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Cheaper water sources for micropropagation of banana (*Musa acuminata*) cv. 'GRANDE NAINE'

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ABSTRACT : Water source in plant tissue culture production laboratories is from distillation units. The costs of these units are a high and regular maintenance is also economically taxing. Hence, to reduce the cost of production of tissue cultured banana, *in vitro* multiplication and rooting was carried out on a medium prepared with different sources of water *viz.*, millipore filter water, aquaguard filter water, double distilled water, single distilled water and autoclaved potable tap water. Cultures grown on MS medium prepared with aquaguard filter water recorded maximum mean number of shoots/culture (12.50), highest mean shoot length (2.77 cm) and maximum mean number of adventitious buds/culture (8.25) followed by millipore filter water and autoclaved potable tap water. Microshoots cultured on MS medium prepared with aquaguard filter water recorded highest rooting percentage (100 %), maximum number of primary roots/ shoots (9.50) and highest root length (7.00 cm) followed by millipore filter water. Cheaper source of water such as aquaguard filter or even autoclaved potable tap water can be used as low cost alternative water source for successful micropropagation of banana 'Grande Naine'.

KEY WORDS : Aquaguard filter water, Grande Naine, Micropropagation, Millipore filter water, Potable tap water

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Bananas (*Musa* sp.) are important staple crops in tropical and subtropical countries. Their trade also creates a considerable income as a cash crop. *In vitro* propagated plants are increasingly becoming the planting material of choice as they are true to type, free from diseases, uniform, vigorous and yields higher than conventionally propagated suckers. However, growers have to face higher cost and pay up to five times more than for suckers (Robinson, 1996). Water is the main component of all plant tissue culture media. Usually in tissue culture research, distilled or double distilled de-ionized water is used. Distilled water produced through electrical distillation is expensive. In some cases, alternative water sources can be used to lower the cost of the medium. If tap water is free from heavy metals and contaminants, it can be substituted for distilled water (Prakash *et al*., 2004). Tap water has been used for *in vitro* propagation of banana (Ganapathi *et al*., 1995) and ginger (Sharma and Singh, 1995). In rural areas, rain water can be collected in clean glass jars and used for tissue culture. In Bangladesh the changeover of water distillation from electrical to gas operated unit reduced the cost from US $ 60 to US $ 5 per month for producing 50-60 litre water per day (Prakash *et al*., 2004).

Considering above facts, various sources of water namely millipore filter water, double distilled water, single distilled water, autoclaved potable tap water, potash alum treated potable tap water and aquaguard filter water were used in the present study to identify cheaper sources of water.

RESEARCH METHODS

Multiple shoot cultures (Fig.1a-f) were established on Murashige and Skoog's (MS) medium supplemented with 2 mg/l benzyl aminopurine (BAP) and 75 mg/l adenine sulphate as reported earlier by Besagarahally (1996). The cultures so established were used for further studies. *In vitro* shoot proliferation and rooting studies were carried out by using media prepared with different sources of water *viz*., millipore filter water, aquaguard filter water, double distilled water,

single distilled water, potash alum treated potable tap water and autoclaved potable tap water. Cultures were incubated for twelve weeks (the cultures were transferred to fresh media after six weeks) maintaining standard culture conditions of 25 ± 2^0 C temperature, 85% RH and photoperiodic cycle of 16 hours light and 8 hours dark period. For rooting, elongated shoots were transferred on to a rooting medium containing 2 mg/l IBA+1mg/l NAA + 2.5 g/l activated charcoal for four weeks maintaining standard culture conditions as mentioned above. The *in vitro* rooted plantlets were acclimatized under green house and shade house for twelve weeks. At the end of experiments, morphological characteristics (number of shoots/explant, shoot length, number of leaves, roots/shoots, fresh weight etc.) were measured. An analysis of variance (ANOVA) was conducted on data concerning shoot and root morphological parameters using the statistical program wax vms fortran.

RESEARCH FINDINGS AND DISCUSSION

Cultures grown on MS medium prepared with aquaguard filter water (Table 1, Fig. 2) recorded significantly maximum number of shoots/explant (12.50), higher shoot length (2.77 cm) and maximum number of adventitious buds/explant (8.25) followed by cultures raised on media prepared with millipore filter water and autoclaved potable tap water (Table 1, Fig. 3). Microshoots cultured on MS medium prepared with aquaguard filter water (Table 2, Fig. 2) recorded 100 % rooting, maximum number of primary roots/shoots (9.50) and higher root length (7.00 cm). It is evident that the explants cultured on MS medium prepared with aquaguard filter water showed superior performance in terms of shoot and root growth as compared to membrane filter water and distilled water. This promotary influence may be attributed to the presence of growth promoting factors in aquaguard water in sufficient concentrations. These findings are in close agreement with the reports of Ziv (2008) who opined that in several plants *in vitro* culture system the use of double distilled water may affect plant growth and structure due to the lack or low levels of silicone.

The results of the present study also suggest that substitution of distilled water which is required in plenty amount for tissue culture works even by autoclaved potable tap water is also possible. Similar findings were also reported in banana (Ganapati *et al*., 1995, Das and Gupta 2009a and b), ginger (Sharma and Singh, 1995), strawberry (Kaur *et al*., 2005) and *Centella asiatica* (Raghu *et al*., 2007).

In the present study, the growth of the multiple shoots on medium prepared autoclaved potable tap water was, however, comparatively slow and the subculture passage was extended to six weeks. This may be attributed to the composition of tap water. These observations are in accordance with the reports of Ganapati *et al*. (1995). Das and Gupta (2009 a and b) reported that the problem of sedimentation in using tap water that had to be solved by pre-boiling water for half an hours and then allowing it for sedimentation for several hours. This boiled water could be safely used for both in multiplication and rooting media. It is, therefore, evident that the quality of tap water was

Table 1: Effect of different water sources on shoot multiplication of banana 'Grande Naine'

Water sources	Mean no. of shoots/explant	Mean shoot length (cm)	Mean no. of adventitious buds/ explant	Mean no. of leaves/shoot
Millipore filter water	7.95	2.57	5.45	2.52
Double distilled water	4.50	2.44	4.60	2.51
Single distilled water	5.60	2.11	6.40	2.30
Autoclaved potable tap water	7.70	2.83	5.86	2.56
Aquaguard filter water	12.50	2.77	8.25	2.26
S.E. ±	1.59	0.14	0.91	
C.D. (P=0.01)	4.78	0.42	2.75	NS

NS =Non-significant

Table 2 : Effect of different water sources on *in vitro* rooting of banana 'Grande Naine'

Water sources	Rooting percentage	Mean no. of roots/shoots	Mean root length (cm)	Mean no. of secondary roots/shoots
Millipore filter water	100	7.90	7.09	36.35
Double distilled water	100	7.30	5.80	23.75
Single distilled water	100	8.95	4.89	19.95
Autoclaved potable tap water	100	7.20	5.11	14.70
Aquaguard filter water	100	9.50	7.00	21.50
S.E.±			0.43	3.41
C.D. (P=0.01)	NS	NS	1.31	10.29

NS=Non-significant

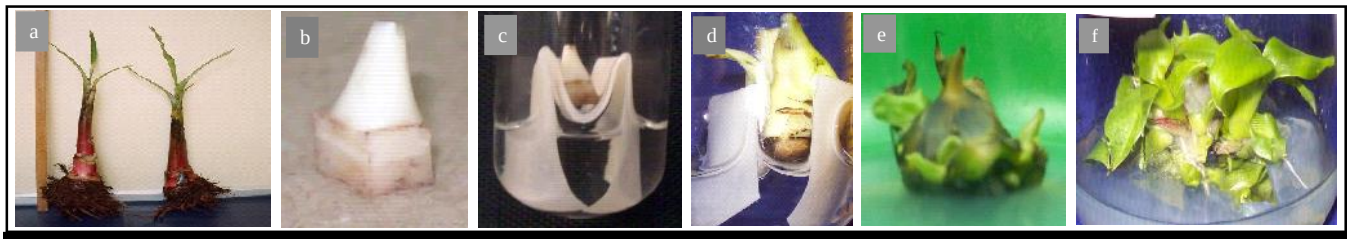

Fig. 1 : Establishment of aseptic culture by shoot-tip culture. a) Sword suckers, b) Shoot-tip, c-d) Liquid shoot-tip culture, e) Aseptic shoot-tip culture on semisolid medium, f) Multiple shoot clump

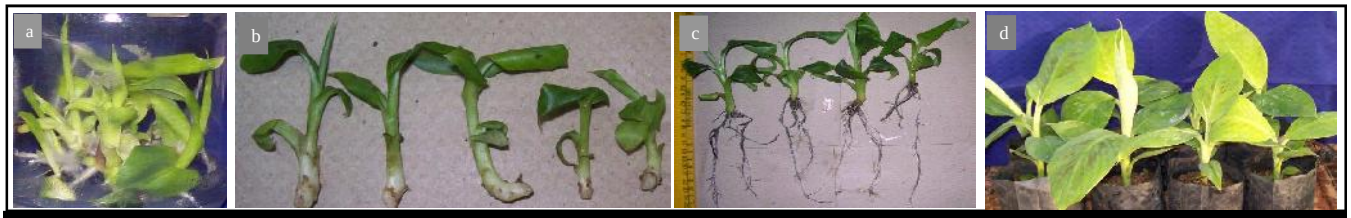

Fig. 2 : Micropropagation of banana 'Grande Naine' using aquaguard filter water. a) Multiple shoot clump, b) Micro-shoots, c) *In vitro* rooted plantlets, d) Hardened plantlets

Fig. 3 : Micropropagation of banana 'Grande Naine' using autoclaved potable tap water. a) Multiple shoot clump, b) Micro-shoots, c) *In vitro* rooted plantlets, d) Hardened plantlets

Fig. 4 : Poor growth and drying of leaf tips of cultures grown on the medium prepared with tap water treated by potash alum

important when used for tissue culture. Mahmood (2004) opined that the chemical quality of tap water might not be the same at all places and the parameters considered suitable for drinking were not necessarily acceptable for plant growth.

Table 3: Differential cost for a litre media with different water sources

Water sources	Price / l medium (Rs.)	Cost reduction over control (%)
Double distilled water	11.77 (Control)	0
Membrane filter water	9.62	18.26
Single distilled water	5.88	50.04
Autoclaved potable tap water	1.00	89.60
Aquaguard filter water	1.00	89.60

However, in the present study no such problem of precipitation was noticed but the cultures inoculated on medium prepared with potable tap water treated by potash alum showed poor growth and there was a problem of drying of leaves tips (Fig. 4). This may be probably due to presence of toxic substances in tap water treated with potash alum.

The results of the present study indicate that the cheaper sources of water such as aquaguard or even autoclaved potable tap water could be best used for cost reduction of micropropagation banana cv. 'Grande Naine' plantlets without compromising with the plantlet quality. With regard to cost (Table 3), both of these cheaper sources of water reduced the cost by 89.60 per cent when compared with double distilled water.

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