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Effect of different factors on *in vitro* growth and shoot proliferation of guava (*Psidium guajava* L.) cv. Allahabad Safeda

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Abstract: In the present experiment an attempt has been made to optimize the effect of different medium, levels of sucrose, pH, adenine sulphate and light intensity for culture establishment and shoot proliferation in guava using nodal segment explants. Culture establishment was greatly influenced by media types. Maximum establishment of explants (74.57%) was recorded on MS medium supplemented with 2.0 mg/l BAP + 0.2 mg/l IBA. In proliferation study, the maximum shoot proliferation was observed in MS + 1 mg/l BAP + 0.25 mg/l GA_3. Sucrose 3% was found to be more favorable for maximum proliferation and growth of shoots moreover, it was reduced gradually as increased or decreased levels of sucrose from 30 g/l. among the various pH levels tested, pH 5.5 recorded maximum number of shoots (8.08) and maximum length of shoots (3.75 cm).In proliferation medium the length of shoot, numbers of shoots and growth rate were increased as increased the adenine sulphate level in the medium. Maximum proliferation was observed on 160 mg/l adenine sulphate in the medium. High light intensity 3000 lux was found to be most suitable for proper growth of regenerated shoots. Low light intensity (1000 lux) resulted in stunted growth. All the above factors significantly influenced shoot multiplication and growth. Thus, optimization of these factors showed significantly increased number of shoots and rapid multiplication. This could be useful for the *in vitro* production of cost effective healthy planting material of guava

Keywords: Adenine sulphate, Light intensity, Multiplication, pH, Shoots

INTRODUCTION

Guava (*Psidium guajava* L.) comes under the family of Myrataceae, originated from tropical America and Peru. It is a major fruit crop of tropical, subtropical and arid region of the country. It is also known as a poor man's fruit. Guava fruits contain higher amount of vitamin C and also rich in iodine and calcium. Leaves and bark used to treat diarrhea and heal the wounds. Among the different cultivars of guava cv. Allahabad Safeda is most popular and delicious fruit. Traditionally, guava is propagated by seeds but due the natural cross pollination (Purseglove, 1968) there are substantial variability exhibits in the seedling plants (Pontikis, 1996). Hence, to overcome this problem, vegetative methods (budding, inarching, grafting, air layering etc.) are used to produce sapling for large scale plantation. However, these vegetative methods are tedious, time consuming and season dependent (Gautam *et al.,* 2010) which is barrier to rapid and mass clonal propagation. Due to slow process of multiplication, availability of true to type planting material of newly released improved cultivars/better clones are often in short supply. Thus, clonal propagation is an urgent necessity for rapid multiplication of desirable clone. *In vitro* cloning as become a rapidly expanding reality nowadays that 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. The major advantage offered by *in vitro* propagation technique in guava are increased rate of multiplication, disease free uniform propaguals, rapid selection and multiplication of elite genotypes and year round availability of planting materials. The technique of tissue culture in guava has been demonstrated earlier (Hari Prakash and Tiwari, 1996; Joshee, 2002; Singh *et al.*, 2002; Ali *et al.,* 2003; Meghawal et *al.,* 2003; Rathore *et al*., 2004; Kumar *et al.,* 2006; Mishra *et al*., 2007; Zamir *et al.*, 2007; Shah *et al.,* 2008 and Rai *et al.*, 2008). Although, these studies are concentrated mostly on evaluation of specific media different plant growth regulators and explants types for *in vitro* plantlet production. Each tissue types have its own requirement of medium formulations and cultural conditions (Narayanswamy, 1996). Success of *in vitro* culture considerably depends upon the various physical factors such as quality and quantity of light, temperature, pho-

toperiod, pH and concentration of carbohydrates. Addition of growth additives like adenine sulphate into medium enhances cell growth and increase shoot multiplication (Rajore *et al.,* 2002; Kaur *et al.*, 1998 and Al-Suliaman, 2010) .The experiment was designed in order to study all the factors like different media, sucrose, pH, adenine sulphate and light intensity for culture establishment and shoot proliferation in guava from nodal segment explants.

MATERIALS AND METHODS

Explants preparation and surface sterilization: Nodal segments were collected from newly emerged shoots having at least 4-5 nodes from 10-15 year old mature guava tree cv. Allahabad Safeda. Shoots were defoliated and washed thoroughly in running tap water for 2 hours to remove traces of dirt. The shoots then cut into small pieces having 2-3 nodes each to facilitate sterilization process. They were kept in a solution of 0.05 per cent bavistin (carbendazim 50 per cent WP) and 0.01 per cent streptocyclin for two hours. Then they were treated with 10 per cent solution of Teepol for 10 minutes. All traces of detergent were removed by repeated washing in double distilled water. Further, sterilization procedures were carried out under aseptic conditions in laminar air flow cabinet. The surface sterilization was made using 0.1 per cent mercuric chloride solution for 5 minutes. Explants thoroughly rinsed at least three times with autoclaved de-ionized distilled water. Sterilized shoot segments were then cut and trimmed into small nodal segment explants of 1-2 cm length quickly inoculated on the nutrient medium.

Culture media and culture condition: The basal medium of MS, B5 and WPM was prepared from the concentrated stock solutions as described by (Murashige and Skoog, 1962; Gamborg, 1968 and Lloyd and McCown, 1980). Medium pH was adjusted to 5.7 by using 0.1 N NaOH or 0.1 N HCl prior to autoclave at 121°C and 15 Ib/in2 for 20 min. Sugar was added at 30gm/l and medium were solidified with 8% agar. Explants were inoculated in 250 ml glass bottles filled with 30 ml medium. Cultures were incubated in a culture room at 26 + 2°C temperature of with 55 ± 5 per cent relative humidity. Light in the culture room was provided using fluorescent tubes with 16:8 hours light/ dark cycle, kept 50 cm above bench surface at 3000 lux.

Standardized the media for culture establishment: Different media viz. MS, B5 and WPM were test to standardize the most suitable culture establishment medium for guava. Sterilized 2-3 cm nodal segments were inoculated into different media supplemented with 2.0 mg/l BAP + 0.2 mg/l IBA. Subculturing of explants was done at one week interval. Observations were recorded every 4-5 days interval. The best medium found in the trail was further used for shoot proliferation study.

Standardize the medium compositions for shoot proliferation: The trial on shoot proliferation was conducted in MS medium supplemented with different concentration of BAP (1.0-2.0 mg/L) in combination with IBA (0.2 mg/l), GA_3 (0.25-50 mg/l) and kinetin (0.1-0.2 mg/l). Small 1-2 cm nodal segments isolated from 5-6 weeks old *in vitro* established shoots were inoculated into medium as per the treatment combinations. Subculturing of explants was done at three weeks interval. The treatment respond well in shoot proliferation was further used to test the effect of different medium pH level, sucrose concentration, adenine sulphate and Light on *in vitro* shoot proliferation in guava.

Optimizing the factors affecting on shoot proliferation: *In vitro* proliferated 5-6 weeks old shoots were cut and separated into 1-2 cm explants for testing the influence of different medium pH levels, sucrose concentrations, adenine sulphate and Light intensity on *in vitro* shoot proliferation. Treatments were allocated in four different batches. MS medium + 1.0 mg/l BAP + 0.25 mg/l GA_3 was used for entire trail. Five different levels (1, 2, 3, 4, and 5 %) of sucrose was tested in the first batch of culture, another batch was made of four level of Adenine sulphate (20, 40, 80, and 100 mg/l). Effect of adenine sulphate was tested with 3.0 % sucrose level in medium, whereas, the pH of medium was maintained at 5.7 for both the cultures. Influence of different pH levels (4.5, 5.0. 5.5, 5.7, 6.0 and 6.5) was tested in third batch of culture. Medium pH was adjusted prior to autoclaving. The effect of light intensity were tested by keeping the culture into three different (1000, 2000 and 3000 lux) light intensity, provided by white fluorescent tube of 36 watt, kept 50 cm above bench surface.

Statistical analysis: Experiments were setup in the completely randomized design and each treatment was repeated three times. Each treatment consisted of 20 explants in ten glass bottles with two explants in each. Means separation was done according to Least Significant Differences (LSD) at 5% level (Pansey and Sukhatme 1985).

RESULTS AND DISCUSSION

Effect of different media on culture establishmen: Establishment of nodal explants was significantly influenced by the medium concentration in guava. Among the different (MS, B5 and WPM) media tested, Maximum establishment of explants (74.57 %) was recorded on MS medium supplemented with 2.0 mg/l BAP + 0.2 mg/l IBA followed by in B5 and WPM (Table 1).Higher number of shoots and internodes/ explants was notice in MS medium (Fig1). Moreover, minimum days for shoot initiation (9.36) were also observed in the same medium. Similarly, Siddiqui and Farooq (1997) was also recorded maximum culture establishment and shoot growth in guava in MS medium, compared to Blaydes, and WPM. Suitability of MS medium for culture establishment and shoot

Table 1. Effect of different media on establishment of explants of guava cv. Allahabad Safeda.

Media Composition	Establishment (%)	Days taken for establishment	Length of Shoot (cm)	Number of internodes/ explant
MS	74.57 (59.69)*	9.36	2.03	2.23
B5	49.43 (44.65)	12.5	0.76	0.76
WPM	45.86 (42.61)	14.64	0.66	0.53
S.Em. ±	0.25	0.16	0.03	0.03
CD at 5%	0.73	0.47	0.09	0.14
CV %	1.34	6.07	6.15	5.42

*Figures in parentheses are arc sine transformed value

Table 2. Effect of different plant growth regulators on proliferation of culture of guava cv. Allahabad Safeda.

Treatments	Proliferation (%)	Length of shoot (cm)	No. of internodes/ shoot (cm)
BAP 2.0 mg/l + IBA 0.20 mg/l + GA_3 0.25 mg/l	61.00 (51.36)*	3.07	2.14
BAP 2.0 mg/l + IBA 0.20 mg/l + GA_3 0.50 mg/l	57.00 (49.02)	2.71	2.51
BAP 1.0 mg/l + GA_3 0.25 mg/l	79.33 (62.96)	3.61	5.51
BAP 1.0 mg/l + GA_3 0.50 mg/l	71.00 (57.42)	3.11	3.51
BAP 2.0 mg/l + GA_3 0.25 mg/l	69.00 (56.17)	2.91	3.07
BAP 2.0 mg/l + GA_3 0.50 mg/l	57.00 (49.02)	2.80	2.52
BAP 2.0 mg/l + IBA 0.20 mg/l + KIN 0.10 mg/l	52.33 (46.34)	2.51	2.21
BAP 2.0 mg/l + IBA 0.20 mg/l + KIN 0.20 mg/l	55.00 (47.87)	2.11	2.51
BAP 1.0 mg/l + KIN 0.10 mg/l	62.33 (52.14)	2.21	3.07
BAP 1.0 mg/l + KIN 0.20 mg/l	73.66 (59.12)	3.07	4.09
BAP 2.0 mg/l + KIN 0.10 mg/l	66.99 (54.93)	2.07	2.51
BAP 2.0 mg/l + KIN 0.20 mg/l	62.33 (52.14)	2.10	2.71
S.Em. ±	0.09	0.03	0.03
CD at 5%	0.26	0.09	0.08
CV %	0.98	5.83	4.84

*Figures in parentheses are arc sine transformed value

Table 3. Effect of different level of sucrose on *in vitro* shoot proliferation of culture of guava cv. Allahabad Safeda.

Sucrose (%)	Proliferation (%)	No. of shoots per culture	Length of shoot (cm)
1	00.00 (00.52)	0.00	0.00
2	43.29 (41.13)	2.26	1.30
3	76.66 (61.09)	7.02	3.78
4	53.33 (46.89)	3.14	1.80
5	26.74 (31.13)	1.10	1.16
S.Em. ±	0.018	0.05	0.04
C.D. at 5%	0.054	0.14	0.13
C.V. %	0.11	3.98	6.22

Figures in parentheses are arc sine transformed value.

Table 4. Effect of initial pH of the medium on *in vitro* shoot proliferation of culture of guava cv. Allahabad Safeda.

Media pH Level	Proliferation (%)	No. of shoots per explants	Length of shoot (cm)
4.5	33.33 (35.25)*	1.44	1.14
5.0	53.41 (46.94)	2.48	1.70
5.5	76.70 (61.12)	8.08	3.75
5.7	73.46 (58.97)	7.02	3.50
6.0	50.24 (45.12)	3.04	1.80
6.5	00.00 (0.52)	0.00	0.00
S.Em. ±	0.02	0.07	0.03
C.D. at 5%	0.73	0.20	0.09
C.V. %	0.13	4.18	3.45

*Figures in parentheses are arc sine transformed value

growth in guava was reported by several other authors (Joshee, 2002; Singh *et al.,* 2002; Ali *et al*., 2003; Rathore *et al.,* 2004; Kumar *et al*., 2006; Mishra *et al.*, 2007; Zamir *et al*., 2007 Shah *et al*., 2008; Rai *et al*., 2008 and Usman *et al.,*2012). Whereas, in contrast of our results, Meghawal *et al.,* (2003) observed maximum explants establishment and shoot growth in WPM with the combination of 2.0 mg/l BAP. Similarly, Shekafandeh and Khosh-Khui (2008) achieved best results on both MS and WPM medium with 0.5-1.5mg/l BA in nodal segment explants of guava. Woody plant medium was found effective in some guava genotypes probably due to the lower concentration of major elements particularly nitrate salts. The variants in response of different media on culture establishment and shoot growth are could be due to the diverse physiological conditions of explants, their endogenous hormonal levels and the types and nutrients composition of media. (Yadav *et al*., 1990).

Effect of different growth regulators on shoot proliferation: Results on the response of different

Table 5. Effect of adenine sulphate on *in vitro* shoot proliferation of culture of guava cv. Allahabad Safeda.

Adenine sulphate mg/l	Proliferation (%)	Culture growth	Length of shoot (cm)
40	33.37 (39.22)*	+	1.28
80	50.20 (61.11)	++	1.58
120	66.70 (58.90)	+++	2.22
160	73.44 (45.09)	++++	3.62
S.Em. ±	0.02		0.04
C.D. at 5%	0.73		0.11
C.V. %	0.13		3.85

*Figures in parentheses are arc sine transformed value.
+ Poor growth, ++ Good growth, +++ Very good growth, ++++ Excellent growth

Table 6. Effect of different light intensity on *in vitro* shoot proliferation of culture of guava cv. Allahabad Safeda.

Light intensity (Lux)	Proliferation (%)	No. of shoots per explants	Length of shoot (cm)
1000	46.70 (46.09)*	2.14	1.18
2000	53.49 (46.98)	4.62	2.48
3000	76.74 (61.14)	8.16	3.58
S.Em. ±	0.02	0.05	0.04
C.D. at 5%	0.07	0.14	0.12
C.V. %	0.11	2.11	3.47

*Figures in parentheses are arc sine transformed value

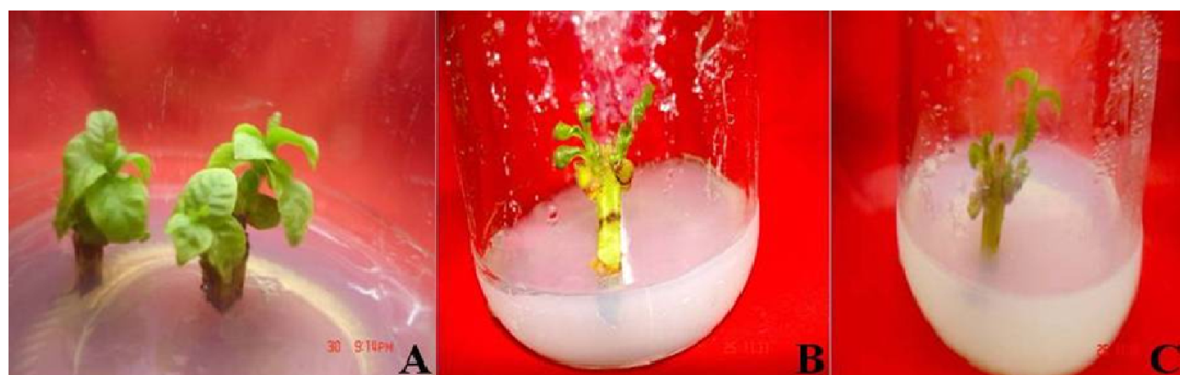

Fig. 1. *(A) Establishment of nodal segment explants on MS medium (B) in WPM medium (C) in B5 medium supplemented with 2.0 mg BAP + 0.2 mg/l IBA after three weeks of inoculation.*

levels of BAP, IBA, GA_3 and Kinetin on shoot proliferation are illustrated in (Table 2). Maximum shoots proliferation per explant (79.33%), length of shoots per explant (3.61cm) and number of internodes per shoot (5.51) were recorded in the treatment MS medium + 1.0 mg/l BAP + 0.25 mg/l GA_3. followed by 1.0 mg/l BAP + 0.20 mg/l KIN and 1.0 mg/l BAP + 0.50 mg/l GA3 . Maximum proliferation of shoots with higher length was obtained on the lower concentration of BAP with GA_3. Similar results were also obtained by (Rodriguez and Diaz Sala, 1991) in pear; (Mishra *et al*. 2007 and Shah *et al*. 2008) in guava. The role of cytokinins in shoot organogenesis is well established by Skoog and Miller (1957). In the present investigation cytokinins in combination with auxins was found less effective in shoot proliferation. However, it produced maximum numbers of proliferated shoots and highest length of shoot in combination with GA_3 (Fig.2A) or Kin. (Fig.2B). these results are in line with (Loha and Rao, 1989 and Shah *et al.,* 2008). This could be due to regeneration potential of cytokine which helped in shoot proliferation, and presence of GA_3 in medium increased intermodal growth resulting increasing the length of shoots. Similarly, Hari Prakash and Tiwari (1996) reported that the application of lower concentration of GA_3 with BA was increased shoot length by increasing the intermodal length in guava.

Effect of levels of sucrose in medium on shoot proliferation: From the perusal of data given in (Table 3) is revel that the shoot proliferation was directly influence by the concentrations of sucrose in the medium. Maximum multiplication (76.66%), number of shoot per explant (7.02) and maximum length of shoot (3.78 cm) was observed in 3% sucrose level (Fig. 2C). Moreover, the growth of shoot was declined gradually with increasing or decreasing levels of sucrose in the medium. Sharp declined in shoot growth was also observed by (Usman *et al*., 2012) with increasing the level of 60g/l sucrose into medium. Carbohydrate is an important element for plant growth, which affects osmotic regulation and photosynthetic activity in plants (Gibson, 2000). Numerous carbon energy sources are used for *in vitro* production in many species. Sucrose is the most widely accepted carbon energy source used in most of the *in vitro* studies (Jain and Babbar, 2003). It is a main and essential part of phloem sap in plants (Ahmad *et al.,* 2007) and has potential to regulate physiological activity and cell differentiation in plants.

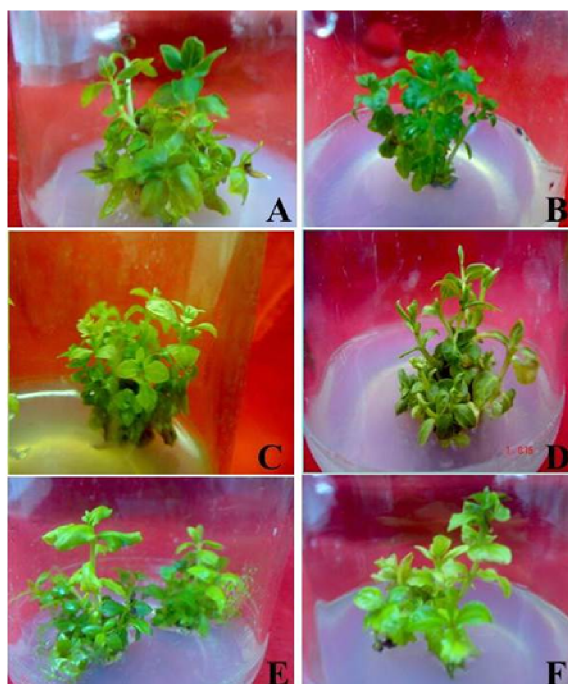

Fig. 2. *(A) Shoot proliferation on MS medium + 1.0 mg/l BAP + 0.25 mg/l GA_3 (B) Shoot proliferation on MS medium 1.0 mg/l BAP + 0.20 mg/l KIN (C) Shoot proliferation in 3 % sucrose on MS medium + 1.0 mg/l BAP + 0.25 mg/l GA_3.(D) proliferated shoots in MS medium + 1.0 mg/l BAP + 0.25 mg/l GA_3 at 5.5 pH (E) 160 mg/l adenine sulphate in MS medium + 1.0 mg/l BAP + 0.25 mg/l GA_3.(F) proliferated shoot at 3000 lux light intensity after three weeks of inoculation.*

However, types and concentration is varying with plants and species (Yaseen *et al.,* 2013). Photosynthetic activity of *in vitro* produced shoots is low (Praveena and Veeresham, 2014) and thus, maintaining an optimum level of sucrose in necessary for increasing shoot growth. In present study the growth was significantly influenced by different concentration of sucrose. Sucrose level 3 per cent in the medium was found optimum for better shoot proliferation. Several authors have reported the importance of the variability in concentration of a particular source of carbohydrates for getting desired response in shoot proliferation in different crops (Gurel and Gulsen, 1998) in Almond, (Asha and Nair, 2010) in *Dioscorea pentaphylla,* (Gabryszewska, 2011) in *Syringa vulgaris* L., (Anchalee, 2012) in *Curcuma longa* L. and (Gauchan, 2012) in Maize. Kumar and Kumar, (1998) reported that the 2 to 3 % sucrose level is optimal for majority of crops for *in vitro* shoot growth. In our study optimum response in guava was obtained at 3 per cent sucrose level. However, in contrast of our finding (Usman *et al*., 2012) obtained the highest number shoots at 45g/l of sucrose in nodal segment explant of guava. Similarly, increasing sucrose level in medium was also produced higher shoot growth in apple rootstock (Yaseen *et al*., 2009) these variant responses could be explained by the different roles played by sugars in plant metabolism. Similar findings were also reported by (Patel, 2008) in papaya and (Shinde, 2008) in grape.

Effect of pH on shoot proliferation: The results on the influence of medium pH on shoot growth and proliferation are presented in (Table 3). Among the different pH levels (4.5, 5.0, 5.5, 5.7, 6.0 and 6.5), maximum shoot growth (76.70%), length of shoot (3.75 cm) and shoots per explants (8.08) was recorded on pH level 5.5 (Fig.2D) Followed by in the pH level 5.7. The trends in number of shoots per explants and length of longest shoot increased as pH level increased up to 5.5, then, subsequently shoot growth was declined. Importance of pH in tissue culture studies was reported earlier by (Gauthert, 1947) who observed drift in pH during the growth of a culture. Majority of fruit crops can grow satisfactory at pH level 5.6 to 5.8 (Conger, 1987 and Skirvin, 1981). In the present study, shoot proliferation and growth of guava was significantly influenced by pH level in medium. Influence of medium pH on shoot growth was reported by several authors in different plant species (Reeves *et al.,* 1983) in peach, (Andersone and Levinsh, 2008) in *Pinus sylvestris.* (De Klerk *et al.,* 2008) in apple and (Shekafandeh, 2010) in almond. The ambient pH could be desired for absorption of various nitrogen sources (Street, 1966). Variant medium pH probably decreases the absorption of nitrogen sources and sucrose uptake by the plants (Bhatia and Ashwath, 2005) which reduced cell differentiation and growth of plants. From the current results it revealed that the higher pH levels (> 5.7) as well as lower (< 5.5) were significantly affecting the shoot growth *in vitro*. The results are coincides with (Walli, 1996) in guava. However, Rai *et al*., (2008) recorded maximum shoot multiplication at 5.8 pH from *in vitro* raised nodal segment of guava. Declining in shoot growth with increasing and decreasing pH levels might be due the well known effect of pH on the availability of nutrients from the medium (Wallihan *et al*., 1997 and Hurley *et al*., 1981).

Effect of adenine sulphate on shoot proliferation: It is evident from the (Table 4) that proliferation and growth of shoots were influenced by adenine sulphate. Maximum shoot proliferation (73.44%) with the highest length (3.62 cm) was obtained at high concentration 160 mg/l adenine sulphate in MS medium + 1.0 mg/l BAP + 0.25 mg/l GA_3 (Fig. 2E). Whereas, lowest concentration of adenine sulphate (40-80 mg/l) was less effective in shoot proliferation. In general, effect of adenine sulphate was seen positive correlation in shoot growth. The results are accordance with Reuveni *et al*. (2004) and Patel (2008) in papaya. Effectiveness of adenine sulpahte in shoot growth and multiplication was reported by several workers in many plant species (Agnihotri *et al*., 2004) in papaya (Nandagopal and Kumara, 2006) in *Cichorium intybus*, (Srivastava and Benergee, 2008) in *jatropa carcus,* (Husain and Anis,

2009) in *Melia azedarach*; (Zibbu and Batra, 2010) in *Thavetia peruviana*, (Gabriela, 2011) in white clover and (Singh *et al.,* 2014) in pomegranate. In the present study adenine sulphate shown synergetic effect in culture growth with low level of cytokinin. Further, shoot proliferation was increased as the adenine sulphate increases into medium from 40 mg/ to 160 mg/l. However, adenine sulphate 160mg/l was found optimum for maximum soot proliferation. This could be due to the potentiality of the adenine sulphate to induced cell growth (Murashige, 1974) resulting increased the number of shoots and accelerated shoot proliferation. Proliferation and shoot growth is controlled by growth regulating substances present in the medium (Imran *et al*., 2012). Similar results were reported by (Siwach and Gill, 2011) in *Ficus religiosa* L. However, Thakur and Kanwar (2008) reported that the adenine sulphate was ineffective on shoot growth from shoot tip and nodal segment explants of pear.

Effect of light intensities on shoot proliferation: Among the different (1000, 2000 and 3000 lux) light intensity examined, the maximum shoot proliferation (76.74%), with maximum number of shoots (8.16) and length of shoot (3.58 cm) was obtained at 3000 lux light intensity, while, at 1000 lux light intensity registered minimum shoot proliferation with small length of shoots (Table 6). The increased proliferation of shoot, number of shoots and length of shoots were observed apparently as increased the light intensity (Fig 2F). Light is the most critical external factor that influences *in vitro* shoot growth and development of plantlets (Gupta and Jatothu, 2013). It is needed to regulate morphogenetic processes, such as initiation of roots, formation of shoots, chlorophyll and asexual embryogenesis, but low light intensity (300-1000 lux) is required to maintain the growth of callus tissue and early organogenesis (Sadhu, 1989). However, higher and lower level of light intensity causes adverse affect on shoot growth by reducing the photosynthesis rates (Gago *et al.,* 2014). Light intensity for *in vitro* shoot growth varies with different crops and species (Chawla, 2002). In present study, 3000 lux light intensity was found optimum for maximum *in vitro* shoot multiplication and growth of guava. Proliferation of shoot was increased as increased the light intensity from 1000 to 3000 lux. The results are in line with (Kumar *et al.,* 2006; Mishra *et al*., 2007 and Rai *et al.*, 2008). Although, in contrast to our results (Zamir *et al*., 2007) found better shoot proliferation at 1500 lux light intensity in guava. Similarly, (Usman *et al*., 2012) reported maximum in *vitro* shoot proliferation at 2500 lux light intensity in cotyledonry explants of guava.

Conclusion

The present study has clearly demonstrated the potentiality of different factors for rapid clonal propagation. The main aim of *in vitro* propagation is to produce maximum numbers of true to type plantlets in shorter period of time at low cast. The optimized level of sucrose (3%), pH level (5.5) and Light intensity (3000 lux) may be utilized further for *in vitro* rapid mass multiplication of guava cv. Allahabad Safeda.

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