot (Biddington and Dearman, 1982).

3.3. Root fresh weight

Results revealed that IAA+GA 100 mg l^{-1} enhanced root fresh weight at 30 days (163 mg, 22.6% increase from control), at 60 days (465 mg, 31.4% increase from control) and at 90 days (495 mg, 16.5% increase from control). Decrease in root fresh

Table 1: Biomass in *Fagopyrum esculentum* at different growth stages under various phytohormone treatments (values are mean±S.E. n=3)

PGRs	Treatment	Shoot fresh weight (g)			Shoot dry weight (g)		
		30 Days	60 Days	90 days	30 Days	60 Days	90 days
IAA+BAP	25 ppm	2.515±0.073	5.527±0.057	8.236±0.086	1.066±0.003	2.271±0.038	3.519±0.025
	50 ppm	2.747±0.014	5.639±0.027	8.428±0.059	1.095±0.020	2.543±0.018	3.606±0.099
	100 ppm	2.872±0.026	5.905±0.091	8.547±0.037	1.106±0.038	2.736±0.073	3.739±0.023
IAA+ ABA	25 ppm	2.112±0.009	4.781±0.084	7.216±0.010	0.829±0.005	1.117±0.063	2.466±0.031
	50 ppm	2.147±0.030	4.985±0.065	7.461±0.015	0.931±0.005	1.216±0.022	2.583±0.072
	100 ppm	2.192±0.026	5.158±0.054	7.944±0.050	0.928±0.010	1.296±0.087	2.789±0.050
IAA+ GA	25 ppm	2.438±0.083	5.215±0.039	8.171±0.028	1.096±0.011	2.784±0.013	3.292±0.050
	50 ppm	2.658±0.030	5.562±0.065	8.395±0.051	1.099±0.030	2.793±0.023	3.055±0.011
	100 ppm	2.866±0.034	5.693±0.026	8.497±0.079	1.059±0.007	2.845±0.051	3.395±0.025
ABA+BAP	25 ppm	2.259±0.088	5.227±0.060	8.055±0.045	1.056±0.016	2.251±0.034	3.637±0.097
	50 ppm	2.467±0.035	5.495±0.079	8.182±0.068	1.041±0.002	2.281±0.088	3.697±0.071
	100 ppm	2.594±0.059	5.586±0.068	8.252±0.014	1.045±0.007	2.528±0.034	3.750±0.086
BAP+GA	25 ppm	2.483±0.013	5.457±0.026	8.150±0.075	1.145±0.010	2.766±0.061	3.465±0.066
	50 ppm	2.678±0.078	5.576±0.042	8.456±0.063	1.147±0.007	2.833±0.046	3.345±0.056
	100 ppm	2.884±0.081	5.799±0.014	8.535±0.059	1.153±0.006	2.866±0.069	3.531±0.042
ABA+GA	25 ppm	2.168±0.010	4.382±0.032	6.752±0.021	1.021±0.003	1.907±0.089	2.368±0.012
	50 ppm	2.269±0.002	5.133±0.019	7.467±0.063	1.033±0.002	2.104±0.035	2.775±0.097
	100 ppm	2.204±0.052	5.211±0.019	7.672±0.085	1.026±0.005	2.214±0.047	2.898±0.087
Control	Distilled water	2.335±0.016	5.101±0.047	7.541±0.055	1.012±0.009	2.049±0.031	2.935±0.034

Table 1: Continue...

PGRs	Treat-ment	Root fresh weight (mg)			PGRs	Treat-ment	Root fresh weight (mg)		
		30 Days	60 Days	90 days			30 Days	60 Days	90 days
IAA+ BAP	25 ppm	114.00±3.85	265.00±2.57	311.00±1.79	ABA+ BAP	50 ppm	119.00±2.92	274.00±1.97	345.00±3.36
	50 ppm	135.00±2.88	354.00±2.69	382.00±4.82		100 ppm	111.00±2.23	244.00±3.15	329.00±3.11
	100 ppm	123.00±3.73	321.00±2.53	424.00±3.24	BAP+ GA	25 ppm	113.00±2.61	252.00±3.19	376.00±2.93
IAA+ ABA	25 ppm	108.00±2.37	338.00±1.69	416.00±3.91		50 ppm	132.00±2.49	276.00±3.16	391.00±2.27
	50 ppm	113.00±4.41	369.00±2.64	449.00±2.86		100 ppm	119.00±2.61	334.00±2.52	403.00±3.23
	100 ppm	103.00±2.92	390.00±3.41	463.00±2.78	ABA+ GA	25 ppm	113.00±2.47	293.00±2.17	338.00±1.59
IAA+ GA	25 ppm	134.00±2.94	359.00±3.73	445.00±1.85		50 ppm	122.00±2.56	391.00±3.60	449.00±1.83
	50 ppm	147.00±2.45	376.00±1.95	479.00±3.26		100 ppm	141.00±2.91	377.00±1.98	451.00±2.63
	100 ppm	163.00±3.28	465.00±3.22	495.00±1.74	Con-trol	Distilled water	133.00±2.90	354.00±4.60	425.00±3.80
ABA+ BAP	25 ppm	121.00±3.53	294.00±2.60	312.00±2.81					

weight was seen in IAA+ABA 100 mg l^{-1} treated plants at 30 days (103 mg, 22.6% decrease from control), ABA+BAP 100 mg l^{-1} treated plants at 60 days (244 mg, 31.1% decrease from control) and IAA+BAP 25 mg l^{-1} treated plants at 90 days (311 mg, 26.8% decrease from control) (Table 1). In case of IAA+GA and ABA+GA higher concentration promoted root fresh weight after 30 days. Combination of BAP+GA, ABA+BAP and IAA+BAP reduced root fresh weight.

3.4. Root dry weight

IAA+GA (100 mg l^{-1}) treated plants showed maximum root dry weight at 30 days (71 mg, 31.5% increase from control) and 60 days (213 mg, 28.3% increase from control) and IAA+ABA 100 mg l^{-1} treated plants at 90 days (253 mg, 24% increase from control) of plant growth. Combinations of BAP+GA (25 mg l^{-1}), IAA+BAP (25 mg l^{-1}) and ABA+BAP (100 mg l^{-1}) showed minimum root dry weight at 30 days (24 mg, 55.6% decrease from control), at 60 days (79 mg, 52.4% decrease from control) and at 90 days (109 mg, 46.6% decrease from control), respectively (Table 2). IAA had synergistic effect on root dry weight especially at 100 mg l^{-1} concentration. GA had promoting effect at higher concentration only after 30 days and IAA+GA, IAA+ABA at 90 days.

High concentration (100 mg l^{-1}) of plant growth regulators was effective to increase fresh and dry weight of roots in *F. esculentum*. IAA and IAA+GA enhanced root fresh weight. Similar to our findings, Zhang et al. (2005) reported that root fresh weight of potato explants enhanced with an increasing of IAA levels either in the presence of GA_3 (treatment IAA+GA) or IAA alone. Naeem et al., 2017 mentioned similar effect of IAA on root fresh weight of *Catharanthus roseus*. IAA+GA, IAA+ABA promoted root dry weight at 100 mg l^{-1} concentration. Enhanced root dry weight of *F. esculentum* with auxin treatment had good root growth and is better able to absorb nutrients leading to healthy plants (Liu et al., 2002). Also, root dry weight increased with GA_3 (10 ppm) treatment in green gram (*Phaseolus mungo*) (Chauhan et al., 2010). Similar to our findings, Latif (2014) observed that ABA reduced the decline in both fresh and dry weights of roots in *Pisum sativum* stimulated by drought stress. Yamaguchi and Street (1977) also observed improved root growth in the presence of ABA especially at low concentrations. GA has been reported to increase root fresh and dry weight in several earlier reports (Idrees et al., 2010; Ahmad et al., 2014). The hairy roots of *Echinacea purpurea* treated with the moderate GA_3 concentration (0.025µM) achieved higher biomass (Abbasi et al., 2012). Root fresh weight of *F. esculentum* decreased in IAA+ABA (100 mg l^{-1}), ABA+BAP (100 mg l^{-1}) IAA+BAP (25 mg l^{-1}) treatments, as well as root dry weight in BAP+GA 25 mg l^{-1} treatments. Our results are similar to the finding of Jeong et al. (2007) in *Panax ginseng* where, dry weight of hairy roots declined with increase in the concentration of both ABA and GA_3.

Table 2: Biomass and leaf relative water content in *Fagopyrum esculentum* at different growth stages under various phytohormone treatments (values are mean±S.E. n=3)

PGRs	Treatments	Root dry weight (mg)			Relative water content (%)		
		30 Days	60 Days	90 days	30 Days	60 Days	90 days
IAA+BAP	25 ppm	53.00±2.23	79.00±1.47	126.00±2.82	66.20±1.71	72.90±1.41	73.90±1.09
	50 ppm	64.00±2.65	156.00±2.95	167.00±2.64	67.60±1.67	79.70±1.24	77.20±1.15
	100 ppm	46.00±2.49	136.00±1.58	215.00±2.18	62.10±1.16	74.60±1.06	74.10±1.24
IAA+ABA	25 ppm	44.00±2.85	136.00±2.55	213.00±1.82	69.50±1.12	77.30±0.25	72.00±0.06
	50 ppm	54.00±2.72	153.00±2.25	225.00±2.52	75.60±1.16	79.90±1.04	77.20±1.50
	100 ppm	41.00±1.92	155.00±1.56	253.00±2.52	73.00±1.10	73.80±1.19	71.80±1.22
IAA+GA	25 ppm	65.00±2.33	149.00±1.56	222.00±2.62	61.70±1.09	74.10±1.26	73.60±1.00
	50 ppm	67.00±1.98	167.00±2.51	238.00±2.72	66.50±1.18	77.20±1.32	74.70±0.57
	100 ppm	71.00±1.42	213.00±2.78	249.00±2.94	64.10±1.52	76.90±1.32	71.40±0.27
ABA+BAP	25 ppm	47.00±2.86	113.00±2.36	129.00±2.73	58.00±1.17	63.60±1.22	68.70±1.43
	50 ppm	36.00±1.75	101.00±2.36	148.00±1.83	62.90±1.20	77.60±1.66	72.80±1.14
	100 ppm	34.00±1.88	81.00±2.37	109.00±2.94	61.00±1.64	73.20±0.38	70.50±1.15
BAP+GA	25 ppm	24.00±2.73	88.00±2.84	136.00±2.97	52.20±2.13	60.50±1.74	61.30±1.45
	50 ppm	35.00±1.96	96.00±2.52	152.00±1.98	56.20±1.31	68.70±1.43	63.30±0.32
	100 ppm	29.00±2.85	121.00±2.46	179.00±2.82	54.40±1.15	66.50±1.53	62.80±1.26
ABA+GA	25 ppm	35.00±2.63	97.00±2.74	151.00±2.87	71.80±0.75	74.40±1.32	70.00±1.43
	50 ppm	43.00±3.33	157.00±3.11	213.00±2.57	79.60±0.82	83.10±1.39	77.20±1.04
	100 ppm	54.00±2.54	152.00±2.76	226.00±2.68	72.50±1.15	78.40±0.41	73.40±1.29
Control	Distilled water	54.00±3.74	166.00±2.39	204.00±2.86	70.00±1.85	78.00±2.41	72.00±1.47

3.5. Number of nodes

Highest number of nodes at 30 days was noted in IAA+BAP (50 and 100 mg l^{-1}) (6.30, 53.7% increase from control). Whereas, IAA+BAP (100 mg l^{-1}) concentration resulted highest number of nodes at both 60 days (24.60, 64% increase from control) and 90 days (27, 44.4% increase from control). Lowest number of nodes at 30 days (3, 26.8% decrease from control), at 60 days (12.30, 18% decrease from control) and at 90 days (15.30, 18.2% decrease from control) was seen in ABA+GA 25 mg l^{-1} treated plants (Figure 1).

It is evident from Figure 1 that IAA+BAP and ABA+BAP had synergistic effect on number of nodes. It was observed that the higher concentration of BAP and IAA+BAP (50 and 100 mg l^{-1}) was effective in promoting maximum number of nodes. Similar to our results, Abubakar et al. (2018) reported highest numbers of nodes in response to 1.5 mg l^{-1} BAP. Bhandari et al. (2009) reported increased number of nodes in *Verbascum thapsus* after IAA (50 ppm) application. Decrease in number of nodes was seen in ABA+GA (25 mg l^{-1}). Rabbani (2001) reported non-significant effect of different GA_3 concentrations on number of nodes in potato. Our results are opposite to findings of Firman (1984) who reported increased node production with GA_3 in potato.

3.6 Internodal length

Internodal length of *F. esculentum* showed a regular increment due to phytohormones action. Highest internodal length at 30 days (4 cm, 21.2% increase from control), at 60 days (5.40 cm, 22.7% increase from control) and at 90 days (5.80 cm, 16% increase from control) was seen in IAA+GA 100 mg l^{-1} treated plants. On the other hand, it declined maximum in ABA+BAP 25 mg l^{-1} (2.10 cm, 36.4% decrease from control) at 30 days, in ABA+BAP 50 mg l^{-1} together with ABA+GA 25 mg l^{-1} (3.60 cm, 18.2% decrease from control) at 60 days and in ABA+BAP 25 mg l^{-1} (3.80 cm, 24% decrease from control) at 90 days (Figure 2).

It is evident from Figure 2 that IAA+GA enhanced internodal length, whereas other phytohormones had retarded effect. Gibberellin is a well known stimulator of cell elongation, expansion as well as elongation of internodes (Huttly and Phillips, 1995). Maximum internodal length was observed in GA (50 and 100 mg l^{-1}) treated plants compared to control.

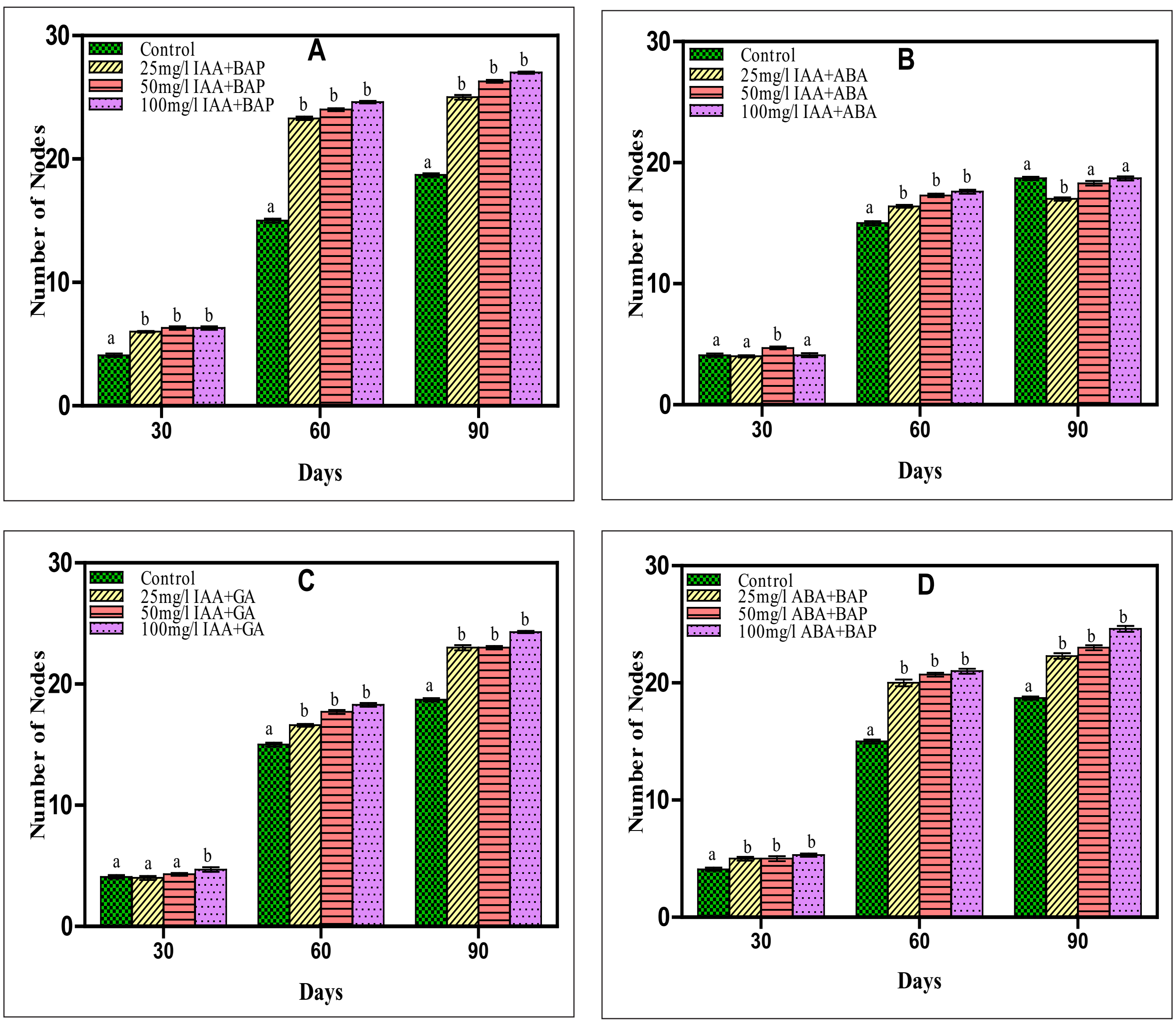

Figure 1: Continue...

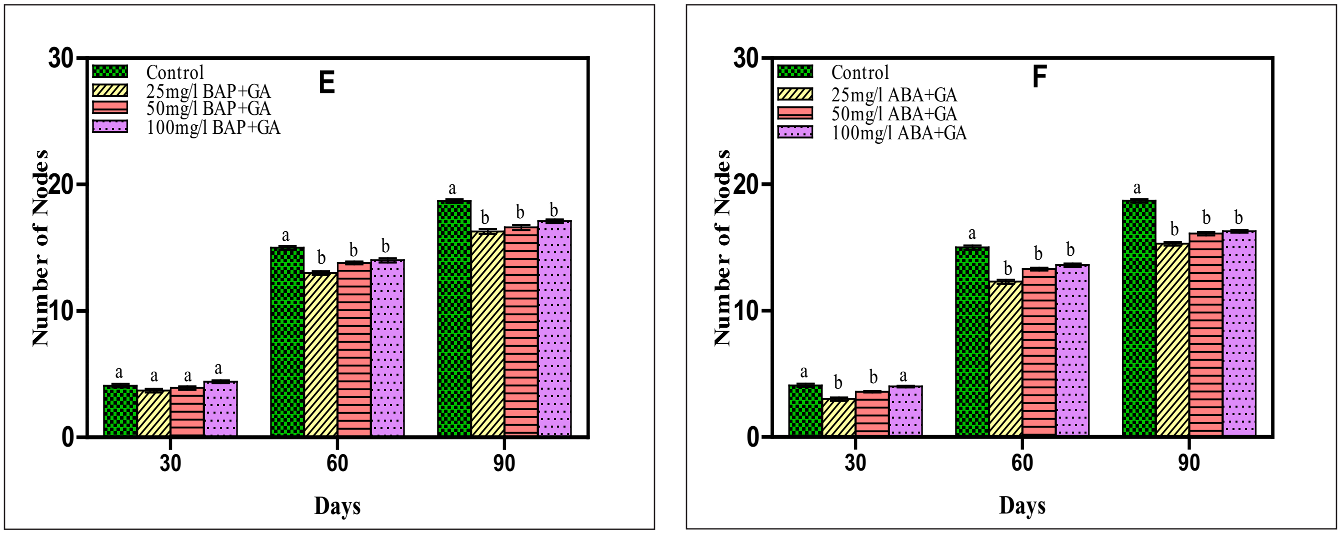

Figure 1: Number of nodes in *Fagopyrum esculentum* on different days under phytohormones treatment i.e., IAA+BAP (A), IAA+ABA (B), IAA+GA (C), ABA+BAP (D), BAP+GA (E) and ABA+GA (F). Values are mean±SE; n=3, analysed by two-way ANOVA followed by Bonferroni's multiple comparison test. Values followed by the same letter are not statistically different (p<0.05) compared with control

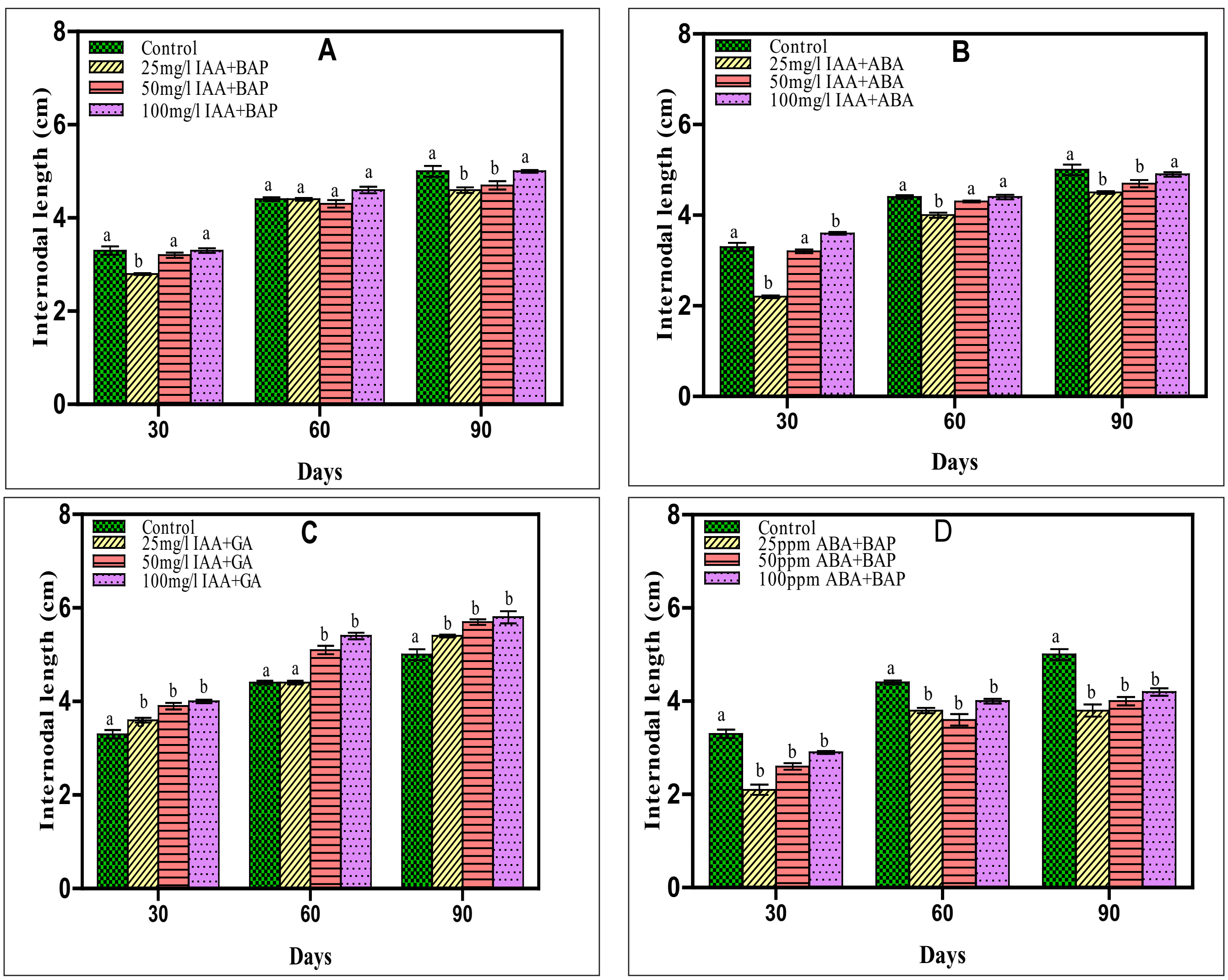

Figure 2: Continue...

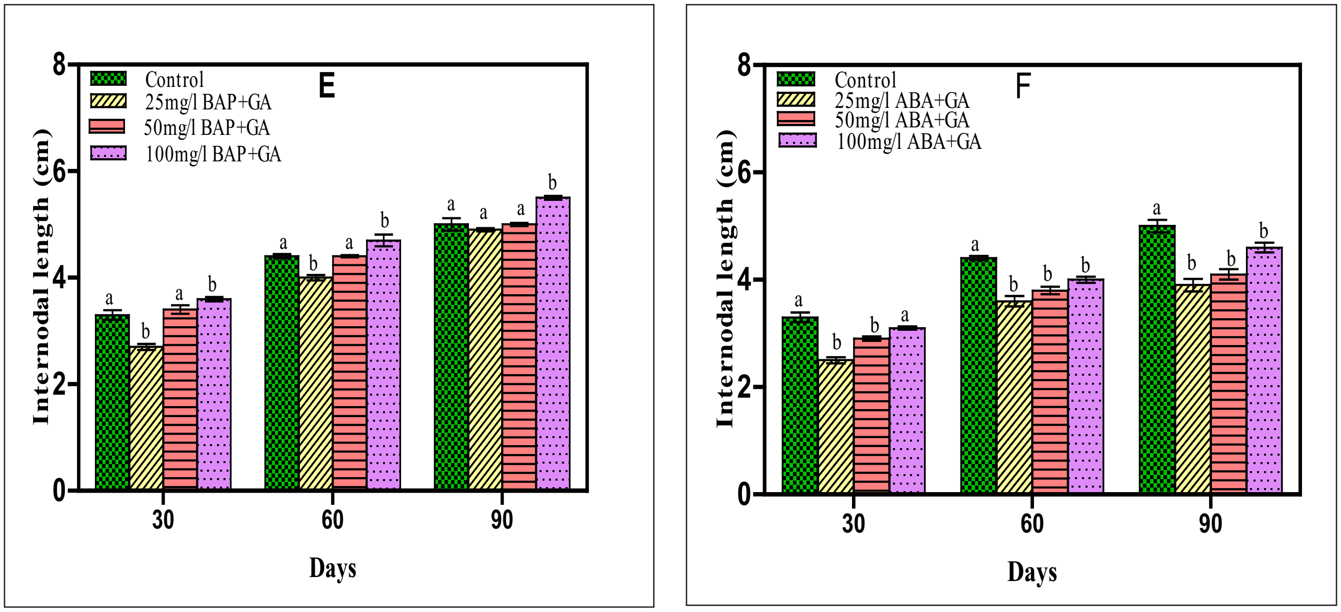

Figure 2: Internodal length in *Fagopyrum esculentumon* different days under phytohormones treatment i.e., IAA+BAP (A), IAA+ABA (B), IAA+GA (C), ABA+BAP (D), BAP+GA (E) and ABA+GA (F). Values are mean ± SE; n=3 analysed by Two-way ANOVA followed by Bonferroni's multiple comparison test. Values followed by the same letter are not statistically different (p<0.05) compared with control.

Similarly internodal length also increased in *lycopersicon* (Rai et al., 2006) and Soybean (Khatun et al., 2016) due to gibberellins. In present study combination of IAA+GA at 100 mg l^{-1} was also effective in promoting internodal length. Earlier Meenakshi and Lingakumar (2011) reported enhanced internodal length in *Mentha arvensis* with IAA and 2,4-D treatment. Mostafa and Abou-Alhamd (2011) concluded that GA (50 ppm) and IAA (2000 ppm) increased internodal length in *Balanites aegyptica.* But according to Ma et al. (2018) IAA is negatively correlated with internodal length. Internodal length decreased in ABA+BAP (25, 50 mg l^{-1}) and ABA+GA (25 mg l^{-1}) treatments. Similar results with ABA treatments were reported in submerged rice (Hoffmann-Benning and Kende, 1992) and floating rice (Azuma et al., 1995). High concentrations of BAP reduced internodal length in *Pyrus calleryana* (Berardi et al. 1993).

3.7 Relative water content

Relative water content (RWC) is used as a most meaningful index for dehydration tolerance and considered a measure of plant water status, reflecting the metabolic activity in tissue. It is evident from the Table 2 that the relative water content of leaf increased up to 60 days of growth and thereafter declined (upto 90 days). ABA+GA 50 mg l^{-1} at 30 days (79.60%, 13.7% increase from control) and 60 days (83.10%, 6.5% increase from control), IAA+BAP 50 mg l^{-1}, IAA+ABA 50 mg l^{-1} and ABA+GA 50 mg l^{-1} at 90 days (77.20%, 7.2% increase from control), promoted relative water content. However, BAP+GA 25 mg l^{-1} reduced the same at 30 days (52.20%, 25.4% decrease from control), at 60 days (60.50%, 22.4% decrease from control) and at 90 days (61.30%, 14.9% decrease from control).

It is evident that combination of ABA with GA and IAA was effective only at 50 mg l^{-1} concentration. Our results are consistent with a previous study (Agehara and Leskovar, 2012), who attributed that ABA improved the relative water content in Muskmelon seedlings throughout water stress and rehydration situation. Earlier studies showed that foliar spray of IAA is effective in maintaining the leaf RWC in barley (*Hordeum vulgare*) (Ashraf and Harris, 2004) and maize plants (Kaya et al., 2010) under water stress and salt stressed conditions, respectively. Perez-Jimeneza et al. (2015) also reported that IAA and ABA promoted water accumulation in leaves of *Capsicum annuum.* In the present study, gibberellic acid treatment improved leaf relative water content in *F. esculentum*. Similar results were reported in *Helianthus annuus* (Sayed and Gadallah, 2013) and wheat (Abo-Hamed et al., 1999). This may be due to the reason that GA increases water uptake and reduces transpiration (Halevey and Mayak, 1981). Present investigation also revealed that BAP alone reduced leaf relative water content, but in combination with IAA it promoted relative water content of leaves. This may be due to the fact that cytokinins regulate stomatal behaviour (Hegele et al., 2008). Decline in relative water content of leaves was noticed in BAP+GA at lower concentration (25 mg l^{-1}). BAP stimulates opening of stomata and transpiration as a result decreased RWC in plants sprayed with 10 μM BAP (Rulcova and Pospisilova, 2001).

4. Conclusion

Need for food and medicines is supposed to continue due to ever-growing world population. Exploitation of underutilized crops can contribute effectively to promote nourishment and biological sustainability. *Fagopyrum esculentum* Moench is one of the essential neglected crops having high nutritive and medicinal value. Combination of PGRs were more effective than solely applied PGRs. Higher concentration was effective than lower concentration of different treatments. The results of the present study call for further research on mechanism of PGRs action by using molecular approaches.

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6. References

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