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Effect of plant bio-regulators and urea on growth of walnut seedling under Kashmir condition

Sanna Manzoor, Umar Iqbal, Amit Kumar*, M. M. Mir and Rasheeba Iqbal

Division of Fruit Science, Sher-e-Kashmir University of Agricultural Sciences and Technology, Kashmir 190 025, India

ABSTRACT

The experiment was carried out by adopting double split plot design with eighteen treatments replicated thrice. The treatments combinations consisted of foliar sprays of gibberellic acid (GA_3) and Indole-3-Butyric Acid (IBA) each at 200 and 400 ppm and urea at 0.3% alone or in combinations. Of the tested treatment combinations, application of 400 GA_3 + 200 ppm IBA + 0.3% urea resulted the highest growth of walnut seedlings in respect of seedling height (66.33 cm), number of leaves per seedling (70.67), leaf area (63.27 cm^2) and stem diameter (17.18 mm) whereas, the longest tap root (52.73 cm) and highest number of secondary roots (33.20) were noticed the plants, sprayed with 200 ppm GA_3 + 200 ppm IBA + 0.3% urea. The maximum percentage of graftable size seedlings (100%) attaining diameter of 10 - 15 mm was obtained in the treatment comprising of GA_3 @ 0 ppm + 200 ppm IBA + 0.3% urea, however application of 400 ppm GA_3 + 200 ppm IBA + 0.3% urea registered 86.7% graftable size seedlings, having a diameter more than 15 mm. Minimum sturdiness quotient of 3.66 was recorded with 400 ppm GA_3 + 200 ppm IBA + 0.3% urea. Based on the results, it can be concluded that four foliar applications of 400 ppm GA_3 + 200 ppm IBA + 0.3% urea on walnut seedlings at an interval of 21 days, starting from 3-4 leaf stage, significantly increased the overall growth of walnut seedlings and thus, led to the production of more number of graftable size plants with in a year.

Key words: *Juglans regia* L., graftable size, seedling, studiness.

INTRODUCTION

Persian walnut (*Juglans regia*) is native to Persia and is extensively grown in temperate areas of the world between 1,200 to 2,150 m amsl. China, USA, Iran, Turkey are the major walnut producing countries of the world, however In India, UT of Jammu and Kashmir is the leading walnut producer with a production of 2.94 lakh MT from an area of 0.86 lakh hectare (Anon., 2). Majority of walnut production in the country comes from seedling origin, having long juvenile period with huge variability in nut size, vigour, quality and a high shell-kernel ratio. The major problems in walnut cultivation are the lack of vegetatively propagated plants of superior quality, suitable rootstocks and inadequate cultural practices. Due to the non-availability of vegetatively propagated plants of walnut in sufficient number, walnut is mostly grown as scattered trees and thus well laid out regular walnut plantations is seldom found (Sharma *et al.*, 16). Use of superior cultivars and appropriate rootstocks together with optimization of production methods are important to improve the quantity and quality of walnut production.

Walnuts are generally propagated on seedling rootstock and the seedling raised from seed require two years to attain a graftable size (Vahdati *et al.*, 18) with an increase in the production cost of nursery plants. To obtain the seedlings ready to graft within the shortest time period (5-6 months), the use of PBR's and nutrients may be the cheapest technology to enhance the quantity and quality of planting materials of walnut. Foliar applications of IBA and GA at early vegetative leaf stages have been shown to increase the rate of crop development because of their positive interaction (Ghodrat *et al.,* 6). Similarly, the role of nitrogen in stimulating vegetative growth is well known in stimulating the growth in fruit trees. Therefore, this experiment was designed to produce walnut seedlings suitable for grafting in one planting year, with the foliar application of PBRs and urea sprays.

MATERIALS AND METHODS

The study was conducted at the experimental fields of Division of Fruit Science, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Shalimar, Srinagar during the year 2019-2020. Temperature ranges from 35°C in summers to a minimum of -7°C in winters. Freshly harvested walnut seeds were sown in well prepared open field at a spacing of 60 cm × 10 cm in the first week of December, and *in-situ* stratified for a period of three months at approx. 4 ± 1°C. The seedlings were foliar sprayed with different treatment combinations of GA_3 (200 and 400 ppm), IBA (200 and 400 ppm) and

*Corresponding author: khokherak@rediffmail.com

urea (0.3 %) at 3-4 leaf stage, and repeated thrice (total spray given four) after 21 day interval. The experiment was laid out according to Double Split Plot Design comprising of eighteen treatment, and replicated thrice with fifteen seedlings per treatment (five seedlings per replication).

The data were recorded on vegetative characters *viz*., seedling height (cm) and length of tap root (cm) with the help of a scale at the end of the growing season. In the end of October, before leaf fall, a total number of leaves per plant was counted, whereas leaf area (cm^2) of ten fully expanded leaves was measured using leaf area meter. Number of secondary roots per seedling was counted at the end of experiment, while seedling diameter (mm) was measured with the help of digital Vernier calipers at 15 cm height above the ground level in the month of December. Sturdiness quotient was calculated by dividing height (cm) of the seedling with seedling diameter (mm). Seedlings diameter in the range of 5-10 mm, 10-15 mm and ≥ 15 mm were counted in the month of December. Data generated from these investigations were analyzed statistically as per the procedure of Gupta *et al.* (7). The level of significance was tested for different variable at 5 % level of significance.

RESULTS AND DISCUSSION

Foliar application of plant growth regulators and urea showed the significant results for all the studied parameters. The longest plant (66.33 cm) was recorded statistically with the foliar application of 400ppm GA_3 + 200ppm IBA + 0.3% urea closely followed by 400ppm GA_3 + 0 ppm IBA + 0.3% urea (63.01 cm), while it was shortest (34.67cm) in untreated control (Table 1). The longest plant may be

Table 1. Effect of plant bio-regulators and urea on height, number of leaves and leaf area of walnut.

Treatment		Seedling height (cm)			No. of leaves/plant			Leaf area (cm^2)		
		U_0	U_1	Sub-mean	U_0	U_1	Sub-mean	U_0	U_1	Sub-mean
GA_3 @ 0 ppm	IBA @ 0 ppm	34.67	46.27	40.47	40.00	46.00	43.00	37.85	39.56	38.70
	IBA @ 200 ppm	44.60	56.73	50.67	43.67	58.33	51.00	41.61	52.35	46.97
	IBA @ 400 ppm	41.60	54.47	48.03	43.00	54.67	48.83	39.69	50.41	45.06
	Sub-means	40.29	52.49	46.39	42.22	53.00	47.61	39.72	47.43	43.57
GA_3 @ 200 ppm	IBA @ 0 ppm	49.27	50.05	49.66	48.33	51.77	50.05	43.29	45.14	44.21
	IBA @ 200 ppm	60.80	62.65	61.72	61.79	68.23	65.01	56.65	61.65	59.15
	IBA @ 400 ppm	58.80	61.30	60.05	62.80	66.00	64.40	56.99	60.01	58.50
	Sub-means	56.29	58.00	57.14	59.56	62.00	59.82	52.30	55.40	53.85
GA_3 @ 400 ppm	IBA @ 0 ppm	51.40	63.01	57.20	51.33	64.00	57.67	46.56	58.90	52.73
	IBA @ 200 ppm	60.60	66.33	63.46	60.23	70.64	65.45	56.70	64.14	60.42
	IBA @ 400 ppm	47.67	47.40	47.53	54.33	56.73	55.53	43.02	45.78	44.40
	Sub-means	53.21	58.91	56.06	55.29	63.80	59.54	48.78	56.31	52.54
Sub-means	IBA @ 0 ppm	45.11	56.00	50.55	46.55	56.44	51.50	42.56	50.94	46.75
	IBA @ 200 ppm	55.00	56.37	55.68	60.66	62.02	61.34	54.43	55.75	55.09
	IBA @ 400 ppm	49.36	53.69	51.52	53.22	56.78	55.00	47.54	51.40	49.47
	Sub-means	49.82	55.35	52.58	53.48	58.41	55.94	48.17	52.69	50.43
$CD_{0.05}$										
Urea			2.59			1.74			2.13	
GA_3			1.08			1.27			1.26	
IBA			0.61			1.19			0.97	
GA_3 × Urea			1.53			1.79			1.78	
IBA × Urea			0.87			1.68			1.37	
GA_3 × IBA			0.87			1.68			1.37	
GA_3 × IBA × Urea			1.23			2.45			2.56	

U0: 0 per cent Urea U1: 0.3 per cent Urea

attributed to the fact that auxin causes acidification of cell walls, which promotes cell elongation, increases the plasticity of cell walls, and also allows the walls to expand due to the force of cell internal turgor pressure (Majda and Robert, 10). Gibberellin promotes growth by stimulating cell division and elongation in the cambium tissue of the internodal region, by increasing mechanical extensibility and plasticity of cell wall, which is followed by hydrolysis of starch to sugar, resulting in lower water potential and allowing water to enter inside the cell (Rastogi *et al.* 14). Rana *et al.* (13) also reported maximum plant height with 1500 ppm GA_3 application in Persian walnut. Application of nitrogen in combination with plant growth regulators also resulted in enhanced height of seedling and which can be explained by the fact that nitrogen is utilized in the formation of protein which is the main component of protoplasm in the plants. Therefore, with an increase in the foliar spray of nitrogen source, the synthesis of amino acids in the plants might have accelerated, which is indirectly exhibited by enhanced growth in walnut seedlings (Dipta, 5). Rattanpal and Singh (15) also observed similar results reported that seedlings treated with urea recorded maximum plant height in Rough lemon.

Maximum number of leaves (70.64) was observed with foliar application of 400 ppm GA_3 + 200 ppm IBA + 0.3% urea which were statistically at par with 400 ppm GA_3 + 200 ppm IBA + 0.3% urea i.e. 68.23, however minimum number of leaves (40.00) was recorded in untreated seedlings (Table 1). The activation of physiological processes and action of GA_3 which stimulates the production of new leaves more rapidly and promotes linear growth and accelerated translocation of food material in the tissue, may be responsible for increase in number of leaves with GA_3 and urea treatment. Nazim *et al.* (11) also reported that treatment with gibberellic acid and auxin resulted in the production of more number of leaves in mango, which could be due to the vigorous growth promoted by these growth regulators, which resulted in a greater number of branches, which in turn allowed the plants to harvest more sunlight, resulting in a greater number of leaves. Rana *et al.* (13) also reported that the number of leaves/ plant increased with the application of gibberellins in walnut. Increase in the number of leaves may also be attributed to the fact that as the number of roots increased under IBA treatment, more number of apical roots also increased which are responsible for synthesis of cytokinin thus lead to the formation of more number of leaves.

Leaf area was significantly influenced by application of plant growth regulators and urea and highest leaf area (64.27 cm^2) was measured in treatment combinations of 400 ppm GA_3 + 200 ppm IBA + 0.3% urea which was statistically at par with treatment combination of 200 ppm GA_3 + 200 ppm IBA + 0.3% urea (61.65 cm^2) whereas minimum leaf area (37.85 cm^2) was attained by untreated seedlings (Table 1). The increase in leaf area might be as a result of increased synthesis of nucleoproteins responsible for enhancing leaf initiation and leaf area due to GA_3 activity at the apical meristem. Urea also leads to overall increase in the actively growing tissues of the plants, resulting in an increase in number as well as size of individual cells. Vishal *et al*. (19) also observed similar results and reported that application of GA_3 resulted in maximum leaf area in strawberry however application of IAA also increase the leaf area in strawberry.

Application of plant growth regulators and urea significantly influence length of tap root, number of secondary roots and seedling diameter (Table 2). Statistically longest tap root (52.73 cm) was measured with 200 ppm GA_3 + 200 ppm IBA + 0.3% urea closely followed by 0 ppm GA_3 + 200 ppm IBA + 0.3% urea (49.25 cm) whereas smallest tap root (29.00 cm) was measured in untreated seedlings, *i.e.* control. The increase in the growth of tap roots with the application of GA_3 may be attributed to its role in stimulation of cambium growth through cell division and expansion (Al-Maathedi *et al.*, 1) because plant growth occurs by two processes i.e. cell division by mitosis which adds new cells and cell elongation of already existing cells by enlargement of the vacuoles. This might also be due to increased synthesis of photosynthates and their translocation to the root zone via phloem, which could be responsible for enhancing the root growth. These results are in agreement with Dipta (5) in walnut who also observed maximum primary root length with GA_3 and IBA, respectively. With the increase in concentration (400 ppm) of both GA_3 and IBA, the length of tap root decreased. This could be due to the fact that auxin induces the synthesis of ethylene which inhibits root growth. However, even if ethylene biosynthesis is specifically prevented, low concentrations of auxin encourage the growth of intact roots, whereas higher concentrations restrict growth. As a result roots may require a minimal concentration of auxin to grow, auxin concentrations that promote elongation in stems and coleoptiles substantially impede root growth (Taiz and Zeiger, 17). Maximum number of secondary roots (33.20) was counted with 200 ppm GA_3 + 200 ppm IBA + 0.3% urea @ which was statistically at par with 200 ppm GA_3 + 200 ppm IBA + 0.0 % urea (32.27) number of secondary roots however minimum number of secondary roots (21.47) was counted in control (Table 2). Lateral roots

Table 2. Effect of plant bio-regulators and urea on length of tap roots, number of secondary roots and diameter of seedlings.

Treatment		Length of tap roots (cm)			No. of secondary roots			Diameter of seedlings (mm)		
		U0	U1	Sub-mean	U0	U1	Sub-mean	U0	U1	Sub-mean
GA_3 @ 0 ppm	IBA @ 0 ppm	29.00	34.07	31.53	21.47	24.40	22.93	6.89	9.87	8.38
	IBA @ 200 ppm	45.33	49.25	47.29	29.87	31.60	30.73	11.51	13.81	12.66
	IBA @ 400 ppm	36.40	42.53	39.46	26.73	27.40	27.07	10.64	12.99	11.81
	Sub-means	36.91	41.95	39.43	26.02	27.80	26.91	9.68	12.22	10.95
GA_3 @ 200 ppm	IBA @ 0 ppm	43.07	47.20	45.13	27.93	30.93	29.43	11.10	13.09	12.10
	IBA @ 200 ppm	49.53	52.73	51.13	32.27	33.20	32.73	16.47	16.57	16.52
	IBA @ 400 ppm	32.20	37.80	35.00	25.67	26.60	26.13	15.42	15.45	15.43
	Sub-means	41.60	45.91	43.75	28.62	30.24	29.43	14.33	14.85	14.59
GA_3@ 400 ppm	IBA @ 0 ppm	35.73	41.60	38.67	26.33	27.53	26.93	11.09	14.65	13.27
	IBA @ 200 ppm	34.40	40.42	37.41	25.87	28.67	27.27	16.32	17.18	16.75
	IBA @ 400 ppm	33.93	34.20	34.06	25.47	25.93	25.70	10.68	13.20	11.94
	Sub-means	34.69	38.74	36.71	25.89	27.38	26.63	12.70	15.01	13.85
Sub-means	IBA @ 0 ppm	35.93	40.95	38.44	25.24	27.62	26.43	9.69	12.39	11.04
	IBA @ 200 ppm	44.13	46.66	45.40	30.00	31.16	30.58	14.80	15.82	15.31
	IBA @ 400 ppm	34.62	37.04	35.83	25.96	26.64	26.30	12.55	13.16	12.85
	Sub-means	38.23	41.55	39.89	27.07	28.47	27.77	12.35	13.79	13.07
$CD_{0.05}$										
Urea			3.41			4.28			0.47	
GA_3			0.54			0.57			0.80	
IBA			0.87			0.80			0.71	
GA_3 × Urea			0.77			NS			0.13	
IBA × Urea			1.24			NS			0.01	
GA_3 × IBA			1.24			1.14			0.24	
GA_3 × IBA × Urea			1.75			1.61			0.76	

U0: 0 per cent Urea U1: 0.3 per cent Urea

emerge from small groups of cells in the pericycle are usually found above the elongation and root hair zone. These pericycle cells are stimulated to divide by auxin. The proliferating cells gradually form into a root apex and the lateral root emerges from the root cortex and epidermis (Taiz and Zeiger, 17). Exogenous application of auxins increases lateral root initiation, according to numerous studies, lateral root development is strongly dependent on auxin and auxin transport. GA_3 and urea promoted secondary roots by enhancing root cell elongation, cell division, auxin metabolism due to GA_3 and more vegetative growth due to nitrogen (Pandiyan *et al.*, 12). Present results are in the accordance with the earlier findings Karunakaran *et al*. (9) also reported that the secondary and fibrous roots increased with application of GA_3 and urea.

Statistically highest seedling diameter (17.18 mm) was recorded with the application of 400ppm GA_3 + 200 ppm IBA + 0.3% urea closely followed by 200 ppm GA_3 + 200 ppm IBA + 0.0% urea (16.57 mm), however lowest seedling diameter (6.89 mm) was observed in control. Plants use nitrogen for the synthesis of proteins, nucleic acids and hormones however, when plants are nitrogen deficient, their growth is stunted and nitrogen has a positive correlation with GA_3, resulting in maximum stem diameter since GA_3 stimulates cell elongation. The beneficial effect of GA_3 was probably due to cell elongation and rapid division of cells following germination. Chiranjeevi *et al.* (4) in Aonla also reported similar findings where seedlings diameter increased with the application of GA_3. Increased diameter may be due to radial growth of stem, which

is achieved by activity of ring of vascular cambium by process of cell division and cell enlargement.

A less rigorous, but non-destructive index is the 'sturdiness quotient', which compares height (cm) over root collar diameter (mm). The sturdiness quotient was significantly influenced by the application of growth regulators and urea (Table 3). The application of all the treatment combinations resulted in sturdiness quotient of less than the recommended threshold value of 6, which is appropriate. Minimum sturdiness quotient (3.66) was recorded with 400ppm GA_3 + 200ppm IBA + 0.3% urea closely followed by 200ppm GA_3 + 200ppm IBA + 0.0 % urea (3.67) and 200ppm GA_3 + 400ppm IBA + 0.0 % urea (3.68) whereas maximum sturdiness quotient (5.04) was recorded under control. A low quotient indicates a sturdy plant with a better chance of survival, particularly on windy or dry sites. A sturdiness quotient of more than 6.0 is considered undesirable (Jaenicke, 8).

Graftable size of walnut seedling rootstock is 12 mm (Sharma *et al.* 16) and in present study (Fig 1) maximum percentage of graftable seedlings (100 per cent) having diameter between 10-15 mm was obtained with the application of 0 ppm GA_3 + 200ppm IBA + 0.3 % urea followed by 0 ppm GA_3 @ 0 ppm + 200ppm IBA + 0.0 % urea where 93.30 per cent of seedlings having 10-15 mm size, however above 15 mm maximum percentage of graftable seedlings (86.70 %) was obtained with the application of 400ppm GA_3 + 200ppm IBA + 0.3% urea followed by 200ppm GA_3 + 200ppm IBA + 0.3% urea having 80.00 per cent of the seedlings. Maximum growth of seedlings and attaining graftable size might be due to the growth promoting effect of GA_3, IBA and urea. Nazim *et al.* (11) in mango and Bandana (3) in apple reported that the combined application of

Table 3. Effect of plant bio-regulators and urea on sturdiness quotient of walnut seedlings.

Treatment		U0	U1	Sub-means
GA_3 @ 0 ppm	IBA @ 0 ppm	5.04	4.72	4.88
	IBA @ 200 ppm	3.82	4.12	3.97
	IBA @ 400 ppm	3.88	4.19	4.03
	Sub-means	4.24	4.34	4.29
GA_3 @ 200 ppm	IBA @ 0 ppm	4.47	4.92	4.69
	IBA @ 200 ppm	3.67	3.72	3.70
	IBA @ 400 ppm	3.68	3.82	3.75
	Sub-means	3.94	4.16	4.04
GA_3@ 400 ppm	IBA @ 0 ppm	4.62	4.64	4.63
	IBA @ 200 ppm	3.82	3.66	3.74
	IBA @ 400 ppm	4.43	4.26	4.34
	Sub-means	4.29	4.19	4.24
Sub-means	IBA @ 0 ppm	4.71	4.76	4.73
	IBA @ 200 ppm	3.77	3.83	3.80
	IBA @ 400 ppm	3.96	4.09	4.03
	Sub-means	4.15	4.23	4.19
$CD_{0.05}$				
Urea			NS	
GA_3			0.21	
IBA			0.11	
GA_3 × Urea			NS	
IBA × Urea			NS	
GA_3 × IBA			0.16	
GA_3 × IBA × Urea			0.22	

U0: 0 per cent Urea U1: 0.3 per cent Urea

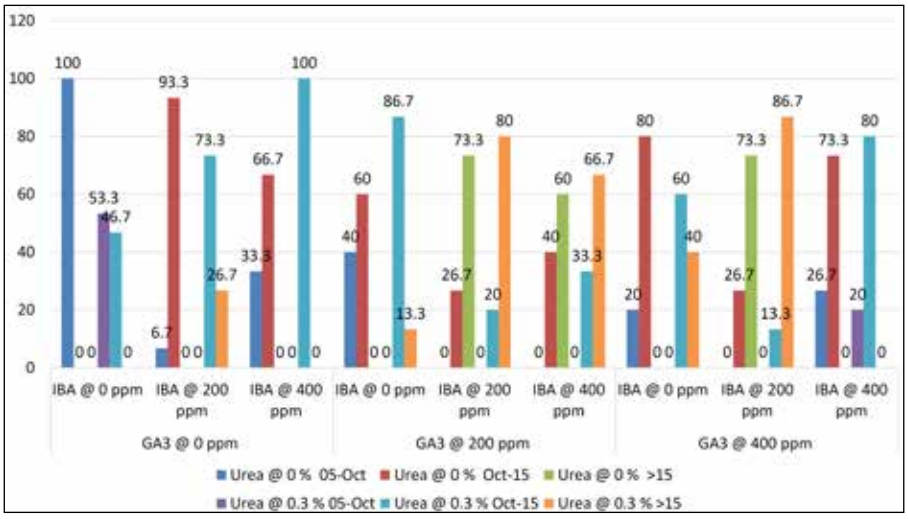

Fig. 1. Effect of plant bio-regulators and urea on graftable seedlings (%) stem diameter

auxins + gibberellins and foliar spray of GA^3 + urea, respectively resulted in maximum per cent of graftable seedlings.

From the present study it is clear that four sprays of these growth regulators (GA_3 and IBA) together with urea starting from 3-4 leaf stage of walnut seedlings, at an interval of 21 days helped to achieve better seedling growth, but the treatment (400ppm GA_3 + 200ppm IBA + 0.3% urea) proved to be the best in terms of growth parameters and percentage of seedlings attaining graftable stem diameter.

AUTHORS CONTRIBUTION

Conceptualization of research (UI, AK, MMM); Designing of the experiments (SM, UI); Contribution of experimental materials (SM, UI, MMM); Analysis of data and interpretation (UI, AK, RI); Preparation of the manuscript (SM, AK, RI); Review and editing (UI, AK).

DECLARATION

The authors declare that they do not have any conflict of interest.

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