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Micropropagation of banana (*Musa acuminata*) through proliferation of axillary shoots*

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Bunchy top disease of banana (*Musa* spp.) caused by Banana bunchy top virus (BBTV) is one of the most important viral diseases and has been considered as a serious threat which is limiting banana production in one quarter of the world's banana growing areas (Dale 1987). The disease was first recognized in Fiji in 1889 and it was introduced in Sri Lanka in 1913 and later into Southern India in 1940 from where the virus spread to various banana-growing areas in India (Vishnoi *et al.* 2009). Bananas are the fourth largest fruit crop in the world, after grapes, citrus and apples. BBTV is one of the most serious diseases of banana (Karan *et al.* 1994). Once established, it is extremely difficult to eradicate or manage. The virus is spread from plant-to-plant by aphids and from place to place by people transporting planting materials raised from infected plants. Some banana varieties, like the Cavendish types, are more readily infected with the virus, but no variety of bananas is resistant. Banana plants that show symptoms rarely bear fruit, and because these are reservoirs of the virus, they must be destroyed (Ferreira *et al.* 1997). Ranasingh (2007) reported that suckers with BBTV symptoms can result in 100% yield loss. To get disease-free planting material, one approach is to import healthy germplasm, but imported germplasm could face acclimatization problems. The other approach is to clean the existing germplasm and multiply shoots at a high rate through the process of micropropagation. A number of countries in the world like Israel (Israeli *et al.* 1995), France (Cote *et al.* 1990), Australia (Drew and Smith 1990), Cuba and many African countries (Vuylsteke 1998) are using this technique for propagation of banana.

In banana (*Musa acuminata* L.), shoots can be easily regenerated through various cultured tissues, such as adventitious buds (Ma and Shii 1972), shoot apices (Doreswamy *et al.* 1983), meristem tips (Alvard *et al.* 1993), floral apices (Alloufa *et al.* 2002), inflorescence apices (Resmi and Nair 2007) and male inflorescences (Darvari *et al.* 2010). Novak *et al.* (1989) has reported somatic embryogenesis and regeneration of banana in liquid medium.

It has been observed that banana shoot multiplication rate is genotype-dependent as well as variable behaviour has been observed among cultures initiated from the same banana genotypes cultured *in vitro* (Israeli *et al*. 1995 and Mendes *et al*. 1999). Further, Mendes *et al*. (1999) reported that in banana *cv.* Maca, multiplication rate tend to decrease with time; after the seventh subculture, new shoots may form at a very low rate.

Therefore, the objective of the present investigation was to study the shoot multiplication rates and number of shoots from sucker-derived shoot apices under *in vitro* conditions during successive subcultures of *cv.* Grand Naine (AAA) for up to seven subculturings.

The elite banana *cv.* Grand Naine was used as an experimental material. Suckers [Fig. 1 (A)] of this cultivar were excised from two-year old healthy banana plants growing in the experimental field of School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana during 2008–09 and 2009–10. The suitable explants were prepared from young suckers (9–11 cm diameter), which were carefully removed from the field by digging a trench around the sucker to completely detach it from the banana mother plants and brought to the laboratory. All the soil was removed by washing them thoroughly under running tap water for 10–15 min. The roots and leaf sheaths of the suckers were removed with the help of a sharp knife. The shoot-tip explants were prepared by removing extraneous corm tissue from suckers. Shoot-tips, containing several sheathing bases enclosing axillary buds measuring about 4.5–5.5 cm in length were obtained. These shoot-tips were first washed with Teepol™ for 4–5 min. and then in running tap water for 5–

*Short note

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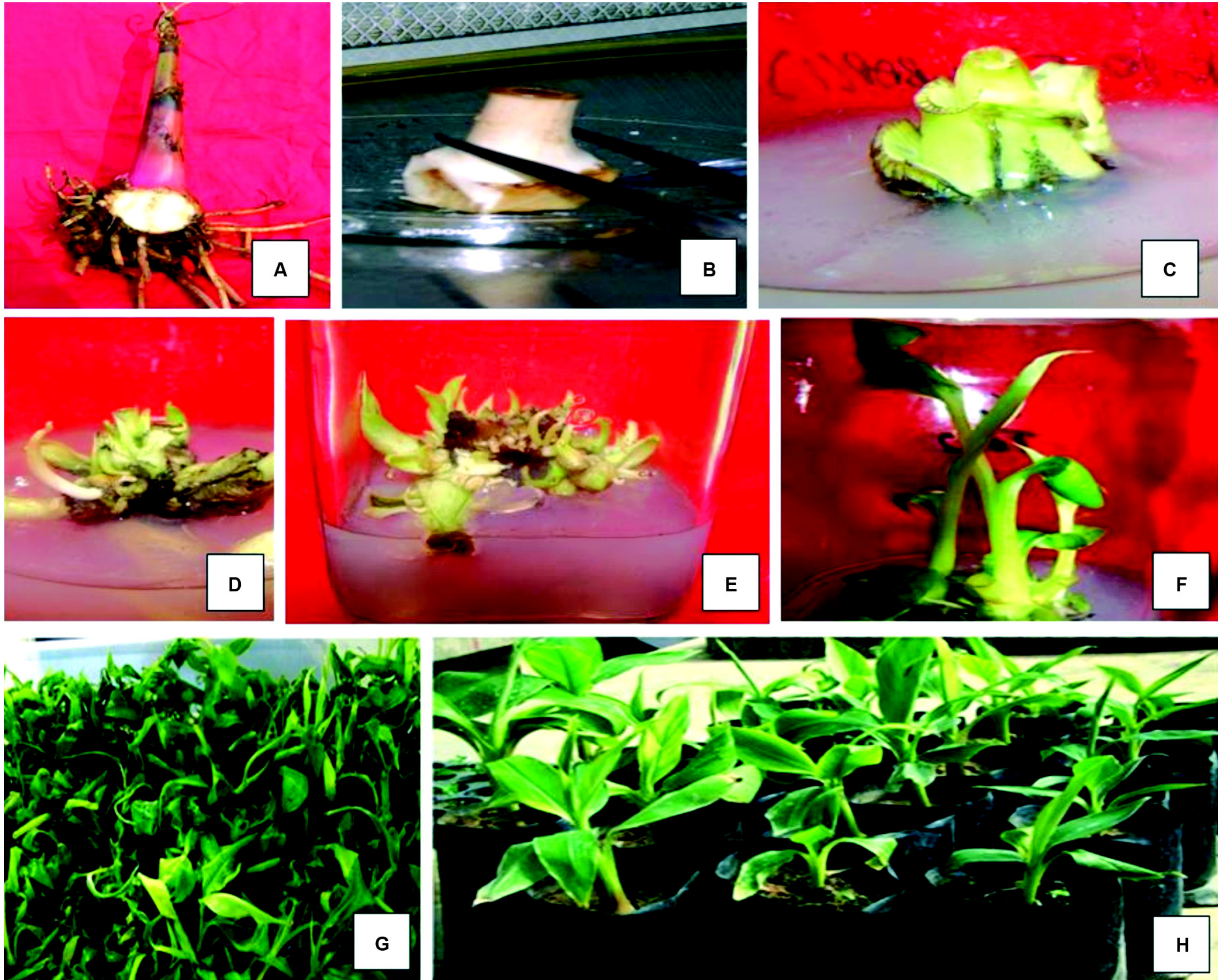

Fig 1 Mass multiplication of banana through micropropagation technique (*A*) Showing suckers of banana *cv.* Grand Naine brought into laboratory, (*B*) shoot-tips used as explants in the present study derived from suckers, (*C*) appearance of green tissue in explant, (*D,E*) axillary shoot proliferation for rapid shoot multiplication, (*F*) shoot elongation on BMM medium (*G*) hardening of plantlets on moist cotton soaked in half MS salts, (*H*) transfer of plantlets from *in vitro* to *in vivo* conditions

10 min. to remove the detergent. These explants were then surface sterilized for 10 min. with 0.1% mercuric chloride. After complete washing with sterile water, explants were trimmed to final size of 3–3.5 cm in the laminar flow cabinet [Fig 1 (B)]. These explants were cultured on Murashige and Skoog basal medium (Murashige and Skoog 1962) containing 5 mg/l BAP (6-benzylaminopurine). After three weeks when growth started, explants were shifted to banana multiplication medium (BMM) (Vuylsteke 1998). Subculturing was done after every three weeks and black tissues were carefully removed before each subculturing. A total of seven subculture cycles were carried out. Number of shoots were counted at the end of each subculture. The shoot multiplication rate was calculated as the number of shoots at the end of subculture divided by the the initial number of shoots.

After the first three weeks of culturing, the external leaf sheaths of shoot-tip explants turned red and then, green [Fig 1 (C)]. Explants showed swelling and unwhorling of leaf sheaths. The base of the explants turned black which may be due to secretion of phenolic compounds. The results on shoot multiplication are given in Table 1. Shoot multiplication occurred through the process of axillary shoot proliferation [Fig 1 (D, E)]. In the presence of high concentration of BAP (5 mg/l) in BMM, apical dominance got inhibited and adventitious buds were formed that resulted in axillary shoot proliferation. Shoot elongation occurred on BMM medium [Fig 1 (F)]. After seven subcultures, the shoots were shifted to rooting medium. Rooting medium was BMM containing 1.0 mg/l IBA (Indole-3-butyric acid). Profuse rooting was observed within two weeks. Tissue culture regenerated

plantlets are usually very fragile and thus, most of these die if directly transferred to the field mainly because of the transplantation shock. Therefore, for improving their survival rate, hardening of plantlets is a very critical step. The plantlets having well developed shoot and root systems were removed from the culture jars and were thoroughly washed under running tap water for one hr. Plantlets were kept over moist cotton soaked in half MS salts under high light intensity for 7–10 days in the incubation room for the elongation of roots and hardening of plantlets [Fig 1 (G)]. Thus, the process of hardening acclimatized the plantlets for their subsequent transfer to soil. The hardened plantlets were then transferred to the normal field soil with farmyard manure in 1:1 ratio in black polyethylene bags and were kept in the glass-house [Fig 1 (H)].

The results showed that one banana shoot tip can produce upto 175.57 mean number of shoots after seven subcultures. Also, all the explants exhibited different response (Table 1) under *in vitro* conditions in terms of shoot multiplication. The explant from sucker number 4 was found to be most responsive as it produced highest number of shoots (190), followed by explant excised from sucker number 6 (189) and explant number 7 (188). The minimum number of shoots (143) were obtained from explant derived from sucker number 1. The differences in growth rate and number of shoots may be due to the different physiological status and response of different suckers. Similar results on variable the rate of shoot multiplication from the suckers of same genotype were also observed by Mendes *et al.* (1999) in banana cultivars, Nanicao and Maca, and by Muhammad *et al.* (2004) in banana *cv*. Basrai. Abdullah *et al.* (1997) reported different rates of multiplication in different *Musa* spp genotypes.

Table 1 Number of shoots and shoot multiplication rate at the end of each subculture from different shoot tips of banana (*Musa acuminata* L.) *cv.* Grand Naine

Sub–culturing	1st	2nd	3rd	4th	5th	6th	7th
Sucker number	Number of shoots (Shoot multiplication rate)						
1	1	2	4	20	80	101	143
	–1	–2	–2	–5	–4	(1.26)	(1.41)
2	2	4	7	21	83	124	150
	–2	–2	–1.75	–3	–3.95	(1.49)	(1.20)
3	1	3	5	10	85	152	163
	–1	–3	–1.66	–2	–8.5	(1.78)	(1.07)
4	3	5	8	17	90	143	190
	–3	–1.66	–1.6	–2.12	–5.29	(1.58)	(1.32)
5	1	3	9	16	81	160	183
	–1	–3	–3	–1.87	–5.06	(1.97)	(1.14)
6	2	4	10	17	74	153	189
	–2	–2	–2.5	–1.7	–4.35	(2.06)	(1.23)
7	2	4	8	22	85	169	188
	–2	–2	–2	–2.77	–3.86	(1.98)	(1.11)
Total	12	25	26	123	578	1 002	1 229
Mean	1.71	3.57	3.71	17.57	82.57	143.14	175.57

In the present investigation, it was observed that the rate of shoot multiplication reached to the maximum level at the end of fifth subculturing (ranging from 3.86 to 8.5), after which the rate decreases. Muhammad *et al.* (2004) recorded the number of shoots for five subculturings in banana *cv.* Basrai. It was also concluded that a fairly good number of shoots can be obtained from different explants with a base size of 9–11 cm diameter.

SUMMARY

An efficient and reproducible micropropagation protocol was developed for mass multiplication of the elite banana (*Musa acuminata* L.) cultivar Grand Naine through axillary shoot proliferation. *In vitro* multiplication of banana was studied. Conventional propagation through suckers has disadvantages like low multiplication rate (5 to 10 in number depending upon variety), lack of uniform plant size and transmission of pathogens from one generation to the next generation. Application of micropropagation technique for large scale production of elite clones is an effective and superior alternative to propagation through conventional cuttings of *Musa* spp. Shoot tips were cultured on MS medium supplemented with 5.0 mg/l BAP. Observations were recorded at the end of each subculture, after three weeks of shifting on a new medium. It was noted that suckers of same genotype exhibited different shoot multiplication rates under *in vitro* conditions. On an average, upto 175.57 shoots were produced from each shoot tip after seven subculturings.

REFERENCES

Abdullah K, Khan I A, Siddiqui S H, Ahmed M and Siddiqui K A. 1997. *In vitro* culture of indigenous and exotic banana clones for maximum multiplication. *Pakistan Journal of Botany* **29**: 143–50.

Alloufa M A I, Macedo C E C, Barroso P A V, Barbalho A D and Oliveira C H B. 2002. Evaluation of the use of inflorescences of banana plant variety 'Pacovan' (*Musa* spp.) as explants source for the culture *in vitro* in the presence of different antioxidant agents. *Ciencia e Agrotecnologia* **26**:1092–6.

Alvard D, Cote F and Teisson C. 1993. Comparison of methods of liquid medium culture for banana micropropagation. *Plant Cell, Tissue and Organ Culture* **32**: 55–60.

Cote F D, Alvard R, Domergue L, Mastache N and Teisson C. 1990. Micropropagation *in vitro* du bananier. *Fruits* **45**: 112–8.

Dale J L. 1987. Banana Bunchy Top: An Economically Important Tropical Plant Virus Disease. *Advances in Virus Research* **33**: 301–25.

Darvari F M, Sariah M, Puad M P and Maziahl M. 2010. Micropropagation of some Malaysian banana and plantain (*Musa* spp.) cultivars using male flowers. *African Journal of Biotechnology* **9**: 2360–6.

Doreswamy R, Rao N K S and Chacko E K. 1983. Tissue-culture propagation of banana. *Scientia Horticulturae* **18**: 247–52.

Drew R A and Smith M K. 1990. Field evaluation of tissue cultured bananas in southeastern Queensland. *Australian Journal of Experimental Agriculture* **30**: 569–74.

Ferreira S A, Trujillo E E and Ogata D Y. 1997. Banana bunchy top virus. *Plant Disease* 12: 1–4.

Israeli Y, Lahav E and Reuveni O. 1995. *In vitro* culture of bananas. *Fruits* **43**: 219–23.

Karan M, Hardy R M and Dale J L 1994. Evidence for two groups of banana bunchy top virus isolates. *Journal of General Virology* **75**: 3541–6.

Ma S S and Shii C T. 1972. *In vitro* formation of adventitious buds in banana shoot apex following decapitation. *Journal of Chinese Society of Horticultural Sciences* **18**: 135–42.

Mendes B M J, Filippi S B, Demetrio C G B and Rodriguez A P M. 1999. A statistical approach to study the dynamics of micropropagation rates, using banana (*Musa* spp.) as an example. *Plant Cell Reports* **18**: 967–71.

Muhammad A, Hussain I, Saqlan Naqvi S M and Rashid H. 2004. Banana plantlet production through tissue culture. *Pakistan Journal of Botany* **36**: 617–20.

Murashige T and Skoog F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* **15**: 473–97.

Novak F J, Afza R, Duren M V, Dallos M P, Conger B V and Xiaolang T. 1989. Somatic embryogenesis and plant regeneration in suspension cultures of dessert (AA and AAA) and cooking (ABB) bananas (*Musa* spp.). *Bio-Technology* **7:** 154–59.

Ranasingh N. 2007. Field diagnosis and management of banana bunchy top disease. *Orissa Review*: 78–80.

Resmi L and Nair A S. 2007. Plantlet production from male inflorescence tips of *Musa acuminata* cultivars from South India. *Plant Cell, Tissue and Organ Culture* **88:** 333–8.

Vishnoi R, Raj S K and Prasad V 2009. Molecular characterization of an Indian isolate of Banana bunchy top virus based on six genomic DNA components. *Virus Genes* **38**: 334–44.

Vuylsteke D. 1998. Shoot-tip culture for the propagation, conservation and distribution of *Musa* germplasm, 82 pp. IITA, Ibadan, Nigeria.