Rooting and acclimatization of *in vitro* raised plantlets of guava cv. Allahabad safeda

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Abstract
In the present investigation the effort was made to develop an efficient protocol for rooting and acclimatization of in vitro raised plantlets of guava cv. Allahabad safeda. In vitro plantlets were generated through nodal segment explant using 1.0 mg/l BA + 0.25 mg/l GA3 in MS medium. Maximum rooting (83.4%) and length of root/shoot (7.5 cm) and minimum days to root initiation (8.0 day) was observed in full strength of MS medium supplemented with 0.4 mg/l IBA. While, maximum length of shoots (8.1 cm) and maximum number of roots (5.52) were observed in treatment (Full MS + 0.2 mg/l IBA) followed by treatment ½ MS + 0.2 mg/l IBA. The plantlets grown in potting mixture containing vermicompost + soil (1:1v/v) showed better survival of plantlet (90.00 %). Survival of plantlets and growth of plantlets were significant influenced by different climate conditions. Maximum survival of plantlets (86.79 %) and length of shoot was maximum (9.33 cm) was reported in net house condition.

Keywords: *In vitro*, Rooting, Acclimatization and Explants

Introduction

Guava (*Psidium guajava* L.) is one of the important fruit crop of the Indian subcontinent and serves as staple food in many countries (Amin and Jaiswal, 1987). Guava fruit is one of the richest natural sources of vitamin C and good source of calcium, phosphorus, iron, dilatory fibres and pectin. The major guava growing states in the country are Uttar Pradesh, Bihar, Madhya Pradesh, Karnataka, Andhra Pradesh, Maharashtra, Gujarat and Chhattisgarh. In Uttar Pradesh, Allahabad district is well known for producing best quality guava in the world (Radha and Mathew, 2007). In general guava is propagated by vegetatively through budding, inarching, veneer grafting, and air layering. However, multiplication rate is slow and difficult to propagate vegetatively on large scale from better genotype. Although, careful realization of treatments against pest, diseases, fungus, bacterial and virus infection cannot be prevented totally. *In vitro* propagation has become a rapidly expanding reality as is now evident from the number of species being successfully propagated through this technique. This has emerged as a potential means of rapid vegetative propagation of plants which can go a long way in solving many problems in fruit crops. *In vitro* propagation technique has increased rate of multiplication, disease free uniform propaguals, rapid selection and multiplication of elite genotypes and year round availability of planting materials. *In vitro* shoot multiplication and proliferation of guava has been successfully demonstrated by many workers i.e. (Amin and Jaiswal, 1987; Joshee *et al*; 2002; Ali *et al*; 2003; and Kumar *et al*; 2006) by using shoot tip and nodal segment explants. However, *in vitro* rooting and acclimatization of plantlets remains a crucial step for success of tissue culture protocols of guava. Acclimatization of *in vitro* plantlets is one of the least explored avenues (Bajpai *et al;* 2003). The ultimate goal of *in vitro* plant propagation is to obtain a large scale of plantlets in a short period of time with high survival rate. High mortality of *in vitro* raised plants during transfer from laboratory to field is a major limitation in large scale application of micro propagation technology. *In vitro* raised plantlets are susceptible to transplantation shocks that cause high mortality during the final stage of micro propagation (Dhawan and Bhojwani, 1986). Acclimatization is essential in the case of *in vitro* produced plantlets as these are not able to adopt directly *in vivo* conditions (Brainerd and Fuchigami, 1981). The success in acclimatization of *in vitro* produced plantlets is mainly dependent upon not only the post-transfer growth conditions but also the pre-transfer culture conditions (Ziv, 1986). *In vitro* plantlets are very poorly adopted to resist the low humidity, high light levels and more variable temperatures prevailing outside (Wainwright, 1988). Thus, light, temperature and relative humidity are the major factors to be controlled during acclimatization to natural environment. Physical, chemical and biological properties of the potting mixtures are also important in the establishment of *in vitro* produced plantlets. However, the survival of *in vitro* plantlets also depends upon the potting mixture used for raising *in vitro* plantlets under greenhouse condition. A few reports are available on *in vitro* rooting and acclimatization in Guava var. Allahabad Safeda. Hence, the present investigation has been under taken to standardize the rooting and acclimatization procedure of *in vitro* raised plantlets of guava from nodal segment explants.

Materials and methods

Plant material and culture condition: Present experiment was conducted at biotechnology laboratory Aspee college of horticulture and forestry, Navsari Gujarat. *In vitro* plantlets were generated by using nodal segment explants from four to five year mature mother plant of guava var. Allahabad Safeda. Surface sterilization of explants was made using 0.1 % mercuric chloride solution for five minutes. Proliferated shoots obtained on MS (Murashige and Skoog, 1962) medium containing with 1.0 mg/l BA + 0.25 mg/l GA_3 with 3% sucrose from nodal segments were used for rooting study. For rooting, ½ MS, MS and White media

with different concentration (0.1, 0.2 and 0.4 mg/l) of both IBA and IAA were tested for root induction. *In vitro* raised plantlets having three to four roots and six leaves were taken out from bottle. The roots were washed thoroughly in tap water to remove adhering agar. Then, plantlets were transplanted in plastic cup containing different types of potting mixtures viz. vermicompost, soil, cocopeat, vermicompost: soil (1:1 v/v) and FYM: soil (1:1 v/v).

Acclimatization of rooted plantlets: Plantlets in cup were covered with plastic cup continuously for one week and kept in air conditioned room. The cover was gradually removed after seven days, initially for three hours followed by six hours and twelve hours in next three days. The cover was removed during night and lights put-off for next three to four days. Subsequently, the period of keeping the plantlets without any cover was gradually increased. Then, after two weeks they were brought in to different climate condition i.e. net house, air condition room, and low tunnel net house for *ex vitro* hardening. Plants were kept in individually as well as in group. Each treatment was replicated three times.

Statistical analysis

Experiments were set up in the (CRD) completely randomized design and repeated three times, each treatment consisted of 50 explants and the means separation were done according to Least Significant Differences (LSD) at 5% level (Panse, and Sukhatme, 1985).

Results and discussion

***In vitro* root induction**: The data on rooting response to different levels of IBA and IAA on half and full strength of MS and White medium are presented in (Table 1). Rooting was significantly influenced by media and auxins. Maximum rooting (83.4%) and number of root/shoot (5.5) with minimum days to root initiation (8.0) was recorded in Full MS + 0.2 mg/l IBA (Fig.1A) followed by treatment ½ MS + 0.2 mg/l IBA. While, maximum length of root/shoot (7.5 cm) was observed in Full MS + 0.4 mg/l IBA. It was noticed that the rooting of *in vitro* shoot on full strength MS medium was found better in respect to all the rooting characters than that observed on half strength MS medium and White medium. These results are in accordance with earlier worker by (Shinde, 2008) in grape. In general, auxins like IAA and IBA are widely use for root induction and considered as an important factor for adventitious root formation from *in vitro* raised shoots (Xiuli *et al.*, 2010). Similarly, Ali *et al.*, (2003) also found that 0.2-0.4 mg/l IBA was effective in inducing adventitious root formation in guava, addition of IBA to the culture medium enhanced root proliferation. Whereas, addition of IAA in the medium found in effective in root growth. Further increase in the concentration of the IBA decreased the rooting percentage and also number of roots. Similar results were also obtained by (Hari Prakash and Tiwari, 1996) in guava. Higher concentration of auxins also inhibits root formation in plants (Blakesley *et al*., 1991). Response of auxins on root induction is dependent on endogenous hormonal level of mother plant. These results are in conformity with earlier workers (Rai *et al*., 2009 and Singh *et al.*, 2002) in guava.

Effect of different potting mixtures: The results obtained on survival of plantlets and plant growth in different potting mixtures is presented in (Table 2). The survival rate of plantlet was significantly influenced by potting mixtures. Maximum survival of plantlets (90.0%) was reported in vermicompost + soil (1:1v/v) followed by treatment FYM: soil: sand (1:1:1v/v), soil, vermicompost and cocopeat. Minimum days for new sprouting (8.5 days) and maximum length of shoots (11.0 cm) was also observed in vermicompost : soil (1:1v/v) (Fig.1.B) followed by treatment FYM : soil : sand (1:1:1v/v) and cocopeat. The potting mixture containing vermicompost: soil (1:1v/v) was found to be the most suitable for better growth and survival of plantlets. Physical, chemical and biological properties of potting mixture are important in the establishment of *in vitro* produced plantlets. Better performance of vermicompost may be attributed to its ability to improve biological properties of soil. Hence, mixing vermicompost and soil resulted in giving grip for roots, ample aeration and efficient organic matter. The participation of organic matter for better establishment of guava plantlets was described by (Amin and Jaiswal, 1987 and Amin and Jaiswal, 1988) they achieved better establishment in pots containing non sterile garden soil and compost. (Papadatou *et al*., 1990) successfully established *in vitro* rooted shoots of guava in peat based compost in pots, whereas, (Hari Prakash and Tiwari, 1996) reported soil, sand, and FYM (1:1:1 v/v) as the potting mixture for better establishment of guava plantlets. (Rai *et al*., 2009) successfully hardened *in vitro* grown guava plantlets in mixture of sand: garden soil (3:1 v/v).

Effect of different climate control conditions on survival of *in vitro* raised plantlets: The survival and growth of plantlets were significant influenced by different climate control conditions (Table 3). Maximum survival of plantlets (86.79%) was reported in net house condition, kept in group (Fig.1C) followed by individual plantlets in net house condition, low tunnel net house with minimum days taken for new sprouting (10.27 days) and maximum length of shoots (11.17 cm) was observed in net house condition (Fig.1D). Whereas, least survival of plantlets was observed in air condition either in individual or group. Similarly, length of shoot was maximum in net house condition either in individual or group. Hardening the *in vitro* raised plantlets, so as to make them to adopt the natural environment, it is a critical process due to their anatomical and physiological peculiarities. On transplanting, excessive water loss from the plantlets was recorded which was attributed to the improper critical and slowness of stomatal response to water stress (Brainerd and Fuchigami, 1981 and Fabbri *et al*., 1984). Therefore, a period of humidity in acclimatization was considered necessary for the newly transferred plantlets to adapt to the natural environment, during which the plantlets undergo a morphological and physiological adoption enabling them to develop typical terrestrial plant water control mechanism (Grout and Aston, 1977 and Sutter *et al*.,1984). In present study maximum plantlets survived when they covered by plastic cup and kept individually under net house. Method of covering the newly transferred plantlets with plastic bag, glass or microscope covers allowed by misting in greenhouse, polyhouse, nethouse for initial period and subsequently removing the cover in a gradual process was successfully adopted by number of earlier workers for hardening the plantlets (Rajeevan and Pandey, 1986 and Rout *et al*., 1989)

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Table 1: Effect of IBA, IAA and strength of media on induction of rooting in Guava cv. Allahabad Safeda

Treatments	Root initiation (days)	Rooting (%)	Length of root (cm)	No. of root/ shoot	Length of shoot (cm)
1/2 MS + IBA 0.1 mg/l	15.2	53.3 (47.0)*	5.0	2.0	6.0
1/2 MS + IBA 0.2 mg/l	9.7	73.4 (59.1)	6.1	2.6	7.2
1/2 MS + IBA 0.4 mg/l	14.2	50.2 (45.1)	6.7	1.9	5.3
1/2 MS + IAA 0.1mg/l	15.0	33.3 (35.4)	2.5	1.0	3.2
1/2 MS + IAA 0.2 mg/l	12.7	30.0 (33.3)	2.1	1.1	4.3
1/2 MS + IAA 0.4 mg/l	0.0	00.0 (0.5)	0.0	0.0	0.0
Full MS + IBA 0.1 mg/l	16.1	53.3 (47.0)	7.0	3.3	6.2
Full MS + IBA 0.2 mg/l	8.0	83.4 (66.1)	6.2	5.5	8.1
Full MS + IBA 0.4 mg/l	11.1	60.1 (50.8)	7.5	3.3	6.0
Full MS + IAA 0.1 mg/l	13.0	45.0 (42.2)	4.0	2.1	4.0
Full MS + IAA 0.2 mg/l	10.0	63.3 (52.7)	3.1	2.3	4.2
Full MS + IAA 0.4 mg/l	17.2	31.6 (35.2)	2.0	1.0	3.0
White Medium + IBA 0.1 mg/l	20.0	40.2 (39.3)	3.0	1.2	3.2
White Medium + IBA 0.2 mg/l	19.1	43.3 (41.3)	3.2	1.5	3.0
White Medium + IBA 0.4 mg/l	21.2	36.6 (37.3)	2.1	1.2	2.1
White Medium + IAA 0.1 mg/l	20.0	26.6 (31.1)	1.8	1.0	3.0
White Medium + IAA 0.2 mg/l	22.0	31.6 (34.3)	2.1	1.2	2.5
White Medium + IAA 0.4 mg/l	0.0	0.0 (0.5)	0.0	0.0	0.0
S. Em. ±	0.15	0.28	0.10	0.06	0.07
C.D. at 5%	0.44	0.84	0.30	0.18	0.20

*Figures in parentheses are arcsine transformed value.

Table 2: Effect of different potting mixtures on acclimatization of guava cv. Allahabad Safeda

Treatments	Survival of plantlets (%)	Days taken for new sprouting	Length of shoot (cm)
Vermicompost	53.33 (46.89)*	12.00	6.00
Soil	63.33 (52.71)	10.50	7.17
Cocopeat	56.44 (48.61)	11.17	6.67
Vermicompost : Soil (1:1v/v)	90.00 (71.54)	8.50	11.00
FYM : Soil : Sand (1:1:1v/v)	76.66 (61.09)	10.17	9.00
S. Em. ±	0.33	0.44	0.24
C.D. at 5%	1.06	1.38	0.77

*Figures in parentheses are arcsine transformed value.

Table- 3: Effect of different climate control conditions on acclimatization of *in vitro* raised plantlets of Guava cv. Allahabad Safeda

Climate control conditions	Survival of plantlets (%)	Days taken for new sprouts	Length of shoot (cm)
Net house	86.79 (68.66)*	10.27	9.33
Air condition	73.61 (59.07)	11.27	6.83
Low tunnel net house	70.00 (56.77)	11.83	6.33
S.Em. ±	0.63	0.15	0.14
CD at 5%	2.00	0.49	0.42

*Figures in parentheses are arcsine transformed value.

Fig.1 (A) Profuse rooting in full MS + IBA 0.2 mg/l (B) *in vitro* rooted plantlets in air condition room after one week of transplanting in plastic cups (C) Two weeks old plantlets in net house (D) *in vitro* rooted plantlet after four in vermicompost : Soil (1:1v/v) medium in net house