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# EFFECT OF ANTIOXIDANTS TO CONTROL BROWNING IN GUAVA SOFTWOOD CUTTINGS

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## ABSTRACT

Guava is the Apple of tropics and becoming the popular fruit of Pakistan. Guava is a nutraceutical fruit enriched with important minerals and vitamins like ascorbic acid. To overcome upon the issue of brown spots on fruit rind of guava soft wood cuttings, a research trial is executed. /the experiment was planned to overcome the problem of brown spots on fruit rind in soft wood cuttings of guava. For this purpose antioxidants *i.e* Vitamin C and L-ascorbate were used as blend mixture with IBA (rooting hormone) used for the initiation of roots in cuttings. A research trial was performed by employing randomized complete block design having three replications including frour treatments. Data subjected to result interpretation revealed that there is success (92.28%) and survival percentage (42.06%) was found maximum in T3 wherease, IBA 0.4% was used in combination with 1.5% citric acid and 1.5% ascorbic acid. In case of vegetative parameters maximum plant height (24.06 cm), number of leaves (25.50), leaf area (51.43mm), number of branches (3.68) and branch length (12.69 cm) were observed in T3 as compared to other treatments. The highest values for root parameters like primary roots (21.17), secondary roots (45.94), root volume (2.26 $cm^3$) and root length (9.65 cm) were found in treatment 3 while lowest values for all treatments were observed in control.

**Keywords:** Citric acid, guava, browning, softwood cuttings, IBA

## INTRODUCTION

Psidium guajava L. is an economically significant fruit that is indigenous to tropical and subtropical zones. The guava has earned the moniker "apple of the tropics" due to its popularity. . Fruit is specie of Myrtaceae family, indigenous to the tropical areas of Central and South America and land under cultivation spread from Mexico to Peru (Samson, 1986: Singh, 2016). Psidium (genus) comprise of 150 species and most of them are fruit producing cultivars. Fruit is extensively cultivated due to its attributes i.e., winter hardy nature, xerophilous and saline resistant. (Samson, 1986). Fruit is enriched in Vitamin C (Rathore, 1976). Guava has high amount of dietary fibers, carotenoids (~250 IU/100 g), polysaccharides and some necessary micro nutrients (i.e., Fe, C and P). Fruit is rich source of oxidation inhibitors i.e., β-carotene, Vitamin A and flavonoids (Jiménez et al., 2001).. Plant organs i.e., edible produce, tree trunk bark, foliage and aerial roots are good source of nutrients and vitamins that are purely for cure of various diseases. Its extract has powerful antioxidant properties and used to treat diseases against liver and cancer. Major role of vitamins present in fruit is to boost up the body immunity. Fruit is also beneficial to cure against diseases related to malnutrition and iron deficiency and also strengthen the metabolism of brain and eye vision and is recommended to treat against obesity or for the one who is concerned with weight loss (Vijaya et al., 2020). . Scientific research reveals that antioxidants (present in fruit) play a leading role in modulating the aging process, potentially leading to improved health span and delayed onset of age-related disease (Feskanich et al., 2000; Gordon, 1996 and Halliwell, 1996). It is Pakistan's 7$^{th}$ most important fruit crop with respect to area and production (FV& Condiments stat, 2021-22). Province Punjab is leading in guava production. The crop has high potential but due to lack of registered varieties, non-uniformity in fruit size, shape and colour, Pakistan exports only 1456.295 tons which is 0.26% of total production. True to type plant production can only be achieved via a-sexual propagation techniques. In order to maintain genetic purity, quality and yield related attributes it is crucial for horticultural crops to propagate through asexual means. Vegetative techniques of propagation, comprising of air layering, stooling, budding, grafting, and root and stem cuttings they all play a crucial role in reproduction and colonal spread of genetically identical fruit crop cultivars (Rai et al., 2010). . Plant propagation technique applied theough cuttings (in Guava) has more successful ratio as compare to all other methods.. It is more practical and viable among all other methods (Kareem et al., 2013). However, guava is not an easy crop in terms of internodal cutting propagation techniques (Rahman et al., 1991). It is commercially propagated through broadcasting/seed sowing but this method has its own limitations (Gautam et al., 2010, Rai et al., 2009).. Unlike many other fruit crops, guava poses significant hurdles to propagation by adopting traditional techniques. Data analysis of conducted trial showed propagation via soft wood cuttings should be substitute technique to propagate elite fruit cultivars (Nitin et al., 2015: AKram et al., 2017 and Shahzad et al., 2019). Exudation of polyphenol extracts that refers to bioactive compounds in all botanical communities act as strong rooting inhibitors. Due to rich source of polyphenol extract compounds in fruit, the twigs and foliage are effected by browning while radicle emergence. This problem is threatening that is directly linked with Quinone formation that drains from base end of cutting that eventually leads to results in browning of concerned part ( Amin and Jaiswal, 1987; Fitch e al., 1990; Siddiqui and Farooq, 1996, 1997; Leon-de-Sierralta et al., 1997; Kumar and Tiwari, 2001; Meghwal et al., 2001; Meghwal et al., 2003; Bisen, 2004; Zamir et al., 2007 and Xiaomei and Yang, 2011).. Polyphenol oxidase (PPO) is a copper-dependent enzyme encoded by nuclear DNA, facilitating the catalytic oxidation of phenolic and quinone substrates (Vaughn et al., 1988, Kim et al., 2001). Beside this PPO plays leading role in mitosis and plant root meristem growth (Gonzalez et al., 1991, Gaspar et al., 1997), , . Also it facilitates the breakdown and decomposition of indole-3-butyric acid (Vanghn et al., 1988 and Kim et al., 2001). Problem regarding to browning of Guava soft wood cuttings can be overcome by using various anti-oxidants like citric acid and ascorbic acid at various concentrations.

Chemicals like ascorbic acid have pivotal role in controlling/inhibiting the exudation of these phenols and ultimately reduces browning (Aditti and Ernst, 1993; Abdelwahd et al., 2008). Treatment of plant cuttings fortified with oxidative stress reducing compounds cuttings with antioxidants like Vitamin Cbefore transplanting may help to inhibit polyphenol transformation and ultimately enhancing plant under stem (root) initiation (Chevre et al., 1983: Tagelsir et al., 2006: Rai et al, 2008 and El-Sharony et al., 2018). Current studies showed that cutting could be an another tool to propagate high quality attributes fruit cultivar and preserve guava genetic pool (Nitin et a., 2015: Akram et al., 2017 and Shahzad et al., 2019). Auxins are rooting hormone which are extensively used to overcome rooting difficulty in various plant species. Indole butyric acid is one of the auxins groups being employed to stimulate root growth in fruit species (Khattak et al., 2002).. (Wahab et al., 2001) reported significant increase in survival of cuttings. It also increases number of roots in guava cuttings. A research had been observed by Yasir (2018) to determine severity level of various concentrations of IBA on guava cuttings. Results showed that IBA @ 2000ppm gave higher survival rate while IBA @ 4000ppm has higher rooting percentage in stem cuttings of guava. According to Bleasdale (1984), auxins accumulation at cutting base facilitates basic root development. To overcome rooting challenges, exogenous application of synthetic auxins can be beneficial. Specifically, Reddy and Singh (1988) demonstrated that treating guava hardwood cuttings with 2500 ppm indole-3-butyric acid (IBA) solution resulted in a significant rooting percentage (87.5%) and survival rate (62.86%)

Keeping in view the role of antioxidants like ascorbic acid and citric acid in controlling browning in various fruit crops, present study was planned to evaluate its effect on guava soft wood cuttings.

## MATERIAL AND METHODS:

Current research trial was laid in duration between September 2020 – October 2021 in fruit experimental area, horticultural research institute; AARI, Faisalabad, Pakistan. One health, vigorous and disease free guava plant was selected as mother plant. Soft wood tip cuttings of 6-8 inches having 2-3 nodes and two healthy leaves were prepared and planted in raised bed under small tunnel (7×4×2.5 ft). Media composition was good soil, silt and compost in ratio of 2:2:1. After 3-4 months the sprouted cuttings were ready to transplant. Filled the polythene bags of size 6×8 inches with media having composition good soil, silt and compost in ratio of 2:1:1. Before transplanting the cuttings were treated with treatments as mentioned below;

T1= Control

T2= 0.4% IBA + 0.5% Citric acid + 0.5% Ascorbic acidT3= IBA 0.4 %+Citric acid 0.1 % +Ascorbic acid0.1 %

T4= 0.4 % IBA + 0.5 % Citric acid + Ascorbic acid 1.5 %

After treatment application the bags were placed in lath house to protect from direct heat. Rsearch trial was performed according to randomized complete block design by applying four treatments (discussed above) by replicating each thrice. Data was collected on various parameters; Success %, Survival%, Plant Height, No. of Branches, Branch Length, No of leaves, Leaf area, No of Primary roots, No of Secondary roots, Root Volume, Root Length. Success percentage was calculated after 45 days of shifting while survival percentage was calculated after 3 months (when plants were able to sale/field shifting). All other parameters were also evaluated after 90 days of shifting. Plant height and branch length was calculated in ft and cm respectively. Twigs sprouted (numbers), foliage in count (numbers), and number of primary and secondary roots was collected in number, diameter of leaf ,root elongation (cm), root system volume. Collected data was subjected to analysis of variance at 5% level of probability. Means were subjected to least significant difference test to check variability among treatment means.

**RESULTS:**

For vegetative propagation of guava, semi-hard wood cuttings are the most reliable one. Commercially most successful method for asexual propagation of guava plants is through cuttings compared to other a-sexual propagation methods. Cuttings were planted in raised bed and after 14 weeks they were ready to shift in ploy bags. Sprouted cuttings were treated with different treatments and shifted to bags filled with media. Success and survival percentage was calculated after 45 and 90 days after shifting respectively. This experiment was conducted during 2020-21 in the months of September and October and prevailing weather data (temperature and relative humidity) during this month is presented in figure. 1 .

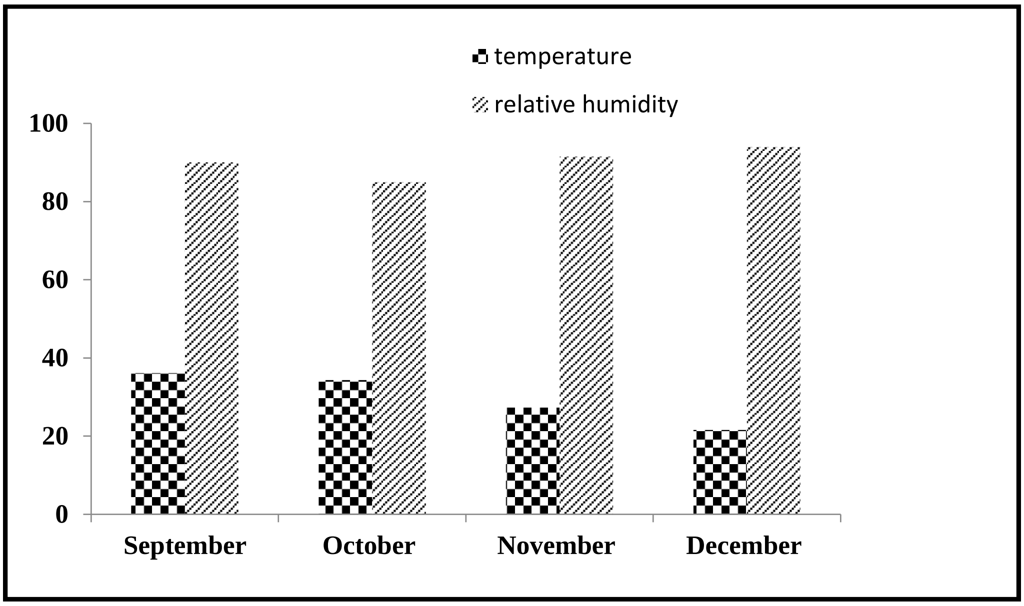


**Figure 1:** Average gprahaical representation recored in month (specific intervals) of the experimental located in Faisalabad, Punjab, Pakistan while data taken days (Sep- December) in 2020-2021 seasons

Data regarding success and survival percentage of guava cuttings analyzed statistically at 5% level of significance showed significant difference between treatment means (Table #1). It is clear from the table that combination having 0.4 % IBA + citric acid and ascorbic acid both (0.1%) and ascorbic acidshowed the maximum percentage of success (92.28%) and survival (42.06%) in guava cuttings. Lowest percentages of these parameters were obtained from T1 which is control treatment. The increased success and survival percentage in T3 is due to positive effect of antioxidants (nutritional supplements ) used with blend mixture of IBA which is a rooting hormone

**Table. 1: Effects of IBA, Citric acid, Ascorbic acid on success percentage, survival percentage and plant height of guava softwood cuttings**

| Cutting Treatment | Success % | Survival % | Plant Height (cm) |
|---|---|---|---|
| T1 | 62.43 d | 5.05 d | 10.77 d |
| T2 | 81.57 c | 32.55 b | 17.92 c |
| T3 | 92.28 a | 42.06 a | 24.06 a |
| T4 | 85.95 b | 30.03 c | 21.55 b |

**T1**=Control, **T2**= 0.4 % IBA + Citric acid and Ascorbic acid (0.5% both) **T3=** IBA (0.4%) + 0.1% Citric acid and Ascorbic acid and **T4**= IBA 0.4 % + Citric Acid 1.5 % +Ascorbic Acid 1.5 %. Anova lettering (values ) obtained by same word (s) in same column are non-significant (P>0.05%) via using least significant difference Test.

In case of vegetative growth parameters of guava soft wood cuttings plant height (24.06 cm), number of leaves (25.50), leaf area (51.43 mm), number of branches (3.68) and branch length (12.69 cm) were recorded maximum in combination where o.4%IBA was used with mixture of 0.1% citric acid and ascorbic acid . However leaf area values are at par with T4 where IBA 0.4 % + Citric Acid 1.5 % +Ascorbic Acid 1.5 % were used.

**Table. 2**: Effects of IBA, Citric acid, Ascorbic acid on vegetative growth measurements of guava softwood cuttings

| **Cutting Treatment** | **Number of Leaves** | **Leaf area (mm)** | **Number of branches** | **Branch Length (cm)** |
|---|---|---|---|---|
| T1 | 17.92 c | 43.42 c | 1.55 d | 4.57d |
| T2 | 20.84 b | 46.73 b | 2.40 c | 7.03 c |
| T3 | 25.50 a | 51.31 a | 3.68 a | 12.69 a |
| T4 | 21.35 b | 51.09 a | 3.11 b | 8.57 b |

**T1**=Control, **T2**=IBA 0.4 % +Citric Acid 0.5 % +Ascorbic Acid 0.5 %, **T3**= IBA 0.4 %+Citric Acid 0.1 % +Ascorbic Acid 0.1 % and **T4**= IBA 0.4 % + Citric Acid 1.5 % +Ascorbic Acid 1.5 %. Anova lettering (values) obtained by same word (s) in same column are non-significant (P>0.05%) via using LSD test.

Data presented in Table # 3 illustrated the significant affect of antioxidants on rooting parameters of cuttings. Among all treatments T3 (IBA 0.4 %+ 0.1% of citric acid and ascorbic acid) exhibited more quantity of primordia (21.71) and emergence roots (45.94) proceeded by T4 where quantity of root primordia and root emergence were 17.30 and 42.45 respectively. Root volume (2.26 $cm^3$) and root length (9.65 cm) was also found highest in T3 while lowest value of these parameters was recorded in control where no rooting hormone and antioxidants was applied.

**Table 3:** Effects of IBA, Citric acid, Ascorbic acid on various rooting parameters of guava softwood cuttings

| Cutting Treatment | No of Primary roots | No of Secondary roots | Root Volume $cm^3$ | Root Length cm |
|---|---|---|---|---|
| T1 | 9.26 d | 23.74 d | 1.45 c | 3.04 d |
| T2 | 13.17 c | 35.85 c | 1.51 c | 7.66 c |
| T3 | 21.17 a | 45.94 a | 2.26 a | 9.65 a |
| T4 | 17.30 b | 42.45 b | 1.91 b | 8.73 b |

**T1**=Control, **T2**=IBA 0.4 % +Citric Acid 0.5 % +Ascorbic Acid 0.5 %, **T3**= IBA 0.4 %+Citric Acid 0.1 % +Ascorbic Acid 0.1 % and **T4**= IBA 0.4 % + Citric Acid 1.5 % +Ascorbic Acid 1.5 %. Anova lettering (values) obtained by same word (s) in same column are non-significant (P>0.05%) via using LSD test.

**DISCUSSION:**

Antioxidants play major role as anti-browning agents in various ways as controlling browning in cuttings and fresh-cut fruit and vegetables. Among various agents naturally occurring agetnts includes citric acid, oxalic acid and ascorbic acid which are predominantly present in fruits and vegetables naturally (Suttirak, W. and S. Manurakchinakorn, 2010).

**Success and Survival %:** As depicted from the results antioxidants treatment significantly increased the success percentage by controlling the browning of cuttings. Browning results from the phenolic exudation in various plant species that inhibit rooting and hence success of cuttings (Strosse et al.,2004; Abdelwahd et al., 2008). Hence with the usage of antioxidants, browning in cuttings can be prevented by scavenging the activities of free radicals of oxygen that will possibly improves the rooting process of cuttings (Zamir et al., 2004). Auxin treatment applied to semi-lignified stem sections of guava also has important affect on success of cuttings. Wahab et al. (2001) reported the most recorded emergence rate of 12.50% while applying IAA in 3000 ppm and 6000 ppm concentrations. Alive rate of sift wood cuttings (sprouted/emerged) must be directly linkage with The survival of sprouted cuttings may be directly linked to the root regeneration in cutting tissue. Auxin's plays vital role increasing root growth of cuttings as research trial results described by many scientists ans results are significant as according to current research.

**Vegetative growth parameters:** Vegetative parameters like number of leaves, number of branches and branch length have significant difference due to various concentrations of antioxidants used along with auxin. Our results are in line with El-Sharony (2018) who recorded maximum leaf numbers in guava cuttings treated with antioxidants in combination with 5000 ppm IBA. Antioxidants along with auxins may have synergistic effect on vegetative growth of cuttings as auxins helps in initiation of roots. Production of more roots helps to increase the absorption area for water and nutrients essential for growth that ultimately influences the vegetative growth indicators. When Auxin applied to Guava cuttings it showed significant effect to increase in foliage amount that is primary site for natural auxin production in plant mechanism along maintaining activities of respiration, transpiration and photosynthesis (Wahab et al., 2001). Mohammad (1998) observed a maximum shoot length of 11.33 cm at a 3000 ppm concentration. Notably, IBA and NAA outperformed paclobutrazol in promoting shoot length. This disparity may be attributed to paclobutrazol's inhibition of gibberellin biosynthesis (Anonymous, 1986), a hormone essential for growth promotion.

**Rooting parameters:** The results revealed that auxin application in combination with ascorbic acid and citric acid (antioxidants) to Guava cuttings will directly results to increase in number of roots (primary and secondary) sprouted by cuttings.. This might be due to the synergistic effect of antioxidants with auxin in root initiation (Sharony et al., 2018).. Auxins help to increase root primordial while antioxidants used to inhibit phenolic exudations that inhibit root initiation by oxidative browning in different plant species. These anti-browning agents are reported to scavenge reactive oxygen species produced during cutting formation ( Abdelwahd et al., 2008). Results that we obtained after research trial ended are in agreement with Rafat et al. (2020) who found significant effect of antioxidants like citric acid in combination with IBA and ethephon on root parameters i.e. root number, root length and root weight of guava cuttings. By treating Guava soft wood cuttings with IBA (4000 ppm) root length is increased by auxin application to high extent. . Auxin treatment promotes healthy root development in guava cuttings by encouraging leaf growth (Wahab et al., 2001). The results obtained are in harmony with the results obtained by (Wahan, 1999) he reported that auxins effects directly on plant root length.This result is agrred with (Lanphear and Mehal, 1963) they reported that use of plant growth regulators helps in maintaining cuttings (root growth) when environmental factors are feasible to withstand (Rahman et al., 2004).

**CONCLUSION:**

Treatment 3 (IBA 0.4 % + 0.1% of both Citric acid and Ascorbic acid ) depicted highest values in maximum parameters of growth and success percentage. It can be concluded that citric acid and ascorbic acid @ 0.15 along with 0.4% IBA can be used to eliminate browning problem in cutting thus enhancing success percentage of guava soft wood cuttings.

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