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Research Note

Effect of Sucrose and Growth Regulator's Level on Ginger Micropropagation

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ABSTRACT

Ginger is most important cash crop of the hilly region of Nepal. However, availability of disease free planting material (rhizome) is the major problem faced by Nepalese farmers. Tissue culture is the only option to produce disease free rhizome of ginger. Suitable culture media combination is most important for the production of planting material in ginger through tissue culture. Therefore, effect of different level of sucrose and growth regulators on micro-propagation of ginger was studied using local collection 'Kaski Local'. Early stage bud was used as explant. MS basal media with different level of sucrose and growth regulators was used as tissue culture media. 30 g/L sucrose, 30 g/L sucrose+5mg/L BA, 30 g/L sucrose+5 mg/L BA+0.5 mg/L NAA, 60 g/L sucrose+5mg/L BA, 60 g/L sucrose+5 mg/L BA+0.5mg/L NAA, 90 g/L sucrose+5 mg/L BA was used in this study. The explants were surface sterilized, cultured and incubated at 25±2°C, 90-95% relative humidity and 14:10 hours light:dark photoperiod for 8 weeks. Increased level of the sucrose increased the rhizome weight, however, addition of NAA produced more positive effect for this. MS basal media with 60 g/L sucrose+5 mg/L BA+0.5 mg/L NAA produced higher rhizome weight.

Keywords: Ginger, *Zingiber officinale*, In-vitro rhizome, Sucrose, NAA, BA, Micropropagation

सारांश

अदुवा नेपालको लागि महत्वपूर्ण नगदेबाली भएतापनि यसमा बीउ (गानो) बाट सर्ने विभिन्न रोगहरुको कारणले गर्दा तन्तु प्रविधिको प्रयोग गरेर साना गानाहरुको उत्पादन गरी बीउको रुपमा प्रयोग गर्न सके नेपालमा अदुवा उत्पादन बढाउन सकिन्छ । त्यसैले कास्की स्थानिय भन्ने अदुवाको जातको तन्तु प्रविधि प्रयोग गरी गानो उत्पादन गर्दा कति मात्रामा चिनी र विभिन्न हर्मोनहरु प्रयोग गर्दा सबैभन्दा राम्रो नतिजा आउछ भनी यो अनुसन्धान गरिएको थियो । अक्जिन (एन ए ए), साइटोकाइनिन (बि ए) र चिनीको उचित प्रयोग गरेमा गानोको आकार बढेको पाइयो र यस प्रविधिलाई बीउको रुपमा प्रयोग गर्न सकिने साना गानाहरु उत्पादन गर्न प्रयोग गर्न सकिने देखियो ।

INTRODUCTION

Ginger (*Zingiber officinale* Roscoe) is important agricultural commodity in Nepal. Area, production and yield of ginger in Nepal is 24226 ha, 276150 MT and 11.40 T/ha respectively (MoAD 2014). It is already the fourth biggest ginger exporter in the world, with about 70% of domestic production exported to India (FAO 2015). It could be one of the options for the farmers to develop their own micro-enterprises in Himalayan regions (Bhatia et al 1999).

Ginger is gaining popularity as high value cash crop in the country. The repeated planting of the rhizomes become the primary source of pathogen inoculum in ginger (Pandey 1997). Though ginger yields excellent cash returns in spite of serious losses from bacterial wilt, fusarium yellows and root knot nematodes, Hosoki and Sagawa (1977) estimated that a three- fold increase in rhizome yield could be attained with sound disease control practices. Dohroo (1989) has reported that about 87% of the field infection with *Fusarium oxysporium* f. sp. *zingiberi* is transmitted through infected rhizomes. The rhizome rot caused by *Pythium* spp is major problem in western hill of Nepal causing yield loss 5-100% (HARP 2001).

Disease transmitted by planting material of these commodities was major production constraint. Niroula (1998) reported the suitable healthy planting material on ginger is one of the problems for this crop cultivation in Nepal. Thus, disease free planting material is most important in ginger.

Ginger plants could be produced all year round in-vitro and used as seed rhizomes (Pandey 1997). He suggested that the in-vitro multiplication of the plants and in-vivo rhizome production should run simultaneously to get higher yield. De Lange et al (1987) reported that they have successfully eliminated root knot nematodes from heavily infected rhizomes through in vitro culture of shoot tips. Bacteria, fungi and viruses elimination are very commonly used in micro-propagation (Sahavacharin 1995).

Since micro-propagation and other tissue culture techniques are most efficient to produce the disease free planting materials, it will be the best option to produce disease free planting material on ginger. High speed mass production and distribution of the disease free planting materials will increase the production and productivity of ginger. Suitable culture media combination is most important for the production of planting material in ginger through tissue culture.

MATERIALS AND METHOD

Effect of different level of sucrose and growth regulators on micro-propagation of ginger was studied using local collection 'Kaski Local' during August-March 2007. Early stage rhizome bud measuring 1.5 to 2 cm was used as explant. MS basal media (Murashige and Skoog 1962) with different level of sucrose and growth regulators was used as tissue culture media. 30 g/L sucrose (T1), 30 g/L sucrose+5 mg/L BA (T2), 30 g/L sucrose+5 mg/L BA + 0.5 mg/L NAA (T3), 60 g/L sucrose+5 mg/L BA (T4), 60 g/L sucrose+5 mg/L BA+0.5 mg/L NAA (T5), 90 g/L sucrose + 5 mg/L BA (T6) was used in this study. The explant was sterilized with 4% sodium hypochlorite solution for 3 minutes. It was washed with distilled water for 3 times and cultured on test tube with 20 ml specified media. It was incubated at 25±2^{o}C, 90-95% relative humidity and 14:10 hours light:dark photoperiod for 8 weeks. Observation was taken after 8 weeks of incubation. Each treatment consists of 25 test tubes with four replications. Completely Randomized Design (CRD) was used for variance analysis using MSTATC.

RESULTS

Cytokinin (BA) increased the plantlet vigor and its proliferation (Figure 1 and Table 1). Villamor (2012) also observed better shoot proliferation only when growth hormone BA was added at concentration of 4-6 mg/l. MS basal media with 60 g/L sucrose+5 mg/L BA produced higher shoot length, leaf no., root no, and weight of total vegetative part (except rhizome). Similar result of increased shoot multiplication was also observed by Kambaska and Santilata (2009) while using 2.0 mg/l BAP + 0.5 mg/l NAA with MS basal medium.

Similarly, MS basal media with 60 g/L sucrose+5mg/L BA+0.5mg/L NAA produced higher rhizome weight followed by MS basal media with 60 g/L sucrose+5mg/L BA and MS basal media with 90 g/L sucrose+5 mg/L BA (Table 1).

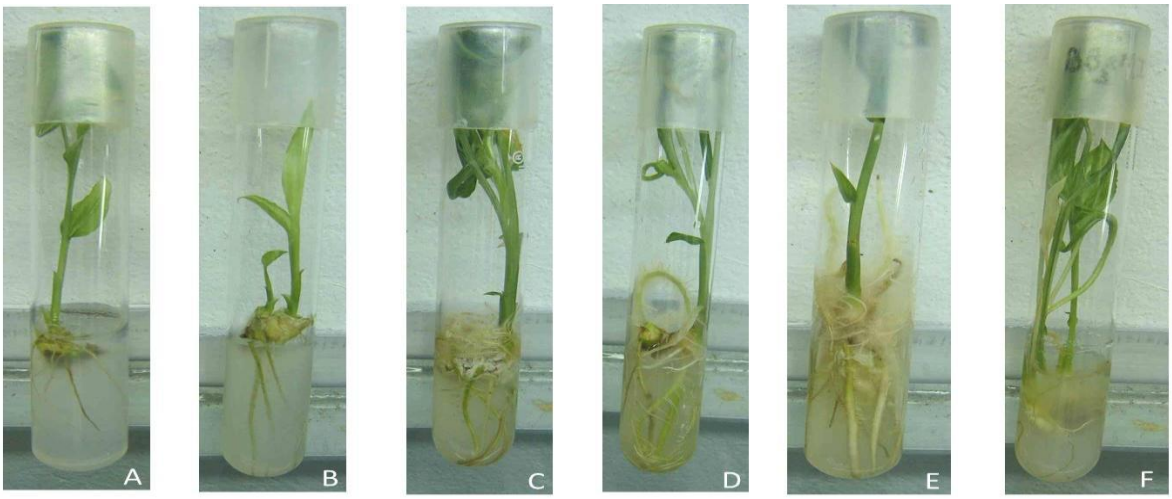

Figure 1. Effect of different level of sucrose and growth regulators on in-vitro plant growth for ginger: 30 g/L sucrose-T1(A), 30 g/L sucrose+5 mg/L BA-T2(B), 30 g/L sucrose+5 mg/LBA+0.5 mg/L NAA-T3(C), 60 g/L sucrose+5 mg/L BA-T4(D), 60 g/L sucrose+5 mg/L BA+0.5mg/L NAA-T5(E), 90 g/L sucrose+5 mg/L BA-T6(F).

Table 1. Effect of different level of sucrose and growth regulators on ginger micro-propagation

Treatment	Shoot No./explant	Shoot length (cm)	Leaf No./shoot	Root No./shoot	Rhizome weight (g)	Wt. of vegetative part (except rhizome) (g)	Root weight (g)
T1	2.14	16.47	6.71	6.00	0.541	0.52	0.315
T2	1.27	17.34	7.43	9.29	0.507	1.71	0.588
T3	1.71	12.43	5.43	6.71	1.111	0.44	0.156
T4	2.0	20.27	9.14	9.71	1.135	1.72	1.142
T5	1.48	18.76	6.29	10.57	1.254	0.99	1.449
T6	2.14	9.47	4.57	8.71	1.135	0.32	0.827
P	0.746	<0.001	<0.001	0.031	0.090	0.014	0.012
LSD, 5%	1.453	4.078	1.525	3.030	0.644	0.888	0.644
CV, %	73.7	23.7	21.2	32.7	57.1	78.6	70

DISCUSSION

Cytokinin enhances shoot proliferation and auxin enhances the root proliferation (Wang and Charles, 1991). The carbon source (sucrose) is important for photosynthesis and development of storage organ (rhizome). Therefore, their combination was essential for growth and development of rhizome in ginger. Zheng et al (2008) also concluded that optimal condition for micro-rhizome production was MS media with 80 g/l sucrose, 1.33-2.35 g/l GA, 0.49-0.66 g/l KT and 0.62 g/l NAA. In this study, suitable combination of BA, NAA and sucrose produced the in-vitro rhizome instead of Pandey (1997) result. MS basal media with 60 g/L sucrose+5mg/L BA+0.5 mg/L NAA produced higher rhizome weight. Therefore, proper combination of sucrose, auxin and cytokinin is important for in-vitro production of seed ginger.

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