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Studies on clonal multiplication of Guava (*Psidium guajava* L.) through cutting under controlled conditions

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Abstract

Nursery experiments were conducted over three years on standardization of clonal propagation technique in *Psidium guajava* L. (guava) through rooting of cuttings. Influence of various factors such as potting mixtures (vermiculite, sand and soil), cutting size (5, 10 & 15 cm) and seasonal changes (summer, rainy & winter), were studied on adventitious root formation in the guava cuttings. The study revealed significantly higher root induction (90±3.87%) in vermiculite followed by sand (50±4.16%). Root formation was retarded when soil was used as potting medium (8.02±1.85%). Accordingly callus formation varied greatly among various potting medium (10.11 to 98%). There is slight difference between 10 and 15 cm long cuttings in terms of root induction (82.8 to 91%). However, it was markedly declined (30%) in smaller cuttings (5 cm long). Rooting potential of cuttings (82.0 to 90%) was marginally effected by seasonal variation. Exogenous application of nutrient solution (half strength Hoagland) promoted formation of strong root system with several brush shaped fibrous roots. The study provided useful information on clonal multiplication of elite germplasm of guava under protected environment for developing commercial nurseries.

Keywords: callusing; cultural practices; Guava; propagation; protected environment.

Introduction

Psidium guajava L. commonly known as guava (Family Myrtaceae) is one of the most important fruit crop of alluvial plains of India. Guavas are now cultivated and naturalized throughout the tropics, and due to growing demand they are also grown in some subtropical regions. It occurs throughout the American tropics, Asia, Africa and Pacific Islands. It has great market potential due to its delicious taste, aroma, sweet flavor and a fine balance of acid, sugar and pectin. Guava (*Psidium guajava* L.) is considered to be one of the exquisite, nutritionally valuable and remunerative crops. Besides its high nutritional value, it bears heavy crop every year and gives good economic returns (Singh et al., 2000). This has prompted several farmers to take up guava orcharding on a commercial scale. Extensive studies have been carried out on guava to investigate yield potential (Srivastava et al., 2002), effect of deblossoming on fruit size and quality (Sardar et al. 1996) and its nutritional status on substandard soil sites (Garg and Khanduja, 1987; Sanyal and Mitra, 1990). Guava has a good potential to grow on wastelands including high pH soils (8.6 to 9.6). Several research and improvement programs on guava have been undertaken at many places like Columbia, Mexico and India to evaluate and select site adapted superior genotypes for vegetative propagation and large-scale cultivation. In India and some other countries, plantation programs are expanding on millions of hectare of substandard soil sites. The demand of quality planting stocks of value added fruit crops like guava is increasing rapidly. Conventional methods of propagation as air layering, inarching or stooling cannot fully meet the increasing demand of planting stock because of dependence on whether conditions and low success rate. Therefore, there is an urgent need to develop cost effective protocols, which are rapid and can provide uniform, high quality genetically predictable stocks in desired quantities within a short period for plantation programs. The scenario is changing from traditional propagation with incorporation of science and technology to nursery management and trade (Singh and Bajpai, 2003). A few departures from conventional rooting methods can significantly enhance the success rate and may reduce the production cost. The present study was aimed to develop novel approach for clonal propagation of guava under controlled nursery conditions without conventional grafting or budding requirements. It is a breakthrough that will be a paradigm shift in guava nursery stock production.

Materials and methods

The study was carried out at Biomass Research Centre, NBRI, Lucknow. Phenotypically superior vigorous and productive mother plants (5 yr-old) having excellent bearing and fruit quality, under degraded soil conditions (high pH 8.6 to 10.6 with poor soil-air-water relationship) were selected as the source material for the study. Juvenile apical shoot cuttings of varying length (5, 10 & 15 cm long), each with 2-4 pairs of leaves, were taken to study. These were thoroughly washed and

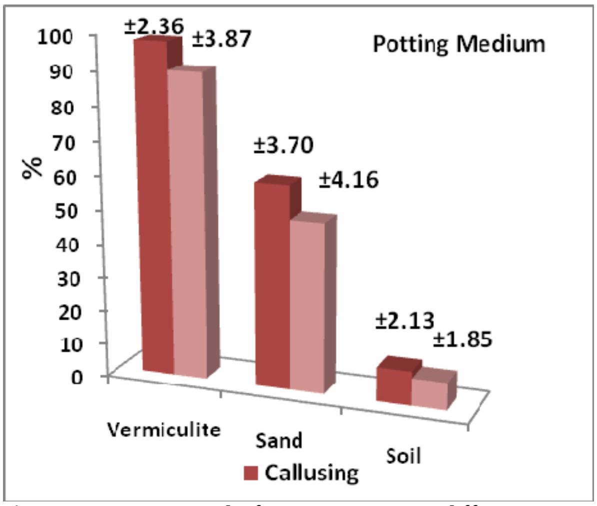

Fig 1. Rooting potential of guava cuttings in different potting media

Fig 2. Formation of brush shaped roots in guava

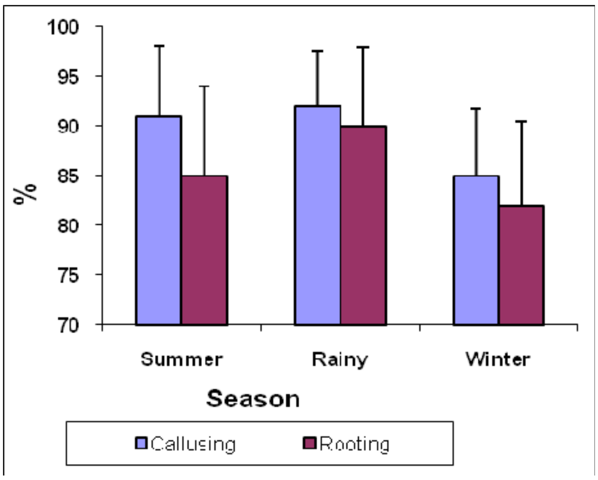

Fig 3. Rooting of cuttings in different seasons

planted in root trainers (15 cm long ribbed cups). Three nursery grade rooting media such as vermiculite, sand and soil were tried separately as potting mixture for root induction. No pre-treatment of auxins or fungicide was given for root induction. The root trainers were kept under poly house with misting facility (atomizers &foggers). The poly house had a covering of 200-micron thick UV proof poly sheet of Indian Petrochemical Limited. The environmental conditions such as humidity (75±10%) and temperature (35±5^{0}C) were maintained. During summer the humidity was controlled using overhead misters. The experiments were repeated for three consecutive years. Data were recorded on cuttings showing formation of root initials (callus formation), development of adventitious root and time required for root induction in different treatments. Statistical analysis (mean values, standard deviations and t test) was done using Excel program (Window 2000).

Result

Influence of rooting medium on root induction

The study showed that most of the cuttings developed the roots within 30 to 40 days. Formation of root initials (callusing) was observed within 20 to 25 days followed by emergence of 3 to 10 roots per cutting with several fine roots. The average success rate in terms of callus formation and rooting induction in different potting mediums varied greatly (Fig. 1). It was significantly high ($p<0.5$) in vermiculite (90±3.87%) followed by sand (50±4.16). Root formation was retarded when soil was used as potting medium (8.02±1.85%). Accordingly root formation varied greatly among various potting medium (10.1 to 98%). The rooted plants showed nutrient deficiency symptoms after 45 to 50 days as rooting medium “vermiculite” or ‘sand’ had no nutrients except the ones transferred through irrigation water. This indicated need for exogenous application of nutrients for developing stronger root system. For this normal half strength Hoagland solution (15 ml) was supplied in each pot on alternate days for 15 days to promote growth of these rooted cuttings. Formation of brush shaped, vigorous and healthy roots were observed in cuttings treated with nutrient solution. The shoots of these plants were healthy and greener as compared to untreated ones ensuring good quality and hardy planting stocks in vermiculite as compared to other potting mediums (Fig. 2).

Seasonal variability in rooting of cuttings

There were marginal differences in callus formation (85 to 92%) and rooting potential of cuttings (82 to 90%) raised during different seasons (Fig.3). However, during extremely hot (June) and cold (January) months, inhibition of root formation (10 to 20%) was recorded due non-availability of actively growing tips of terminal shoot cuttings. Number of roots per cutting ranged from 3 to 12. It was irrespective of planting season.

Influence of cutting length on rooting induction

Size of cuttings showed significant effect on rooting induction (Fig. 4). Both callus formation (32%) and root induction (30%) was significantly ($p<0.05$) declined in small cuttings (5 cm long), while these differences were insignificant among 10 and

15 cm long cuttings. No additional significant gains could be obtained by increasing the size of cuttings from 10 cm (82.8%) to 15 cm (91%). It indicates that 10 cm is the optimum size for root induction in guava cuttings.

Hardening of planting stock

The acclimatization and survival of the young plants raised under poly house condition are very important and a limiting factor in nursery stock production. The young rooted plants (15 to 20 cm long) were shifted in perforated poly-bags filled with a mixture of sand + soil + leaf mold in 1:1:1 ratio. These were kept in partial shade under net houses (Fig. 5) with 50 to 75% shading intensities (depending on weather condition) for a month. Sprinklers were used to irrigate young plants under net house condition. These plants were gradually exposed to lower humidity under open nursery before transferring them to field condition.

Discussion

During the past few years, interest has grown in clonal propagation and marketing of horticultural plants particularly major wasteland fruit crops such as *Emblica officinalis* (Aonla), *Psidium guajava* (Guava), *Ziziphus mauritiana* (Ber), *Syzygium cumini* (Jamun), *Carrisa carandas* (Karando), *Tamarindus indica* (Imli) etc. In recent years, guava is getting popularity in the international trade due to its nutritional value and processed products (Singh, 2005). However, the greatest handicap in guava plantation is discriminate multiplication of plants from unreliable sources by nurserymen (Singh et al., 2005). Non-availability of quality planting materials and consequent substitution of poor quality seedlings have adversely affected the guava production and productivity. The initial planting material is the basic requirement on which the final crop depend both in quality and quantity (Singh et al., 2005). Breeding programs for perennial plants like fruit trees are time consuming because of their slow growth rate and long generation time. In the present context, rapid methods of propagation become very important when planting materials are limited due to the scarcity of a clone or verities or due to sudden expansion in acreage. Adventitious root formation is a key component of clonal propagation of selected woody plants (Smart *et al*., 2001). In fruit trees, several vegetative propagation techniques as air layering, root cuttings and stooling, have been tried with varying success rate to increase productivity and gains by clonal propagation and selection (Morton, 1987, Pathak & Dwivedi, 1996). Though these techniques are still not commercially viable due to varying rate of success, absence of tap root system and cumbersome process. The technique was developed by us is simpler, rapid, less labour intensive and economical, as no growth hormones are required for root initiation except for manipulation of cultural environments. It is useful as compared to conventional method of propagation (grafting/budding) of guava because of higher success rate, independence of season and climate, small size of cuttings, use of juvenile shoot cuttings, disease free nature and production of large number of uniform true to mother type plants in a short period of time. It is possible to root several thousand cutting in relatively a small area and the

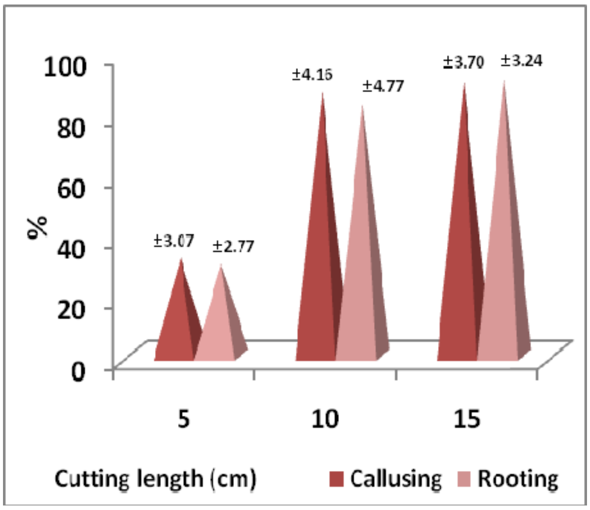

Fig 4. Influence of cutting size on rooting induction in guava

Fig 5. Hardening of planting stock under net house

Fig 6. Fruiting in Guava plant after two year of growth

same can be transported to the planting site easily. Rooting behavior of a species may be effected by a number of external factors like season, temperature, light, water, humidity and aeration, composition and pH of rooting medium (Hartman and Kester, 1975). During present study rooting was marginally effected by external environmental conditions because cuttings were raised under controlled humidity and temperature. However, relatively poor root formation recorded during extremely hot and cold conditions was due to low activity of cambium to proliferate in unfavorable and adverse environmental conditions (Goel and Behl, 1994). The important internal factors that affect rooting are nutritional, genetic, metabolic and developmental status of cutting. In the present experiment juvenile apical shoot cuttings of guava were selected for rooting induction. Goel and Behl (2004) suggested use of juvenile top cuttings for rooting induction in hard wood species such as Neem because physiologically mature tissues have lower rooting potential, take a long time to initiate roots and develop fewer roots than does physiologically juvenile material. Selection of optimum sizes of cutting for rooting induction is very crucial in case of precious super materials. The studies carried out by most of the earlier workers revealed 20-22 cm as the optimum size for rooting induction in woody plants (Puri and Swamy, 2000). We have achieved success in standardizing the macro-propagation technique where small cuttings (10 or 5 cm long) could be multiplied easily under protected environment nursery depending on the availability of mother stock. Field-testing of sample plants were carried out to prove their worthiness on substandard soils, which were stressed due to high pH (8.6 to 10.5) and poor soil-air-water relationship. Plants started fruiting after two growing seasons (Fig. 6). However, profuse fruiting was recorded after fourth year.

Conclusions

The study revealed potential of clonal propagation technique in guava for nursery stock production at commercial scale. The selection of appropriate site adapted phenotypes and elite genotypes with excellent fruit bearing and flesh quality and manipulation of cultural environment are important to improve the quality of planting stock for optimum gains. Further the propagation technique of nursery stock production is simpler and cheaper than *in vitro* culture and can be used even by unskilled nursery growers.

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