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IMPROVING ROOTING ABILITY AND SURVIVAL PERCENTAGE OF IBA TREATED GUAVA SOFTWOOD CUTTINGS IN DIFFERENT SEASONS

Malik Mohsin Abbas [1], Qamar Shahzad Anjum * [2], Abdul Aziz [3], Muhammad Maaz Aziz [4], Hira Faiz [5] and Muhammad Ikhlaq Khan [6]

[1,6] Principal scientist,
[2,5] Scientific Officer,
[3] Chief Scientist/Director,
[4] Senior Scientist, Horticultural Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan

*Corresponding author's email: qamar.aari@gmail.com

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ABSTRACT

Present study was carried out to findout the best planting time (February, March, August & September) for survival and root induction of indole butyric acid (IBA) treated (@ 0.2%, 0.4%, 0.6 %) guava softwood cuttings (GSWCs) during 2018-19 to 2020-21 . Study was conducted at Horticultural Research Institute, Ayub Agricultural Research Institute, Faisalabad, Pakistan. Guave cuttings (15 cm long) having 4-5 nodes with healthy leaves were taken from 5-10 years old plant (cv. *Gola*). Cuttings were dipped in IBA solutions for five minutes, while untreated cuttings served as control. Cuttings were planted in raised beds (3ft x 4ft x 0.75ft) in low tunnel (5ft x 6ft x 3ft) covered with polythene sheet , maintaining inside relative humidity (80-85%) and temperature (25-28°C). Treatment of cuttings with 0.4% IBA and plantation during September showed highest survival percentage (57.3%), branch length (13.8 cm) and number of leaves per plant (25.8) at 60 days. Highest number of roots per plant (26.0) recorded at transplantation stage (90 days from planting) were also found for same treatment. Minimum values for success percentage (5.3%), increase in branch length (2.8 cm), number of leaves per plant (4.0) and number of roots (8.5) were recorded for control during March. Overall, treatment of guava softwood cuttings with IBA improved survival percentage as compared to control for both seasons. The study validated the potential of clonal propagation of guava through softwood cuttings to preserve genetic purity and productive traits.

KEYWORDS: *Psidium guajava;* softwood cuttings; clonal propagation; IBA; Pakistan

INTRODUCTION

Guava (*Psidium guajava* L.) the "poor man's fruit" or "apple of the tropics" is classified as an evergreen shrub or small tree belonging to *Myrtaceae* family with native origin of Caribbean, Central America and South America region . It is considered of hardy nature and well adapted to climatic conditions of tropical and subtropical areas. Although it can be grown up to 1500 meters altitude above sea level and can sustains healthy growth upto 10°C; however, guava plants are susceptible to frost damage. Guava plant bears fruits twice every year in Pakistan (June-August and October-March). It starts bearing fruit after 2-3 years of plantation in field, however commercial production (60-100 kg per plant) commences after about 8-10 years. Plant can bear heavily for 15-25 years and subsequently yield potential decreases; however yield and quality of fruit depends upon varietal traits and orchard management practices (Anon, 2020). Multiple bioactive metabolites (vitamin C, quercetin, guaijaverin, catechin & naringenin etc.) are present in fruit, which increase its pharmacological importance in addition to nutritional value. Guava fruit contains ample quality of carbohydrates (9.1-17 mg), crude fiber (0.9-1.0 g), calcium (17.8-30 mg) and thiamine (0.03-0.04 mg) per 100g of fruit (Uzzaman *et al.,* 2018).

Pakistan is ranked as 4th country on world level with reference to guava production after India, China and Thailand. Regarding total guava production in the country, share of Punjab province is 81.8% (4,47,890 tonnes), Sindh 9.4% (51,451 tonnes), Khyber Pukhtunkhwa 8.3% (45,503 tonnes) and Baluchistan 0.5% (2,702 tonnes). Similarly, out of total area under guava cultivation in Pakistan, 83.1% (46,650 hectares) lies in Punjab, 9.6% (5,394) in Sindh, 6.4% (3,594 hectares) in Khyber Pakhtunkhwa and only 0.9% (503 hectares) in Baluchistan (Anon, 2016; GoP, 2020). In Punjab, areas of Sharqpur, Sheikhupura, Kasur, Lahore, Sahiwal, Faisalabad and Gujranwala, while Shikarpur, Dadu, Larkana and Hyderabad in Sindh and Mardan,

Kohat and Hazara in Khyber Pukhtunkhwa are major growing areas of this fruit. Need for mass multiplication of uniform planting material with desirable attributes *i.e.,* high yield, good fruit quality, disease resistance and enhanced shelf life with nutritional enrichment has increased to meet the increasing demand of guava (GoP, 2020).

Commercially, guava plants are multiplied through seeds in Pakistan and clonal multiplication by cuttings, layering or grafting is limited to research organizations only rather than commercial nurseries in private sector. Due to open pollination, maximum of 35% out-crossing has been reported in guava which results in considerable variation of desirable characteristics of daughter plants raised from seeds (Nakasone and Paul, 1998). Additionally, seedling propagation results in segregation and recombination of productive traits which mostly results in decrease of potential yield and desired quality (Ali *et al*., 2003; Kanupriya *et al*., 2011). Clonal propagation through cuttings is comparatively easy yet development of root system after plantation of cuttings is dependent on multiple factors *i.e.,* media formulation, age and tenderness of planting material, termite attack, fungal infestation and micro-climatic conditions during initial growth stages etc .

Indole butyric acid (IBA) being growth stimulator has gained much importance to facilitate root initiation of cuttings. IBA treatment of cuttings increases endogenous level of carbohydrates (45%) and total phenolics (100%) in plants which results in growth enhancement (Abdel-Rahman *et al*., 2020). Planting time is also an important factor determining survival and success of growth initiation of planted cuttings. Therefore, this study was designed to findout the best planting time and IBA concentration for improving success percentage of guava softwood cuttings.

MATERIALS AND METHODS

Guava soft wood cuttings of commercial variety "*Gola*" were prepared from 5-10 years old plants from Experimental Progeny Garden, Horticultural Research Institue, Ayub Agricultural Research Institute, Faisalabad, Pakistan during 2018-19 to 2020-21 (31.4504° N& 73.1350° E). Cuttings (15 cm long) having 4-5 nodes were taken from healthy branches with no apparent disease symptoms. All leaves of cutting were clipped except two apical leaves. Initially, quick dipping was given to all cuttings with copper oxychloride solution to avoid fungal attack during growth. Then cuttings were dipped in 0.2% (2000 ppm), 0.4% (4000 ppm) and 0.6% (6000 ppm) IBA solutions for five minutes, while untreated cuttings were kept as control. Cuttings were planted in raised beds (3ft x 4ft x 0.75ft) at 5cm x 5cm distance (plant-plant/ row-row distance). Media used for preparation of beds was mixture of sand, silt and farm yard manure (2:2:1). Three fourth portion of cuttings was buried in soil facing slanting cut upward. Low tunnel (5ft x 6ft x 3ft) was fixed on bed and completely covered with polythene sheet for first ten days maintaining inside relative humidity (80-85%) and temperature (25-28°C); however later on, polythene sheet was often removed according to micro-climate conditions inside tunnel. Sprinkler irrigation was applied according to need of soil. Experiment was repeated during February, March, August and September to find the most suitable time for plantation. Research was conducted during 2018-19, repeated for next two years *i.e.,* 2019-20 and 2020-21 and data presented here were mean value of three years. The experiment was laid out according to Randomized Complete Block Design with two factor factorial arrangement (factor-1: IBA concentrations & factor-2: time of plantation). In each month, total 320 cuttings were planted as 20 cuttings per treatment and replicated four times. The data for success percentage (%), branch length (cm) and number of leaves per plant were recorded after 60 days of plantation. After 90 days, sprouted cuttings were uprooted gently and transplanted in polythene bags (6 inches x 9 inches) filled with mixture of soil and silt (1:1). Number of roots per plant was counted at this stage. Collected data were analyzed statistically by using software STATISTIX 8.1. Analysis of Variance Technique (Steel *et al*., 1997) was used to findout the significance of data at P value ≤0.05, while Tuckey's test was applied for comparison of treatment means.

RESULTS AND DISCUSSION

Success percentage (%)

Interaction of IBA concentrations and planting months for success percentage was statistically significant. Minimum success percentage (5.3%) was recorded for untreated (control) guava cuttings when planted during March (Fig. 1). Pre-planting treatment of guava cuttings with different concentrations of IBA improved their post-planting survival rate during the months under study. Maximum success percentage (57.3%) for survival of planted cuttings was recorded in response to treatment with 0.4% IBA when planted during September followed by the results of cuttings treated with same concentration while transplanted during August (45.3%), February (30.3%) and March (30%). Results of improved success percentage and root initiation of guava cuttings are well aligned with the findings of Ibironke (2016), who observed better adventitious

root formation from IBA treated bougainvillea cuttings. These results are also in accordance with Elhaak *et al*. (2015) and Chauhan *et al.* (1992) who recorded considerable improvement in rooting ability of rosemary cuttings with reduced transplanting shock. According to the findings of Henrique *et al*. (2006), treatment of pinus cuttings with IBA 4000ppm concentration (0.4%) demonstrated highest rooting percentage and root initiation. Similar findings for positive effect of IBA treatment have also been reported by Tolera (2016) during micro propagation of sugarcane. Fathi *et al*. (2017) found highest rooting percentage (78%) of bougainvillea cuttings treated with 4000ppm (0.4%) IBA. Better root initiation of guava cuttings in response to IBA treatment could be ascribed to possible conversion of total sugars to reducing sugars (Henrique *et al*., 2006), increased accumulation of total free phenols and total soluble sugars (Akakpo *et al*., 2014). Root initiation period was also decreased for cuttings treated with different concentrations of IBA that was possibly due to better growth and dry matter accumulation (Chowdhuri, 2017; Parmar *et al*., 2016). Kaur (2015) found 4000ppm (0.4%) IBA as best concentration for treatment of peach (*Prunus persica* L.) cuttings in relation to reduction in sprouting time. Similar results for improving sprouting success and root initiation had been reported by Patel *et al*. (2017) for fig cuttings treated with 4000 ppm (0.4%) IBA.

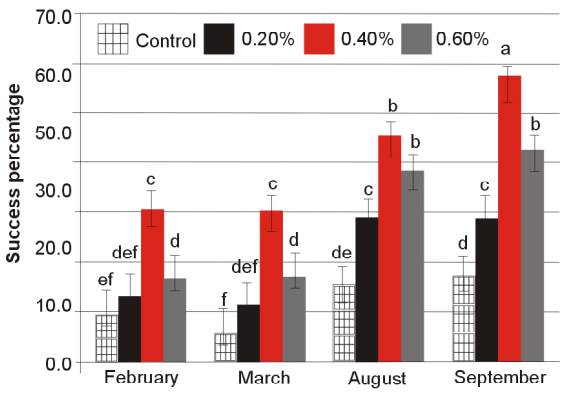

Fig. 1. Effect of IBA on success percentage (%) of guava softwood cuttings

Branch length after 60 days (cm)

Statistical analysis of data for branch length showed significant interactive effect for IBA concentrations and planting time. Results demonstrated that untreated cuttings produced least branch length (2.8 cm) when planted in the month of March (Fig. 2). Untreated cuttings planted in August produced 5.5 cm, September produced 4.8 cm and in February produced 2.9 cm branch length. Treatment of cuttings with 0.4% IBA showed best results for aforementioned months. Maximum length of main branch (13.8 cm) was found of cuttings planted in September treated with 0.4% IBA. Furthermore, maximum branch length of guava cuttings planted in August, February and March was 12.5 cm, 10.2 cm and 5.82 cm, respectively in response to this IBA treatment. Better growth of guava softwood cuttings in response to IBA treatment is also pertinent to improvement in callus formation and tissue differentiation of vascular tissues (Kaur and Kaur, 2016), enhanced transport of carbohydrates within plant body which increased branch length. Earlier findings of Abdel-Rahman *et al*. (2020) also strengthen our results, who reported almost double increase in branch length of IBA treated conocarpus cuttings as compared to untreated cuttings. This increased growth is related to better accumulation of carbohydrates, total phenolics, IAA and GA after IBA treatment (Abdel-Rahman *et al*., 2020; Abdul-Hafeez, 2020). This result ties well with previous study wherein Karim and Muhammad (2020) found significant increase in stem length of IBA treated pomegranate cuttings.

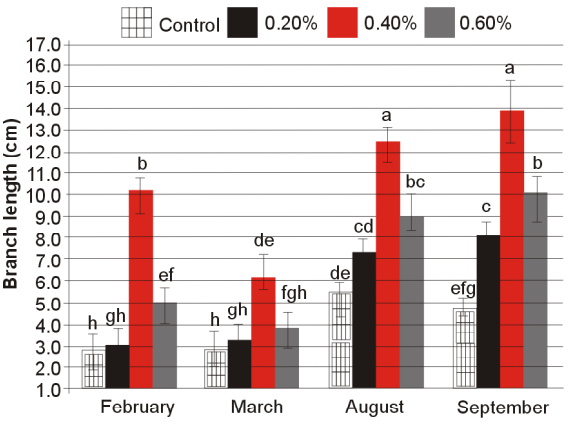

Fig. 2. Effect of IBA on length (after 60 days) of guava softwood cuttings

Number of leaves after 60 days

Statistical analysis of data recorded for number of leaves per plant showed significant interaction of both factors under study. Guava cuttings from control treatment produced minimum number of leaves as compared to IBA treated cuttings (Fig. 3). Minimum number of leaves (4.0) was produced by untreated cuttings planted in March. Whereas, untreated guava cuttings planted in August, September and February produced 10.8, 11.8 and 4.8 leaves per plant, respectively, recorded after 60 days from planting. Guava cuttings planted in September produced maximum number of leaves (25.8) recorded in response to 0.4% IBA treatment. Similarly, treatment with 0.4% IBA also produced greater number of leaves per plant for August (23.5),

February (9.5) and March (8.0) plantation. These results are in line with findings of Abdel-Rahman *et al*. (2020), who reported almost four times increase in number of leaves of IBA treated conocarpus cuttings than untreated cuttings. A similar pattern of results was also obtained by Karim and Muhammad (2020) for pomegranate cuttings treated with IBA. Better vegetative growth of IBA treated cuttings might have resulted from hydrolysis of bioactive compounds, IAA biosynthesis, nitric oxide signaling and robust meristematic activity, which provided necessary energy for growth initiation (Al-Barazi and Schwabe, 1982; Elhaak *et al*., 2015; Fattorini *et al*., 2017) of guava cuttings.

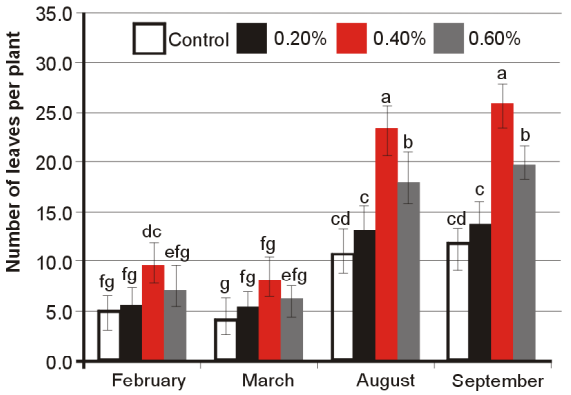

Fig. 3. Effect of IBA on number of leaves per plant (after 60 days)

Number of roots after 90 days

Statistically significant interaction of IBA concentrations and month of planting was recorded for number of roots per plant. It was evident that number of roots developed by untreated cuttings during 90 days was minimum (8.5) planted during March than produced by cuttings with IBA treatment (Fig. 4). Untreated cuttings planted in August, September and February developed average number of roots per cutting as 12.8, 13.5 and 9.8 respectively. Maximum number of roots per plant (26.0) was produced in response to treatment with 0.4% IBA for September plantation followed by March (24.8), August (24.5) and February plantation (19.3). Increased root number, root length and root diameter might have resulted in better later growth of guava plants as earlier reported in pomegranate (Kaur and Kaur, 2016). Biochemical changes in plant metabolic machinery with manipulation of source sink relationship, translocation of metabolites, peroxidase enzymatic activity, IAA-oxidase activity, polyphenol oxidase activity and total phenolic contents accumulation can also be ascribed to better root formation mechanism (Dash *et al*., 2011). Number of roots increased due to possible accumulation of soluble sugars and proteins translocated from upper parts of plant (Shao *et al*., 2018). These results are in line with reported by Abdel-Rahman *et al*., (2020), who found 2-3 times increased root number in IBA treated conocarpus cuttings. Intercellular conversion of IBA to IAA caters energy requirement of growing tissues (Li *et al*., 2016) at apex points, thereby number of roots had been improved in this study with possible up-regulation of auxin signaling, cellular influx and efflux with involvement of coding protein synthesis (Wei *et al*., 2014; Li *et al*., 2016). Similarly at molecular level, up-regulation of genes involved in adventitious root formation *i.e.,* auxin-inducible *GH3* (*GRETCHEN HAGEN 3*) family, members of the auxin-inducible *ARF* (*AUXIN RESPONSE FACTOR*) family and tryptophan-dependent IAA biosynthesis gene *YUCCA* etc. might have played role for root growth with better nutrient absorption from soil as observed under this study and is validated by earlier study of Li *et al*. (2016) for *Arabidopsis thaliana*.

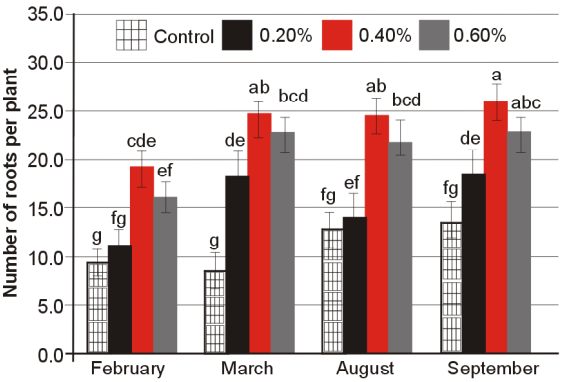

Fig. 4. Effect of IBA on number of roots per plant (after 90 days)

CONCLUSION AND RECOMMENDATION

Guava softwood cuttings treated with 0.4% IBA (4000ppm) and planted during September showed maximum improvement in survival percentage (5.3% to 57.3%), branch length (2.8 cm to 13.8 cm), number of leaves per plant (4.0 to 25.8) and number of roots per plant (8.5 to 26.0) as compared to untreated cuttings. Therefore, guava softwood cuttings dipped into 0.4% Indole Butyric Acid (IBA) solution for five minutes is recommended better sprouting success.

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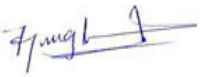
CONTRIBUTION OF AUTHORS

Sr. No.	Author's name	Contribution	Signature
1.	Malik Mohsin Abbas	Conducted the research and collected the data	
2.	Qamar Shahzad Anjum	Helped in data collection	
3.	Abdul Aziz	Reviewed the manuscript	
4.	Muhammad Maaz Aziz	Analysed the data statistically	
5.	Hira Faiz	Collected the literature	
6.	Muhammad Ikhlaq Khan	Helped in results and discussion write-up