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Biomass Production on the Hard Wood Stem Cutting of Pomegranate (*Punica granatum* L.) added with IBA and NAA with and without GA_3

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ABSTRACT: Pomegranate is quite important fruit very rich in vitamins and minerals possessing high medicinal values. Unfortunately it has not received the attention of the scientist and growers and there is lack of genuine planting material. The quality of fruits from seedling plants is very poor therefore, an experiment was conducted at the Garden, Department of Fruit Science, Chandra Shekhar Azad University of Agriculture and Technology Kanpur (U.P.) in the year of 2019- 20 and 2020-21. Growth regulators e.g. IBA and NAA each at four levels i.e. 3000, 4000, 5000, and 6000 ppm with and without 2000 ppm GA_3 along with a control (Water spray) making 18 treatments in all were tried in a Randomized Block Design replicating thrice. Application of NAA 5000 ppm in association with GA_3 2000 ppm maximized the shoot parameters significantly recording 7.55 shoots per cutting, 4.78 number of branches per shoot, 21.46 cm length of shoot and 5.52 mm diameter of shoot. The next effective treatment boosting the above growth parameters was observed to be NAA 4000 ppm + GA_3 2000 ppm revealing 7.31, 4.71, 20.04 cm and 5.30 mm values respectively. Untreated plants (control) showed significantly poorest performance in all the above attributes recording 2.96, 3.07, 12.15 cm and 2.19 mm respectively. Biomass production as the term of leaves was also promoted by the treatment of NAA 5000 ppm in association with GA_3 2000 ppm exhibiting 42.99 number of leaves having their length and diameter 6.21 cm, and 1.80 mm respectively. The treatment also enhanced the fresh (5.13 g) and dry (541.92 mg) weight respectively. Untreated cuttings (Control) expressed significantly poorest values to the tune of 23.48, 4.46 cm, 1.22 cm, 2.55 g and 280.73 mg in the above leaf parameters respectively.

Keywords: IBA, NAA, GA_3, shoot parameters and leaf attributes.

INTRODUCTION

Pomegranate (*Punica granatum* L.) is popularly called as Anar is regarded as fruit of "Paradise'. In India its mostly grown in Maharashtra, Gujarat and Uttar Pradesh. The total production of the country was 2329000 MT with an area of 264000 hectare in (2019-20. NHB). It is a drought and salinity tolerant crop and adapts well in marginal land of arid and semiarid area where cool winter and hot summer prevails (Sarkhosh *et al.,* 2006). Pomegranate is highly nutritive and medicinal rich fruit having ample of sugar, calcium, phosphorus, iron, vitamin-C, riboflavin, thiamine, antioxidant and polyphenol (Pawar and Singh, 2020). It is commercially propagated by hard wood cuttings but percentage of rooting and survival is quite meagre and the surviving are lean and thin having biomass. However, erratic efforts have been made to regeneration it with the help of growth regulators and their varying concentration (Sharma *et al.,* 2009; Kaur and Kaur, 2016). Root promoting hormone undoubtedly play important role in the callus formation producing more number of longer and thicker roots shortening the days required for sprouting at the same time provided. They are treated with proper hormone and their concentration (Kaur *et al.,* 2016; Kumar and Singh, 2020). NAA, besides influencing other physiological activities of plants has been found help full in root initiation and root development (Ghosh *et al.,* 2017; Pop *et al.,* 2011). IBA produce rooting in stem cuttings and air layers due to its ability to activate cambium regeneration, cell division and cell multiplication which ultimately affect the biomass production on the treated cuttings (Sharma *et al.,* 2009; Punasya *et al.,* 2018). GA_3 has also been exploited in the citrus and other fruit crops and it may promotes flowering in different fruit crop also (Kumar *et al.,* 2017; Ahmad *et al.,* 2018). Propagation of plants with stem cuttings is influenced with a number of factors e.g. physiological condition of mother plants cuttings types preparation time planting medium, selection of hormone and its optimal concentration and the variety (Aytekin and Caliskam, 2009; Rao *et al.,* 2021). In view of the above a

systematic experiment was planned to regenerate pomegranate plant with the help of optimal concentration of growth regulators so that healthy plants having greater biomass production obviously with better survival may be obtained.

MATERIALS AND METHOD

The experiment was conducted during the two consecutive years i.e. 2019-2020 and 2020-2021 at the at the Garden of the Department of Fruit Science, Chandra Shekhar Azad University of Agriculture and Technology Kanpur (U.P.) India with the view of regenerating pomegranate plants of Kandhari variety by hard wood stem cutting with the aid of growth regulators. Growth regulators IBA and NAA each at 3000, 4000, 5000 and 6000 ppm were tried with and without GA_3 2000 ppm. There were thus, 18 treatments in all replicated thrice in a Randomized Block Design. Hard wood cuttings of pencil thickness of 20 cm long having 4 to 5 buds were planted after treating with respective concentration of growth regulators as per treatment. Cultural practices, irrigation, manuring and plant protection measures were adapted as per requirements. The data obtained during both the years of study were subjected to statistical analysis. Pooled analysis was also made to confirmed the authenticity (Panse and Sukhatme, 2000).

RESULTS AND DISCUSSION

A. Shoot Parameters

(i) Number of shoots per cutting and number of branches per shoot: NAA 5000 ppm in association with GA_3 2000 ppm enhanced the number of shoot per cutting to the maximum being at par and followed by NAA 4000 ppm + GA_3 2000 ppm treatment exhibiting 7.55 and 7.31 number of shoots respectively. Control produced significantly poorest 2.96 number of shoots per cutting. This treatment registered 155.07 % more shoots when compared to control. Similarly, the number of branches per shoot was noted significantly higher when the cutting were treated with NAA 5000 ppm + GA_3 2000 ppm which produced 4.78 branches closely followed by NAA 4000 ppm + GA_3 2000 ppm revealing 4.71 branches remaining at par in between. The poorest (3.07) number of branches were recorded under control. It is obvious from the mean values that NAA 5000 ppm + GA_3 2000 ppm recorded 55.70 % more branches as compared to control. It could be possible due to exogenous application of NAA in present investigation meeting the necessary hormone of demand which promoted number of shoots and branches. When GA_3 2000 ppm was combined with different levels of NAA, it improved physiological activities by altering auxin status of tissues. These findings are similar to the reports of Deb *et al.*, (2009) in lemon, Damer *et al.*, (2014) in pomegranate, Hakim *et al.*, (2018) in pomegranate and Kruthika *et al.*, (2020) in mulberry.

(ii) Size of shoots (Length and diameter): The treatment of NAA 5000 ppm + GA_3 2000 ppm significantly induced longest shoots measuring 21.46 cm closely followed by NAA 4000 ppm + GA_3 2000 ppm (21.04 cm). Both the treatments remaining at par in between proved significantly superiors than the rest of treatments. All the individual treatments (Table 1) of NAA despite its varied concentrations elongated the shoots significantly than the different doses of IBA.

Table 1: Effect of IBA and NAA with or without GA_3 treatment on shoot parameters of pomegranate stem cuttings on the pooled analysis.

Symbols	Treatments	Number of shoots per cutting	Number of branches per shoots	Length of shoots (cm)	Diameter of shoots (mm)
T_1	Control (Water spray)	2.96	3.07	12.15	2.91
T_2	IBA 3000 ppm	3.95	3.20	13.35	3.14
T_3	IBA 4000 ppm	4.63	3.44	14.79	3.37
T_4	IBA 5000 ppm	4.42	3.34	14.30	3.24
T_5	IBA 6000 ppm	4.18	3.25	13.15	3.20
T_6	NAA 3000 ppm	5.61	3.87	17.30	4.12
T_7	NAA 4000 ppm	6.29	4.04	18.16	4.41
T_8	NAA 5000 ppm	6.51	4.11	18.71	4.60
T_9	NAA 6000 ppm	5.94	3.96	17.76	4.37
T_{10}	GA_3 2000 ppm	6.63	4.01	19.22	4.72
T_{11}	IBA 3000 ppm + GA_3 2000 ppm	4.86	4.25	19.32	4.78
T_{12}	IBA 4000 ppm + GA_3 2000 ppm	5.53	4.45	19.50	4.90
T_{13}	IBA 5000 ppm + GA_3 2000 ppm	5.27	4.39	19.41	4.90
T_{14}	IBA 6000 ppm + GA_3 2000 ppm	4.79	4.33	19.28	4.79
T_{15}	NAA 3000 ppm + GA_3 2000 ppm	6.86	4.53	19.77	4.98
T_{16}	NAA 4000 ppm + GA_3 2000 ppm	7.31	4.71	21.04	5.30
T_{17}	NAA 5000 ppm + GA_3 2000 ppm	7.55	4.78	21.46	5.52
T_{18}	NAA 6000 ppm + GA_3 2000 ppm	7.09	4.69	20.16	5.17
	C.D. at 5%	0.44	0.45	0.69	0.32

The cuttings under control showed significantly shortest length of shoot (12.15 cm) than the rest of treatments. Treatment NAA 5000 ppm + GA_3 2000 ppm causing maximum length of shoot showed 76.62% greater length than control.

The thickness of the shoot behaved similar to their length and treatment of NAA 5000 ppm in association with GA_3 2000 ppm inducing significantly maximum 5.52 mm diameter. Second effective treatment in this regard was NAA 4000 ppm + GA_3 2000 ppm recording 5.30 mm thickness of shoot being at par in between individual. Among treatments GA_3 2000 ppm proved more effective than all the other individual treatments of it either IBA or NAA barring NAA 4000 and 5000 ppm. The maximum, improvement on diameter of shoot was 89.69 % over control due to NAA 5000 ppm + GA_3 2000 ppm treatment. The maximum top growth judged in terms of shoot length and diameter on the cuttings might be attributed to greater number of roots owing to auxin treatment which could have favoured cell division and cell elongation which might have helped better roots development there by resulting in longer shoots of greater diameter. It may also be ascribed to higher cell activity, more synthesis of food material having greater photosythates hence more shoot length and diameter (Devi *et al.,* 2016) in phalsa. Auxin and GA_3 both produced similar of response which suggest the action of two substances which may be interconnected. GA_3 however, exert its physiological effect by altering the auxin status of the tissues (Kefford, 1962). The results of present investigation are in line with the reports of Hakim *et al.,* (2018) in pomegranate and Deb *et al.,* (2009) in lemon, Seiar, (2017) in pomegranate, Dahale *et al.,* (2018) in fig, Sujin *et al.,* (2020) in guava and Joseph and Sobhana (2020) in passion fruit.

B. Leaf Parameters

(i) Number of leaves their length and width: The biomass production in term of number of leaves their length and diameter enhanced significantly when cuttings were treated with NAA 5000 ppm + GA_3 2000 ppm exhibiting 42.99 leaves, 6.21 cm length and 1.80 cm diameter respectively. The next effective dose boosting the above parameters was observed to be NAA 4000 ppm in association with GA_3 2000 ppm recording 38.94, 6.08 cm and 1.74 values. Untreated plants i.e. control registering significantly poor leaf attributes indicating 23.48 leaves, 4.46 cm and 1.22 cm length and width respectively. Application of GA_3 applied alone proved effective than the individual concentrations of IBA and NAA in the above traits (Table 2). Data demonstrated that treatment of NAA 5000 ppm + GA_3 2000 ppm exerting on leaf number their length and width brought about 83.09, 39.24 and 47.54 % improvement over control. The improvement caused by individual treatment of NAA 5000 ppm or their combination with GA_3 2000 ppm might have activated cell division, influencing elongation of shoots under. Besides exogenous application of chemicals enhancing hydrolysis of nutritional reserves could have brought causing improvement in leaf parameters. Earlier it was thought that auxin stimulates growth depleting tissues, whereas, GA induces growth in the plants. However now it has been concluded that auxin and GA control separate processes in cell elongation may act preferential in younger cell compared with auxin and induce cell division and cell elongation, whereas, auxin act by promoting cell extension (Yang and Dang, 1993). The results of present investigations are in accordance with the reports of Ahmad *et al*., (2018) in Rangpur lime, Kumar and Singh (2020) in kagzi lime, Chauhan and Maheshwari (1970) in peach and Shukla *et al.,* (2010) in peach, Kiruthika *et al.,* (2020) in mulberry and Hakin *et al.,* (2018) in pomegranate.

(ii) Fresh weight and dry weight of leaves: The fresh weight and dry weight of leaves were recorded significantly maximum when the cutting were treated with NAA 5000 ppm + GA_3 2000 ppm exhibiting 5.13 g and 541.92 mg weight respectively being significantly greater than rest of treatments. It was However, followed by treatments of NAA 4000 ppm + GA_3 2000 ppm and expressing 4.64 g fresh weight and 511.06 mg dry weight of leaves. When individual treatment of NAA and IBA were compared in between NAA 5000 ppm recorded maximum i.e. 3.14 g and 345.05 mg fresh and dry weight respectively. Individual treatment of GA_3 2000 ppm also caused greater fresh and dry weight registering 3.09 g and 342.94 mg values respectively. The plants under control recorded minimum weight of both the attributes giving 2.55 g and 280.73 mg values. The maximum enhancement obtained 101.18 % and 93.04 % occurred on fresh weight and dry weight of leaves due to NAA 5000 ppm + GA_3 2000 ppm over control. The improvement is fresh and dry weight of leaves may be ascribed to the highest number of leaves in associated with increased number and length of sprouts of cutting which in turn depends on hydrolysis of reserve food materials thereby causing proper shoot and root balance. In the present investigation auxin and GA_3 promoted the biomass on the treated cuttings. Application of both the growth regulators i.e. NAA 5000 ppm and GA_3 2000 ppm fortified the top growth which could have supplied photosynthates and metabolites causing longer and thicker roots which obviously expressed greater fresh and dry weight of leaves. The findings are in agreement with the reports of Chauhan and Maheshwari (1970) in peach and Hakim *et al.,* (2018) in pomegranate.

Table 2: Effect of IBA and NAA with or without GA_3 treatment on leaves parameters of pomegranate stem cuttings of pomegranate on the pooled analysis.

Treatments		Number of leaves per cutting	Length of leaves (cm)	Width of leaves (cm)	Fresh weight of leaves (g)	Dry weight of leaves (mg)
T_1	Control (Water spray)	23.48	4.46	1.22	2.55	280.73
T_2	IBA 3000 ppm	24.13	4.57	1.29	2.59	286.96
T_3	IBA 4000 ppm	25.95	4.77	1.39	2.80	308.06
T_4	IBA 5000 ppm	25.55	4.74	1.33	2.76	304.23
T_5	IBA 6000 ppm	24.64	4.72	1.31	2.69	296.19
T_6	NAA 3000 ppm	26.03	4.96	1.48	2.84	312.46
T_7	NAA 4000 ppm	27.45	5.06	1.52	3.00	329.54
T_8	NAA 5000 ppm	28.29	5.13	1.57	3.14	345.05
T_9	NAA 6000 ppm	26.35	5.08	1.53	2.98	327.72
T_{10}	GA_3 2000 ppm	29.02	5.05	1.51	3.09	342.94
T_{11}	IBA 3000 ppm + GA_3 2000 ppm	30.82	5.15	1.59	3.26	358.34
T_{12}	IBA 4000 ppm + GA_3 2000 ppm	33.98	5.26	1.62	4.05	344.45
T_{13}	IBA 5000 ppm + GA_3 2000 ppm	36.07	5.23	1.56	3.97	432.40
T_{14}	IBA 6000 ppm + GA_3 2000 ppm	34.67	5.20	1.53	3.94	435.39
T_{15}	NAA 3000 ppm + GA_3 2000 ppm	35.02	5.79	1.68	4.18	461.89
T_{16}	NAA 4000 ppm + GA_3 2000 ppm	38.94	6.08	1.74	4.64	511.06
T_{17}	NAA 5000 ppm + GA_3 2000 ppm	42.99	6.21	1.80	5.13	541.92
T_{18}	NAA 6000 ppm + GA_3 2000 ppm	38.23	6.12	1.72	4.61	507.21
	C.D. at 5%	38.23	0.39	0.08	0.21	10.05

CONCLUSION

It can be concluded that genuine healthy plants of pomegranate may be regenerated by hard wood stem cuttings treated with NAA 5000 ppm + GA_3 2000 ppm as it was obtained by higher biomass produced. However, the cuttings treated with IBA 4000 ppm + GA_3 2000 ppm exhibited better rooting performance. It is therefore suggested that systematic trials should be conducted is future using NAA 5000 ppm + IBA 4000 ppm + GA_3 2000 ppm. So that the beneficial role of all the three growth regulators may be ascertained in rooting and shooting attributes together.

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Conflict of Interest. Nil.

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