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Efficacy of different levels of IBA and NAA on rooting of Phalsa (*Grewia asiatica* L.) cuttings

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Abstract
Vegetative propagation of Phalsa (*Grewia asiatica* L.) through cutting is the most convenient and the cheapest method to obtain true to the type fully developed plants in considerably lesser time. The effect of various concentrations of IBA and NAA on the rooting and shooting of hardwood cutting of Phalsa was assessed in an experiment conducted at the AICRP on tropical fruit crops, Mondouri, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia (West Bengal), India. The experiment was laid out in Completely Randomized Design (CRD) with three replications. Maximum rooting (70.55%), root number (40.85) and root length (27.47 cm) is observed that hard wood cutting treated with IBA 200 ppm by dipping in prepared solution for 24 hours. Field survival of the plants develop through hard wood cuttings treated with IBA 200 ppm (37.82%) followed by NAA 200 ppm (30.47%) was found superior over all the treatments.

Keywords: IBA, NAA, phalsa, rooting, survival

Introduction
Phalsa (*Grewia asiatica* L.), also known as "Star Apple", belonging to family Tiliaceae, is an important small minor fruit crop of India. It is native to the Indian sub-continent and South-East Asia. The genus *Grewia* has 140 species, of which only *Grewia asiatica* L. is of commercial importance. Phalsa cultivation is the most suitable for marginal lands, particularly for the utilization of the wastelands in arid and semi-arid regions of the world. It is a small bush and hardy in nature and preferred for dry land horticulture and silvi-horticulture. It is a hardy fruit crop and can withstand diversity of soil and climatic conditions, where some fruit crops cannot be grown successfully (Pujari, 2012) [14]. It is basically a small statured fruit crop but it is also used as a folklore medicine. Singh *et al*., (2015) [19] reported that unripe Phalsa fruits alleviates inflammation and is administered in respiratory, cardiac and blood disorders, as well as in fever reduction. Ripe fruits of Phalsa are consumed fresh, as desserts, or processed into refreshing fruit and soft drinks enjoyed in India during hot summer months as it has cooling tonic and aphrodisiac effects which overcomes thirst and sensation as well as they are rich source of vitamin A and C with fair amount of minerals major being Phosphorus and Iron. The bark is used as a soap substitute in Burma. The leaves are believed to have antibiotic properties hence, applied on skin eruptions and they are known to have antibiotic action (Singh and Tomar, 2015) [22]. Phalsa is commercially propagated by seeds in India. But the seed lose viability greatly within three months, when stored in ambient condition and hence, fresh seed from fully ripe fruits should only be sown. Propagation by cutting is the easiest and widely employed method which is usually followed in easy-to-root species. In cuttings, growth substances applied exogenously are found beneficial to enhance early and good root formation. Various classes of growth regulators such as auxins, cytokinins, gibberellin and ethylene influence root initiation in cuttings. Of these, auxins have greater effect on root formation in cuttings. In addition to these groups, various growth retardants and promoters may have less direct part in adventitious root formation. IBA is used for inducing the root formation in cuttings of woody plants. Roots induced by IBA showed a highly increased number of vascular strands in relation to its concentration. The use of NAA stimulated the development of more fertile branches (Singh and Bahadur, 2015) [18]. Hence, use of growth regulators would help for rapid multiplication in propagating Phalsa cuttings. As little work has been done pertaining to rooting in Phalsa cuttings, this experiment was carried out with the objective to standardize the growth regulator treatment for better rooting.

Materials and Methods

Study Area

The experiment was conducted at polyhouse of ICAR-AICRP on Fruit, Mondouri, Bidhan Chandra Krishi Viswavidyalaya, Mohanpur, Nadia (West Bengal), during monsoon in the year 2016.

Preparation of stem cuttings

Healthy and uniform hardwood cuttings were obtained from 4-5 year old plants. From the selected branches, 25 cm long having 4 to 5 nodes and 1.0-1.2 cm thick cuttings were taken from hardwood portion of the branches. Cuttings were dipped in fungicide solution for 2-3 minutes and subsequently washed in distilled water and kept in shade for 10 minutes before giving hormonal treatment. After that cuttings were briefly dipped in the hormonal solution and were planted in polythene bags filled with substrate (Sand: Soil: FYM @ 1:2:1). In this study, there were four levels of IBA and NAA treatments (T_1- IBA 100 ppm, T_2- IBA 200 ppm, T_3- IBA 300 ppm, T_4- IBA 400 ppm, T_5- NAA 100 ppm, T_6- NAA 200 ppm, T_7- NAA 300 ppm, T_8- NAA 400 ppm and T_9- Control) with three replications and each replication consisted of forty cuttings. The polybags were then kept in the polyhouse and watered regularly.

Observation recorded

Observations were recorded daily up to 60 days after planting (DAP). The observation recorded were –

Rooting success percent

It was calculated by using this formula

$$\frac{\text{Total number of cuttings success}}{\text{Total number of cuttings planted in all replicates}} \text{ X } 100\ (\%)$$

Days taken for sprouting

The treated cuttings were observed daily under each treatment for its sprouting. The number of days required for first sprouting was recorded

Number of leaves

The number of leaves in each cutting on the 60th day after planting in the polybag was recorded

Leaf area (cm^2)

The total leaf area in each cutting on 60th day after planting was measured by graphical method and expressed in centimeter square.

Number of roots

The total number of adventitious roots per cutting was counted under each treatment on 60th day after planting.

Root length (cm)

The total length of the longest root per cutting in each treatment was recorded by vernier calipers on 60th day after planting.

Fresh weight of the roots and shoots (g)

The total fresh weight of the roots and shoots per cutting was taken immediately after removal from the polybag in each treatment by destructive method. The weight was measured with the help of an electronic balance and expressed in grams on 60th day after planting.

Number of cuttings rooted

Total Number of cuttings

Dry weight of the roots and shoots (g)

The total roots and shoots of each sample of the treatment was collected and they are oven dried at a temperature of 55^{0}C and then the dry weight was recorded with the help of an electronic balance and expressed in grams on 60th day after planting.

Root to shoot ratio

The root to shoot ratio was calculated from the weight of the roots to weight of the shoot on dry matter basis and expressed in ratio by using the following formulae.

$$\text{Root/Shoot ratio} = \frac{\text{Dry matter weight of the root (g)}}{\text{Dry matter weight of the shoot (g)}}$$

Survival Percentage (%)

The total number of rooted cuttings survived under each treatment was recorded at the end of experimental period and survival percentage of rooted cuttings to that of diseased cuttings was calculated and expressed in percentage on 60th day after planting.

Experimental design and statistical analysis

The experiment was laid out in Completely Randomized Design (CRD) with 9 treatments and 3 replications. Analysis of variance (one way classified data) for each parameter was performed using op stat software (online version). The statistical analysis was done by following Completely Randomized Design (CRD) as per Gomez and Gomez (1983) [6]. The significance of different sources of variation was tested by error mean square by Fischer-Snedecor's 'F' test at probability level of 0.05 percent.

Results and Discussions

Rooting Success percent

A perusal of Table 1 indicated that the rooting success percentage was influenced significantly by
IBA and NAA treatment. The highest percentage (70.55%) was recorded under T_2 (200 ppm of IBA) followed by T_6 (200 ppm of NAA) (65.79%) and the minimum survival percentage of cutting (20.83%) observed under T_9 (Control). The reason might be that application of auxins has been found to stimulate cambial activity thereby resulting the mobilization of reserve food material to the site of root initiation as stated by Gurumurthy *et al.* (1984) [7]. Natural and synthetic auxins when applied exogenously to the stem cuttings generally increase the development of pre-existing root primordial increases the more number of roots per cutting which further helps in sprouting and growth as reported by Haissing (1974) [8]. The enhanced hydrolytic activity in presence of applied IBA might be responsible for the increased percentage of rooted cuttings. High carbohydrate and low nitrogen have been reported to favour root formation (Singh and Tomar, 2015) [22].

Days taken for sprouting

Results (Table 1) represent that days taken for sprouting of cutting of *Grewia asiatica* L. ranged from 9.79 to 29.74 DAP. Minimum duration (9.79 DAP) was obtained in T_2 followed by T_6 (12.55 DAP) and maximum duration (29.74 DAP) was obtained in T_9 treatment. The data on days taken for sprouting was statistically significant under all the treatments. This might be due to better utilization of stored carbohydrates; nitrogen in the hardwood with the auxin application enhanced the auxin concentration in the cell and increased the cell division which results on quick callus formation in the cutting as stated by Chauhan *et al.* (1971) [4] in plum. Similar results were reported by Patil *et al.* (2000) [13] in grape and Chandramouli (2001) [3] in stevia.

Number of Leaves

Data regarding number of leaves are presented in table 1, which states that number of leaves was significantly affected by IBA and NAA concentrations. The average number of leaves (9.23) was highest under T_2 (200 ppm IBA) treatment followed by T_6 (200 ppm NAA) treatment (8.12). The average number of leaves on new sprout per cutting (1.92) was lowest under T_9 (Control) treatment. Favourable climatic conditions play an important role to increase the number of leaves. The application of IBA and NAA might have played some role in augmenting the number of leaves per cutting (Singh and Singh, 2002) [20]. These findings are agreed with the findings of Singh *et al*. (2015) [18].

Leaf Area

Results (Table 1) represent that leaf area ranged from 9.15 to 14.46 cm^2. Maximum leaf area (65.87%) was obtained in T_2 followed by T_6 (13.76 cm^2) and minimum success (9.15 cm^2) was obtained in T_9 treatment. The relative humidity and optimum light intensity which are the important factors in the development of leaf coupled with auxin content activated the synthesis of more carbohydrates in the leaves which might have resulted in elongation of leaves through cell division and cell elongation that resulted in more leaf area per cutting when compared to all other treatments. The results are in agreement with the findings of Baghel and Saraswat (1989) [1] in pomegranate.

Table 1: Effect of IBA and NAA on Phalsa cuttings

Treatments	Rooting success (%)	Days taken for sprouting	Number of leaves	Leaf area (cm^2)
T_1 (IBA 100 ppm)	60.11 (50.81)	14.25	7.00	13.01
T_2 (IBA 200 ppm)	70.55(57.11)	9.79	9.23	14.46
T_3 (IBA 300 ppm)	48.69(44.23)	19.08	5.07	11.72
T_4 (IBA 400 ppm)	54.22(47.40)	16.72	3.11	10.48
T_5 (NAA 100 ppm)	35.13(36.33)	24.17	6.13	12.53
T_6 (NAA 200 ppm)	65.79(54.18)	12.55	8.12	13.76
T_7 (NAA 300 ppm)	40.14(39.30)	21.93	4.36	11.01
T_8 (NAA 400 ppm)	28.06(31.97)	26.52	2.22	9.85
T_9 (Control)	20.83(27.14)	29.74	1.92	9.15
S.E(m) ±	0.022	0.028	0.032	0.029
C.D	0.064	0.083	0.096	0.087

Number of Roots

The effect of auxins on the number of roots that are observed in the hardwood cuttings treated with IBA 200 ppm concentration (40.85) followed by NAA 200 ppm (38.12) concentration performed better over all other treatments (Table- 2). This might be due to enhanced hydrolysis of carbohydrates caused by auxin treatment. Rajarama (1997) [16] and Krishnamurthy (1981) [11] opined that auxins would bring about various physiological changes, but the mechanism by which these changes are brought about this is not fully understood except for the effect on cell elongation. The more number of roots per cutting under optimum concentration of IBA may be attributed to the increased rate of respiration, accumulation of higher level of amino acids at their bases in the auxin treated cuttings than untreated cuttings. Similarly, nitrogenous substances accumulating in the basal part of treated cuttings, apparently which are mobilized in the upper part and translocated as aspargine as reported by Strydom and Hartmann (1960) [23] might caused the more number of roots under IBA treated cuttings.

Root Length

The effect of auxin on the length root observed in the hardwood cutting treated with IBA 200 ppm concentration recorded highest (27.47 cm) compared to cuttings raised under control (6.08 cm) (Table-2). Jadhav (2007) [10] recorded increased root length in IBA treated cuttings in phalsa. This might be attributed that the action of auxin activity which might caused hydrolysis and translocation of carbohydrates and nitrogenous substances in the cellular level at the base of cuttings and resulted in accelerated cell elongation and cell division under favourable environmental condition as reported by Singh *et al.* (2003) [21].

Fresh Root Weight

In the hardwood cutting treated with IBA at 200 ppm (3.37 g) and NAA 200 ppm (3.06 g) concentration over control (0.58 g) recorded maximum fresh weight of the roots. The fresh weight of the root is directly proportional to number of roots in each cutting. The increase in number of roots per cutting might have directly influenced the fresh weight of the roots as reported by Milind (2008) [12] in stevia and Singh and Tomar (2015) [22] in Phalsa.

Dry Root Weight

The dry weight of the roots per cutting was maximum in hardwood cuttings on the application of auxin with IBA at 200 ppm (0.98 g) followed by NAA 200 ppm (0.87 g) concentration. The higher dry weight of the roots might be attributed to higher root length which accumulates more stored carbohydrates and more number of roots increased their volume per cutting of the roots in hardwood cuttings were observed than semi hardwood cuttings as stated by Hartman (1990) [9]. Similar results were also reported by Singh and Tomar (2015) [22] in Phalsa, Camellia *et al.* (2009) [2] in *Jatropha curcas* and Singh *et al*. (2015) [19] in phalsa.

Table 2: Effect of IBA and NAA on Phalsa cuttings

Treatments	Number of roots	Root length (cm)	Fresh root weight (g)	Dry root weight (g)
T_1 (IBA 100 ppm)	35.25	20.10	2.85	0.85
T_2 (IBA 200 ppm)	40.85	27.47	3.37	0.98
T_3 (IBA 300 ppm)	31.07	15.23	2.32	0.68
T_4 (IBA 400 ppm)	26.35	10.16	1.86	0.44
T_5 (NAA 100 ppm)	33.47	17.46	2.55	0.75
T_6 (NAA 200 ppm)	38.12	24.85	3.06	0.87
T_7 (NAA 300 ppm)	29.12	12.08	2.07	0.55
T_8 (NAA 400 ppm)	21.48	8.46	1.47	0.35
T_9 (Control)	17.24	6.08	0.58	0.20
S.E(m) ±	0.028	0.031	0.025	0.023
C.D	0.085	0.092	0.076	0.069

Fresh and dry shoot weight

The effect of auxins on fresh (18.75 g) and dry weight of the shoot (9.98 g) in the hardwood cuttings treated with IBA at 200 ppm followed by NAA 200 ppm (17.58 g and 8.75 g) recorded maximum. This may be due to the fact that hardwood cuttings produced more number of sprouts, leaves, increased leaf area, leaf chlorophyll content, more starch, total sugar and C/N ratio which resulted in maximum fresh and dry weights of the shoot as stated by Purohit and Shekarappa (1985) in pomegranate. The similar results were also reported by Shukla and Bist (1994) [17] in pear, Singh and Tomar (2015) [22] in Phalsa and Singh *et al*. (2015) [19] in phalsa.

Root shoot ratio

A perusal of Table 3 indicated that the root shoot ratio was influenced significantly by IBA and NAA treatment. The highest ratio (0.10) was recorded under T_2 (200 ppm of IBA) and T_6 (200 ppm of NAA) and the minimum value (0.05) observed under T_9 (Control).

Survival Percentage

In the present study it is noted that the treatment of hardwood cuttings with IBA at 200 ppm concentration gave the maximum survival percentage (37.82%) among all other treatments. This might be due to increased length, maximum number of primary roots and early sprouting resulted in more thickness of the roots, perhaps the ability of regenerating further new fibrous roots from main roots, which probably absorb more nutrients and water from the soil under low transpirational losses. Similar results were also reported by Singh and Bahadur (2015) [18] and Singh *et al.* (2015) [19] in phalsa. The effect of auxins might be due to the slow translocation property or slow destruction of auxins by auxin destroying enzyme system as reported by Debnath and Maiti (1990) [5] and also by the early formation of roots and more utilization of reserved food materials of the treated cuttings.

Table 3: Effect of IBA and NAA on Phalsa cuttings

Treatments	Fresh shoot weight (g)	Dry shoot weight (g)	Root shoot ratio	Survival (%)
T_1 (IBA 100 ppm)	16.33	8.22	0.10	25.76 (30.49)
T_2 (IBA 200 ppm)	18.75	9.98	0.10	37.82(37.94)
T_3 (IBA 300 ppm)	14.08	7.08	0.10	16.71(24.12)
T_4 (IBA 400 ppm)	12.35	6.11	0.07	7.25(15.61)
T_5 (NAA 100 ppm)	15.13	7.84	0.10	21.63(27.70)
T_6 (NAA 200 ppm)	17.58	8.75	0.10	30.47(33.49)
T_7 (NAA 300 ppm)	13.28	6.68	0.08	10.18(18.60)
T_8 (NAA 400 ppm)	11.09	5.30	0.07	5.32(13.33)
T_9 (Control)	10.08	4.05	0.05	2.15(8.42)
S.E(m) ±	0.030	0.024	0.004	0.033
C.D	0.090	0.071	0.011	0.100

Conclusion

From the above discussion, it may be concluded that various levels of IBA and NAA had a large impact on the success, survival and rooting in cuttings of Phalsa (*Grewia asiatica* L.) within a short period of time. IBA @ 200 ppm followed by NAA 200 ppm were found to be the best treatments may be recommended for the commercial vegetative propagation of Phalsa by stem cuttings.

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