Research Journal of Chemical and Environmental Sciences
Res J. Chem. Environ. Sci. Vol 7 [3] June 2019: 01-08
Online ISSN 2321-1040
CODEN: RJCEA2 [USA]

Website: www.aelsindia.com/rjces.htm

RJCES

ORIGINAL ARTICLE

In vitro Plantlet Regeneration in Guava (*Psidium guajava* L.) cv. Hisar Safeda and Hisar Surkha

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ABSTRACT
An efficient in vitro regeneration protocol for obtaining plants from nodal and shoot tip explants of guava cv. Hisar Safeda and Hisar Surkha has been developed. Exudation of phenoloics from the explants was controlled by fortification of the medium with activated charcoal of 6.0 g/l in addition to a pre-treatment of explants with 0.2% ascorbic acid and 0.4% citric acid for 20 minutes. Maximum numbers of shoots were obtained from shoot tip as explants with high frequency of shooting response (70 %) on MS medium fortified with 5.0 mg/l BAP and 0.1 mg/l of NAA. Continuous trials throughout the year showed that April-June was best season for culturing of explants. A liquid MS medium fortified with 3.0 mg/l NAA showed maximum rooting response in terms of number of roots and root length. Vermiculite was found best as a pot mixture for hardening. The regenerated plants survived with 70-75 % success. There was no significant effect of cultivars on different parameters considered during the study.
Keywords: in vitro regeneration, Psidium guajava, explant

Received 27.03.2019 Accepted 15.05.2019

INTRODUCTION

Guava (*Psidium guajava* L.) also called as the apple of the tropics is an important fruit crop of tropical and sub-tropical regions of the world [1]. It is one of the richest natural sources of vitamin C, a premium source of calcium, phosphorus, iron and pectin. Fruit, leaves, roots and bark of guava are used in local medicines to treat diarrhoea, gastroenteritis and dysentery [2]. Therefore it has an immense potential in areas where most of the other fruit crops fail to give commercial production.

Guava industry is facing the major agronomic and horticultural problems such as susceptibility to many pathogens, severity of wilt disease and stresses, short fruit shelf life, low yield and high seed content etc [3]. Propagation from seeds is not practical because guava seeds require long time for seedling emergence as well as have poor and uneven seed germination, [4]. Natural cross pollination is common in guava which results in considerable variability in planting whereas vegetative methods are tedious, time consuming, season dependent, give low survival percentage and resultant trees are susceptible to wind damage [5]. Due to having long juvenile period, self incompatibility and heterozygous nature, conventional breeding techniques have limited scope of improvement of guava. Some of the most serious problems of the guava industry can be resolved by biotechnology. Transfer of useful traits such as resistance to insects, diseases and stresses between plant species has played an important role in crop improvement [6]. An efficient *in vitro* regeneration system is requires for the crop improvement through genetic transformation [7]. Therefore micropropagation seems to be a potential alternative for cloning of selected trees with desirable qualities. The present study was done for development of a cost effective micropropagation protocol of *Psidium guajava* L. cv. Hisar Safeda and Hisar Surkha via direct organogenesis from nodal and shoot tip explants.

MATERIALS AND METHODS

Mother Plant and explants

The plants of Guava cv. Hisar Safeda and Hisar Surkha were selected from the farm block of Horticulture, CCS Haryana Agricultural University Campus, Hisar on the basis of performance (yield, growth and productivity). The nodal and shoot tips segments were taken as the explants for establishment of

cultures. Small segments of young shoots were excised and washed thoroughly to remove the superficial dust particles in running tap water.

Removal of Phenolics

For removal of phenolics different treatments such as fortification of media with antioxidants such as PVP (2-5 g/l), Ascorbic Acid (0.1-0.5 %), Citric Acid (0.1-0.5 mg/l), PEG (0.1-0.5 %) and Charcoal (2-6 g/l) were tried.

Surface Sterilization

Nodal and shoot tips explants (1-2 cm long) were excised from the shoots and then treated with 2% (v/v) Teepol solution for ten minutes with constant stirring followed by washing in running tap water. The explants were then kept in 2% Bavistin for half an hour. Final sterilization was done using 0.1% Mercuric Chloride for 7.5 minutes and 70% ethanol for 2-3 minutes under aseptic conditions in Laminar Flow Systems (Yorco sales Pvt. Ltd., New Delhi). The surface sterilized nodal and shoot tip explants were aseptically trimmed at their edges and immediately inoculated to prevent the drying of the cut edges of the explants.

Culture medium and culture conditions

The excised nodal and shoot tip explants were cultured on MS [8] medium (containing 3% w/v sucrose, 0.8% w/v agar and analytical grade chemicals), supplemented with various concentration of growth hormones cytokinins, viz. BAP, Kn, Zeatin and/or Auxins, viz. 2,4-D, IAA and NAA alone and in combination (Table 1). The pH of the medium was adjusted at 5.8 and autoclaved. The effect of different treatments was quantified on the basis of the percent response of explants showing and the number of regenerates per node after 5 weeks. Each treatment was carried out in 24 culture bottles containing 3 explants per bottle and the cultures were incubated at 25±2°C under white fluorescent light with a photoperiod of 16h light (intensity of 2000 lux) and 8h of darkness.

Shoot proliferation and multiplication

The four weeks old axillary shoots, proliferated from the nodal and shoot tip explants were harvested and transferred on culture initiation medium (MS + BAP 5.0 mg/l + NAA 0.1 mg/l) for further multiplication. Total numbers of developing shoots were counted at the end of five weeks of subculture. The growth of shoots was assessed by measuring the length of shoots in the cluster.

Rooting and acclimatization

In vitro developed shoots (4-5 cm or longer) were excised from the cluster and further cultured on MS basal and MS (solid or liquid) medium that was supplemented with various auxins, viz. IAA, IBA and NAA to induce rooting. Plantlets having well-developed roots were taken out from the culture vessels. The roots were transferred into small sterilized sand containing pots after washing thoroughly in running tap water by using fine brush. Half strength MS salt solution was supplied in the pots and covered with polythene bags to maintain high relative humidity. These pots were placed in growth chamber at 25±2°C under 16h photoperiod. For the acclimatization of plantlets to natural humidity and sun light, the polythene bags were removed gradually (3-4 h daily) after two weeks. The transparent polythene bags were removed permanently after new leaf emergence. Plantlets were transferred along with sand to the pots filled with garden soil after 6 weeks and kept in greenhouse for the normal growth.

For each treatment, 24 explants were used and each experiment was repeated thrice. The results are expressed as a mean of three independent experiments. The data were subjected to Completely Randomized Block Design.

RESULTS AND DISCUSSION

Exudation of phenolic compounds

All the given treatments considerably reduced the exudation of phenolic compounds both in shoot tip and nodal segment explants. In both the cultivers, lowest phenolic exudation was found in activated charcoal (6g/l) supplemented medium. When explants were treated with 0.2% ascorbic acid and 0.4% citric acid for 20 min. before culturing, the degree of phenolic exudation was minimum (data not shown). PEG (0.2%) was found to be least effective to control the phenolic exudation.

Browning of explants due to exudation of phenolic compounds from the cut ends of plant tissues proves lethal to the establishment of cultures. Explants browning generally results from peroxidase activity at the cut ends of explants. The pre-existing total phenols are associated with browning incidence and ultimately have a profound impact on explants survival. A treatment with antioxidant compounds such as ascorbic acid, citric acid, activated charcoal, polyvinilpyrrolidone and PEG has been reported for several micropropagation systems [9, 10, 11]. High level of polyphenols, tannins, and products of lipid peroxidation is found in guava cultivars like other woody plants. The oxidized products can prevent explant development as well as lead to death of the explants [12].

Culture establishment and multiplication: *Nodal explants*

The maximum % bud break (58.82%, 62.20% in Hisar Safeda and Hisar Surkha respectively) was found on EM_9 (5.0 BAP+0.1 NAA) medium which was at par (55.92%, 57.80% in Hisar Safeda and Hisar Surkha respectively) with EM_8 (3.0 BAP+0.1 NAA) medium (Fig. 1A). The minimum shoot regeneration was observed on EM_4 (10.32%, 13.20%, in Hisar Safeda and Hisar Surkha respectively) medium however no regeneration of shoot was observed in the medium with no growth regulators. When explants were cultured on EM_9 (5.0 BAP + 0.1 NAA) medium, the minimum time for shoot initiation was recorded 18.93 days and 18.40 days (Hisar Safeda and Hisar Surkha respectively) for shoot initiation (Table 1).

It was recorded that BAP in combination with NAA was found significantly superior over other combinations for number of shoots. Maximum number of shoots (4.00, 4.33 in Hisar Safeda and Hisar Surkha respectively) were produced when (EM_9) 5.0 mg/l of BAP along with 0.1 mg/l of NAA supplemented to medium (Table 1) followed by 5.0 mg/l of BAP along with 0.1 mg/l of IAA (EM_{15}) supplemented to medium. Perusal of data reveals increased shoot length when cytokinins added alone or in combination. Shoot length was observed maximum (6.20 cm, 6.0 cm in Hisar Safeda and Hisar Surkha respectively) on MS medium supplemented with 5.0 mg/l BAP along with 0.1 mg/l of NAA (EM_9). Further increase in concentration of cytokinins decreased the shoot length (Table 1). No shoot formation was observed in control that was without growth regulators. Kinetin was least effective on shoot length. Non-significant differences were found between the cultivars of guava.

Analysis of effect of two types of cytokinin (BAP, Kinetin,) and combination (BAP and Kinetin) indicated that when BAP added in combination with NAA was better than the others in reducing the number of days for shoot induction in both shoot tip and nodal segment explants [13]. The probable reason for this may be that BAP suppressed apical dominance and stimulate the growth of lateral buds. Among different cytokinins tested BAP gave better response than the other cytokinins for shoot regeneration of guava cultivars. These findings are also in agreement with Ali *et al*, [14] and Zamir *et al*, [15] who found BAP better than other cytokinins for shoot proliferation phase of guava. Being synthetic in nature, BAP supply provides thus breaking the apical dominance and stimulates growth of new shoots.

Culture establishment and multiplication: *Shoot tip explants*

The maximum % bud break (68.15%, 69.73%, in Hisar Safeda and Hisar Surkha respectively) observed on EM_9 (5.0 BAP+0.1 NAA) medium whereas similar response was observed (61.42%, 64.80%, in Hisar Safeda and Hisar Surkha respectively) with EM_8 (3.0 BAP+0.1 NAA) medium (Table 2). BAP was found better than kinetin. BAP in combination with NAA was found better than BAP with IAA. Kinetin in combination of auxin also increased the shoot regeneration percentage than kinetin alone. Kinetin was found least effective in shoot regeneration. No shoot regeneration was found in control, EM_0 (without growth regulators) in both the cultivar of guava which is followed by EM_4 (1.0 Kin.).

The result evident that the addition of cytokinins either individually or in combination decreased the time required for shoot initiation in both the cultivars of guava. BAP alone performed better than BAP along with kinetin on number of days taken for shoot initiation, BAP in combination of NAA gave better response than BAP in combination of IAA for days taken for shoot initiation. Kinetin in combination with auxin also reduced the time for shoot initiation (Table 2). The minimum time required (18.30 days, 18.10 days in Hisar Safeda, Hisar Surkha respectively) for shoot initiation was observed with EM_9 (5.0 BAP + 0.1 NAA) medium which was at par with EM_{15} (5.0 BAP + 0.1 IAA).

The numbers of shoots were significantly affected with combination of cytokinins and auxins. Combination of BAP and NAA was found superior. Kinetin in combination with auxin (NAA or IAA) improved the multiplication rate than kinetin alone. In the same manner, BAP in combination with auxin also increased the number of branches than BAP alone. BAP in combination with NAA showed better response than BAP in combination with IAA. BAP and kinetin also improved the branching but after certain cytokinin level (5mg/l) it reduced the branching. The maximum number of shoots were recorded (3.67, 4.33 in Hisar Safeda and Hisar Surkha respectively) when cultured on EM_9 (5.0 BAP + 0.1 NAA) medium (Table 2).

Table 1. Effect of different growth regulators on axillary bud proliferation from nodal explants of guava cv. Hisar Safeda & Hisar Surkha

Media Code	Hormonal Composition (mg/l)	Hisar Safeda	Hisar Surkha	Hisar Safeda	Hisar Surkha	Hisar Safeda	Hisar Surkha	Hisar Safeda	Hisar Surkha
		% bud break		No of days		No of shoots		Shoot length	
EM_0	Control	0.00	0.00	-	-	-	-	-	-
EM_1	1.0 BAP	19.45	22.10	23.80	23.80	1.33	2.33	3.87	3.70
EM_2	3.0 BAP	24.45	26.73	21.90	21.70	2.33	2.67	4.40	4.10
EM_3	5.0 BAP	31.55	35.10	20.90	21.20	3.67	3.67	5.40	5.30
EM_4	1.0 Kinetin	10.32	13.20	25.30	25.30	1.33	1.67	1.90	1.60
EM_5	3.0 Kinetin	16.52	19.17	23.80	24.20	1.67	2.00	2.40	2.10
EM_6	5.0 Kinetin	23.35	26.63	21.87	22.40	2.33	3.00	3.00	2.70
EM_7	1.0 BAP + 0.1 NAA	46.35	49.40	22.87	23.40	2.67	2.67	4.80	4.70
EM_8	3.0 BAP + 0.1 NAA	55.92	57.80	21.60	21.60	3.33	3.33	5.70	5.40
EM_9	5.0 BAP + 0.1 NAA	58.82	62.20	18.93	18.40	4.00	4.33	6.20	6.00
EM_{10}	1.0 Kinetin + 0.1 NAA	33.62	32.27	23.70	22.90	2.00	2.33	2.80	2.40
EM_{11}	3.0 Kinetin + 0.1 NAA	35.98	39.20	22.30	22.80	2.00	2.67	3.30	3.10
EM_{12}	5.0 Kinetin + 0.1 NAA	41.65	43.73	21.10	22.40	3.00	3.33	3.90	3.40
EM_{13}	1.0 BAP + 0.1 IAA	20.42	22.60	22.90	23.40	2.33	2.67	3.90	3.80
EM_{14}	3.0 BAP + 0.1 IAA	28.42	31.83	21.70	21.63	3.33	3.67	4.50	4.30
EM_{15}	5.0 BAP + 0.1 IAA	35.98	37.70	20.27	21.03	3.67	3.67	5.90	5.90
EM_{16}	1.0 Kinetin + 0.1 IAA	19.35	22.50	23.90	22.90	2.00	2.33	2.63	1.70
EM_{17}	3.0 Kinetin + 0.1 IAA	27.75	30.50	22.90	22.10	2.33	2.33	3.10	3.00
EM_{18}	5.0 Kinetin + 0.1 IAA	33.82	34.70	22.70	22.40	2.67	3.00	3.30	3.33
EM_{19}	1.0 BAP + 0.5 Kinetin	33.45	36.60	21.90	22.30	2.33	2.67	4.20	4.00
EM_{20}	3.0 BAP + 0.5 Kinetin	48.25	51.83	20.63	21.37	3.33	3.33	5.70	5.27
EM_{21}	5.0 BAP + 0.5 Kinetin	42.95	44.87	19.13	19.43	3.00	2.00	4.40	4.00
EM_{22}	1.0 BAP + 1.0 Kinetin	42.55	45.70	21.13	22.00	3.00	2.67	5.30	5.27
EM_{23}	3.0 BAP + 1.0 Kinetin	48.85	52.80	19.47	20.13	3.00	3.67	5.90	5.40
EM_{24}	5.0 BAP + 1.0 Kinetin	31.25	32.30	20.13	19.93	2.67	2.67	3.23	2.90
	CD at 5%	1.68	0.22	0.25	0.27	1.21	1.24	0.31	0.27
	SE (m)	0.59	0.07	0.09	0.09	0.42	0.43	0.11	0.09

The experiment conducted revels that cytokinins had significant effect on shoot length, when used alone or in combination with auxin; BAP and NAA were superior over other combinations. BAP gave better results than kinetin. BAP proved most effective among all cytokinins.

Shoot length was found to be maximum (6.2 cm, 5.9cm in Hisar Safeda and Hisar Surkha respectively) on MS medium supplemented with 5.0 mg/l of BAP along with 0.1 mg/l NAA (EM_9) followed by 5.0 mg/l of BAP along with 0.1 mg/l of NAA (EM_{15}) supplemented to the MS medium (Table 2, Fig. 1C). Further increase in concentration of cytokinines reduced the shoots length. Kinetin found least effective on shoot length for both the cultivars of guava.

Shoot tip explants were found better than nodal segment explants for promoting shoot induction. Better response of shoot tip explants over other explants was also noted by [16, 17] in peach and almond cultivars respectively. Rai *et al.* [2] observed the superiority of BAP for shoot induction and concluded that it may be attributed to the ability of plant tissues to metabolize BAP more readily than other synthetic plant growth regulators or to the ability of BAP that may induce production of natural hormones, such as zeatin, within the tissue [18].

Shoot formation in different seasons: in vitro

The data presented in Table 3 reveals that new sprout had significant effect on shoot formation. As temperature of season increased, the exudation of phenolic compounds decreased. The combination of both parameters had significant effect on shoot length. Maximum shoot formation (80.40%, 81.20% in Hisar Safedam and Hisar Surkha respectively) was observed during April-June (Fig. 1B) where as minimum number of shoot were recorded (15.2%, 17.4% in Hisar Safeda and Hisar Surkha respectively) during November-December.

Explant establishment is greatly influenced by seasonal changes [19]. The actively growing season is found to be more responsive for bud break [20]. Maximum explants died due to heavy leaching of phenols in the spring season. This is perhaps due to presence of higher phenolic and water content in growing shoots. Our observations seem to be in conformity with those of Bhat and Dhar [21].

Rooting and acclimatization of plant

It was observed that results of rooting % as well as number of roots per shoot were found better in liquid medium than semi-solid medium. Highest rooting percentage was recorded (54.30, 54.60 in Hisar Safeda and Hisar Surkha respectively) when (RM_7) 3.0 mg/l of NAA was added in liquid medium (Table 4, Fig. 1D). In case of semi solid medium, the maximum rooting response (45.53, 44.10 in Hisar Safeda and Hisar Surkha respectively) was achieved with (RM_{14}) 3.0 mg/l of NAA supplemented to semi-solid medium. NAA produced better results than IBA in liquid as well as semi-solid medium. The maximum number of roots formed (4.82, 4.87 in Hisar Safeda and Hisar Surkha respectively) when 3.0 mg/l NAA (RM_7) was supplemented to liquid medium, along with root length of 4.95, 4.85 cm in Hisar Safeda and Hisar Surkha respectively (Table 4).

The effect of different pot mixtures on different cultivars on percent survival of plantlets *in vivo* indicated that vermiculite showed maximum survival of plantlet followed by soil: sand: FYM and least was recorded in soil sand mixture. The maximum survival was observed (75.5, 76.6 in Hisar Safeda and Hisar Surkha respectively) when plantlet were transferred to vermiculite followed by soil : sand : FYM and lowest was recorded (56.7, 57.4 in Hisar Safeda and Hisar Surkha respectively) in soil sand mixture (Table 5, Fig. 1E).

Similar observations have been made by Shah *et al*: [22] and Zamir *et al*; [15] in guava. The different effectiveness among auxins may be related to the different affinity of each auxin to the auxin receptor involved in the rooting process [23], which could be specific for each cultivar, or to different rates of auxin turnover. Our study indicated that there were significant differences when effects of IBA with NAA combination were compared in guava cultivars. These findings are in agreement with the results of Tereso *et al*; [24].

Table 2. Effect of different growth regulators on bud proliferation from shoot tip explants of guava cv. Hisar Safeda & Hisar Surkha

Media Code	Hormonal Composition (mg/l)	Hisar Safeda	Hisar Surkha	Hisar Safeda	Hisar Surkha	Hisar Safeda	Hisar Surkha	Hisar Safeda	Hisar Surkha
		% bud break		No of days		No of shoots		Shoot length	
EM_0	Control	0.00	0.00	-	-	-	-	-	-
EM_1	1.0 BAP	31.82	33.60	23.30	23.10	1.67	2.33	3.90	3.60
EM_2	3.0 BAP	38.05	38.80	21.90	22.10	2.33	2.67	4.20	4.00
EM_3	5.0 BAP	42.58	45.10	19.10	19.07	3.67	3.67	6.10	5.30
EM_4	1.0 Kinetin	16.95	18.93	24.50	24.20	1.33	1.67	1.57	1.40
EM_5	3.0 Kinetin	25.88	27.70	23.10	23.10	1.67	2.00	2.20	1.90
EM_6	5.0 Kinetin	30.62	32.73	21.10	21.03	2.33	3.00	2.80	2.60
EM_7	1.0 BAP + 0.1 NAA	49.68	51.77	22.30	22.10	2.67	2.67	4.67	4.60
EM_8	3.0 BAP + 0.1 NAA	61.42	64.80	21.00	21.00	3.33	3.33	5.60	5.40
EM_9	5.0 BAP + 0.1 NAA	68.15	69.73	18.30	18.10	3.67	4.33	6.20	5.90
EM_{10}	1.0 Kinetin + 0.1 NAA	32.45	35.27	22.30	22.10	2.00	2.33	2.60	2.33
EM_{11}	3.0 Kinetin + 0.1 NAA	40.42	42.70	21.70	21.50	2.33	2.67	3.00	2.93
EM_{12}	5.0 Kinetin + 0.1 NAA	46.55	48.50	21.03	21.03	3.00	3.33	3.43	3.37
EM_{13}	1.0 BAP + 0.1 IAA	23.48	25.47	23.00	22.90	2.33	2.67	4.10	3.90
EM_{14}	3.0 BAP + 0.1 IAA	30.95	32.30	21.90	21.60	3.33	3.67	4.60	4.40

EM_{15}	5.0 BAP + 0.1 IAA	37.05	39.23	19.10	19.00	3.67	3.67	5.60	5.50
EM_{16}	1.0 Kinetin + 0.1 IAA	24.15	26.33	23.00	22.60	2.00	2.33	1.70	1.53
EM_{17}	3.0 Kinetin + 0.1 IAA	28.35	30.97	22.50	22.10	2.33	2.33	2.30	2.20
EM_{18}	5.0 Kinetin + 0.1 IAA	37.25	39.60	21.10	21.10	2.67	3.00	2.97	2.77
EM_{19}	1.0 BAP + 0.5 Kinetin	34.35	36.80	22.23	22.10	2.33	2.67	3.80	3.60
EM_{20}	3.0 BAP + 0.5 Kinetin	49.08	51.40	20.83	21.50	3.33	3.33	5.20	4.90
EM_{21}	5.0 BAP + 0.5 Kinetin	46.08	47.50	19.50	19.80	3.00	2.00	5.50	5.10
EM_{22}	1.0 BAP + 1.0 Kinetin	39.15	41.80	21.50	21.93	3.00	2.67	4.70	4.60
EM_{23}	3.0 BAP + 1.0 Kinetin	49.65	51.10	20.83	20.80	3.00	3.67	5.60	5.20
EM_{24}	5.0 BAP + 1.0 Kinetin	31.25	32.80	20.17	20.10	2.67	2.67	3.80	3.40
	CD at 5%	0.52	0.44	0.25	0.26	1.21	1.24	0.47	1.81
	SE (m)	0.18	0.15	0.08	0.09	0.42	0.43	0.16	0.63

Table 3. *In vitro* response of guava cv. in different months of the year

Month	Hisar Safeda (% shoots)	Hisar Surkha (% shoots)
February-March	60.30	61.50
April-June	80.40	81.20
July-August	25.60	26.60
September-October	51.30	52.30
November-December	15.20	17.4
CD at 5%	0.38	0.44
SE(m)	0.09	0.13

Table 4. Effect of different concentrations of growth regulators on rooting of *in vitro* regenerated shoots of guava cv.

Media Code	Hormonal Composition (mg/l)	Hisar Safeda	Hisar Surkha	Hisar Safeda	Hisar Surkha	Hisar Safeda	Hisar Surkha
		% Rooting		No of roots/shoot		Root length (cm)	
			Liquid medium				
RM_0	Control	0.00	0.00	0.00	0.00	0.00	0.00
RM_1	0.5 IBA	28.60	29.10	1.53	1.63	1.76	1.85
RM_2	1.5 IBA	38.87	39.43	2.65	2.75	2.75	2.83
RM_3	3.0 IBA	42.40	42.77	3.22	3.23	3.37	3.45
RM_4	4.0 IBA	46.30	46.87	3.53	3.57	3.62	3.73
RM_5	0.5 NAA	38.10	38.53	2.71	2.75	2.89	2.92
RM_6	1.5 NAA	44.17	44.63	4.36	4.39	4.38	4.47
RM_7	3.0 NAA	54.30	54.60	4.82	4.87	4.95	4.85
			Semi Solid Medium				
RM_8	0.5 IBA	20.70	21.10	1.35	1.39	1.23	1.28
RM_9	1.5 IBA	33.47	33.83	2.35	2.42	2.17	2.33
RM_{10}	3.0 IBA	38.50	38.67	2.92	2.95	2.87	2.97
RM_{11}	4.0 IBA	42.33	42.80	3.08	3.19	3.13	3.16
RM_{12}	0.5 NAA	33.60	34.20	2.39	2.40	2.24	2.33
RM_{13}	1.5 NAA	38.70	39.23	3.96	3.95	3.64	3.78
RM_{14}	3.0 NAA	43.53	44.10	4.34	4.31	3.82	3.97
	CD at 5%	0.23	0.19	0.06	0.04	0.05	0.04
	SE (m)	0.08	0.06	0.02	0.01	0.01	0.01

Table 5. Effect of pot mixtures on guava cv. percent survival

Pot mixtures	Hisar Safeda	Hisar Surkha
Vermiculite	75.50	76.60
Soil sand mixture	56.60	57.30
Soil :Sand : FYM mixture	63.70	64.40
CD at 5%	1.26	1.81
SE(m)	0.30	0.15

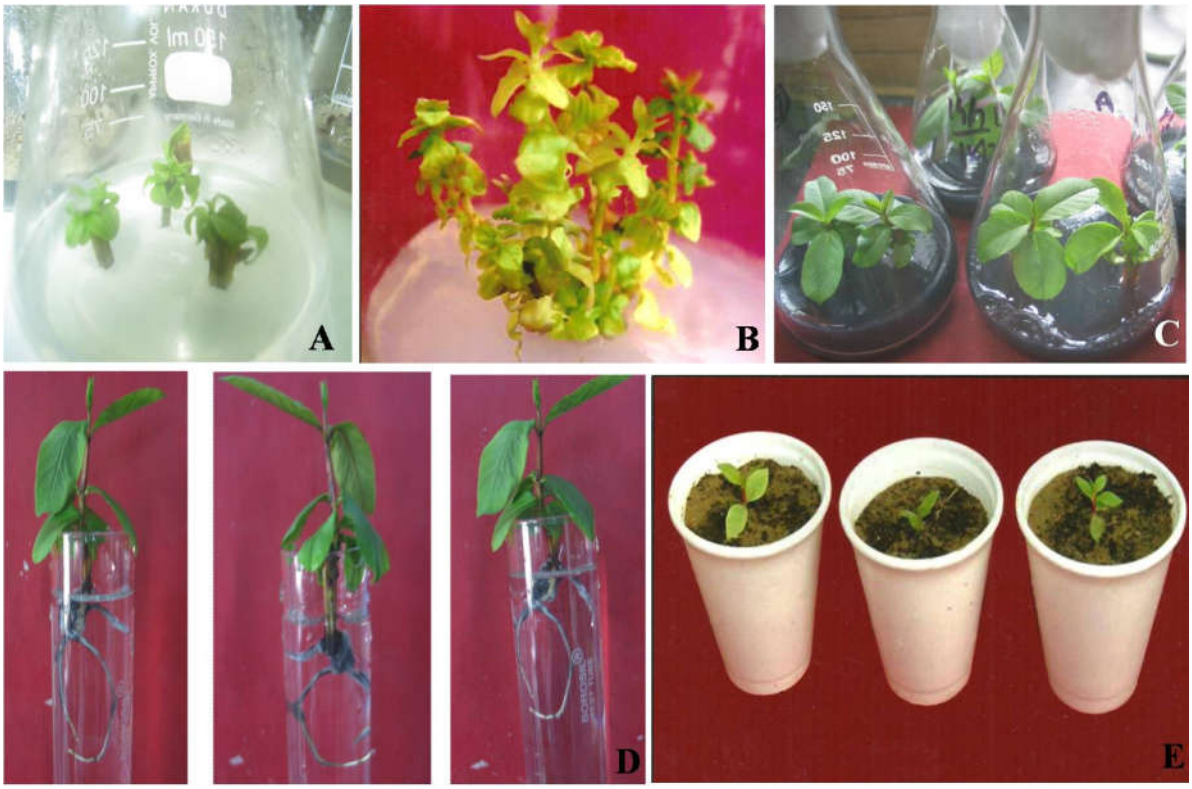

Figure. 1(A-E) *In vitro* propagation of *Psidium guajava*. A. Nodal explants with axillary shoots on MS medium fortified with BAP (5.0 mg/l) + NAA (0.1 mg/l); B. High rate of shoots multiplication on the same medium; C. Well developed regenerated shoots; D. Rooting of *in vitro* regenerated shoots on liquid MS medium with 3.0 mg/l NAA; E. Hardening of plantlets in greenhouse with a mixture of vermiculite.

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CITE THIS ARTICLE

A Kumar, S Kumar, R. C. Yadav, and M Yadav. *In vitro* Plantlet Regeneration in Guava (*Psidium guajava* L.) cv. Hisar Safeda and Hisar Surkha. Res. J. Chem. Env. Sci. Vol 7 [3] June 2019. 01-08