Hibiscus syriacus Plant Regeneration From Callus©

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The regeneration ability of callus derived from three different plant fragments of eastern hibiscus (*Hibiscus syriacus*) was investigated. Callus was initiated from three different, 1-month-old seedling fragments (root, hypocotyl, and cotyledon) on Murashige-Skoog medium supplemented with 2,4-D or NAA, and BA, 2iP, or kinetin. The largest mass of callus was observed on medium containing 2,4-D and 2iP. The highest number of shoots, leaves, and roots was achieved from callus induced on NAA and BA medium and regenerated on medium with 0.1 mg liter^{-1} of BA.

INTRODUCTION

Hibiscus syriacus (Malvaceae) is a woody ornamental, used in the landscape as a single shrub, in groups of shrubs, and to form hedges. It is grown in a wide range of latitudes due to its low soil requirements, heat and cold tolerance, and decorative characteristics (Egolf, 1988). In vitro propagation of the species is difficult because of the presence of endogenous pathogens and limited ability to proliferate shoots. Other Malvaceae species, such as, cotton (*Gossypium hirsutum*; Gupta et al., 1997), okra (*Abelmoschus esculentus*; Mangat and Roy, 1986; Roy and Mangat, 1998), and kenaf (*H. cannabinus*; Cristofari et al., 1988) have been propagated in vitro with variable success.

Murashige and Skoog (MS) medium enriched with BA and kinetin were successfully used for callus induction in okra from hypocotyl and cotyledon explants. However, a combination of the two cytokinins with IAA or NAA failed to produce callus on cotyledon explants and only a small number of hypocotyl cultures developed callus (Mangat and Roy, 1986; Roy and Mangat, 1998). In studies on in vitro propagation of *Agave sisalana*, regenerable callus from rhizome and stem explants was derived on media with various salt combination, enriched with 2,4-D (0.1 to 2.0 mg liter^{-1}) or BA (0.1 to 5.0 mg liter^{-1}; Nikam, 1997).

The objective of this experiment was to test two different auxins (2,4-D and NAA) and three different cytokinins (BA, 2iP, and kinetin) for their potential to produce callus and regenerate plants from explants of *H. syriacus* 'Aphrodite'.

MATERIAL AND METHODS

Seeds of *H. syriacus* 'Aphrodite' were aseptically germinated on half-strength MS medium (Sigma M5524), supplemented with sucrose (15 g liter^{-1}) and solidified with agar (8 g liter^{-1}). Callus was initiated from three different, 1-month-old seedling fragments (root, hypocotyl and cotyledon), about 1 cm long. The explants were

cultured on media with two different auxins (2,4-D and NAA, both 0.3 mg liter^{-1}), three different cytokinins (BA, 2iP, and kinetin, all 0.1 mg liter^{-1}) and without hormones. To all callus induction media (pH 5.8), myo-inositol (100 mg liter^{-1}), thiamine (0.4 mg liter^{-1}), sucrose (30 mg liter^{-1}), nicotinic acid and pyridoxine (both 0.5 mg liter^{-1}) and agar (8 g liter^{-1}) were added. Callus induction was carried out in 100 mm × 200-mm petri dishes placed in a growth chamber at 24 ± 2°C and 16-h photoperiod.

Callus was regenerated on McCown media (Sigma M6774), supplemented with KNO_3 (800 mg liter^{-1}), glycine (2 mg liter^{-1}), MS vitamins (Sigma M7150), sucrose (30 mg liter^{-1}), three different cytokinins as BA (0.1 and 1.0 mg liter^{-1}), kinetin and

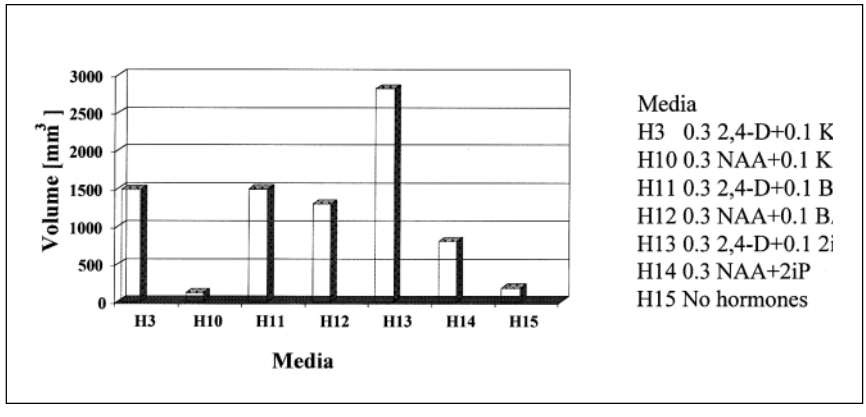

Figure 1. Volume of callus produced by *Hibisucs syriacus* explants cultured on media with 2,4-D and NAA and three different cytokinin types.

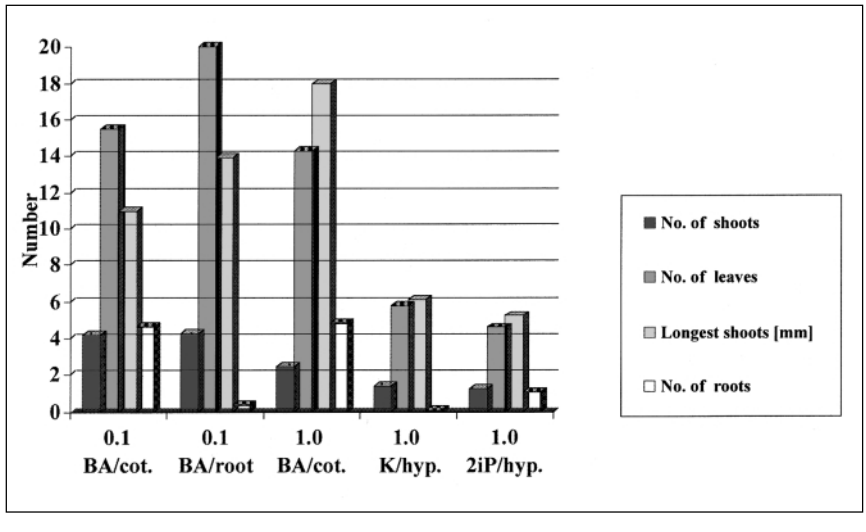

Figure 2. Number of shoots, leaves, and roots observed on regenerated cultures of *Hibiscus syriacus*.

2iP (both 1.0 mg liter^{-1}), and solidified with agar (8 g liter^{-1}). The first subculture was carried out in petri dishes; the following passages were to test tubes. The cultures were grown in a growing room, with a 16-h photoperiod.

Callus growth was evaluated after approximately 70 days after initiation as were the number of shoots, leaves, and roots after 180 days of cultivation on regeneration media. The subculture interval was 45 days. Each treatment had 5 petri dishes with 6 explants per dish. After passage to test tubes, 25 explants per treatment were evaluated.

RESULTS AND DISCUSSION

The largest callus volume (2822.2 mm^3) was observed on medium containing 2,4-D and 2iP (0.3 mg liter^{-1} and 0.1 mg liter^{-1}), respectively, (Fig. 1). The smallest amount of callus was induced on NAA and kinetin medium (120.8 mm^3) and on medium without hormones. Hypocotyl cultures were the most effective explants for callus production.

After two passages to regeneration media, the most prolific and healthy looking cultures were observed on media with 0.1 mg liter^{-1} of BA and 1.0 mg liter^{-1} of kinetin and 2iP. Only cultures grown on those media were transferred to test tubes containing the same media and 1½ months later, they were evaluated for number of shoots, leaves, and roots produced. The highest number of shoots (4.2 per culture) was observed on cultures grown on 0.1 mg liter^{-1} of BA (Fig. 2). The cultures originated from callus induced on cotyledon and root explants, grown on NAA and BA (0.3 and 0.1 mg liter^{-1}, respectively) medium. Malvaceae species are difficult in micropropagation. The number of primary shoots produced in cotton (*G. hirsutum*) on MS medium with much higher BA content (~5 mg liter^{-1}) was 0.5 to 4.4 per culture (Gupta et. al., 1997). The longest shoots (17.9 mm) were developed on cultures regenerated on medium with 1.0 mg liter^{-1} of BA.

The highest leaf number (20 per culture) was observed on callus initiated from root explants, cultured on NAA and BA (0.3 and 0.1 mg liter^{-1}) and regenerated on BA-supplemented medium (0.1 mg liter^{-1}). Also, this medium combination promoted the highest number of roots (4.8 and 4.6 per culture) irrespective of the cytokinin concentration during regeneration. In studies in okra regeneration, Roy and Mangat (1988) observed 3 to 6 leaves per shoot on explants grown on media with 1.0 mg liter^{-1} of BA and various concentrations of silver nitrate.

Regenerated plants were planted into growing trays [perlite and vermiculite (1 :1, v/v) and 30% (wt. per vol.) of MS basal salts] and after 30 days of cultivation in the growing room, they were transplanted to a greenhouse in 4 × 4-inch pots and kept under misting for 4 days. One and a half months later, 100% of plants developed additional leaves and elongated shoots.

NAA and BA callus induction medium and BA supplemented regeneration medium were the most suitable for micropropagation of *H. syriacus* 'Aphrodite' and offered a great potential for mass propagation of eastern hibiscus.

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The Use of Shoot Tip Culture in Foundation Plant Materials Service Programs©

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Foundation Plant Materials Service (FPMS) is a self-supporting service department in the College of Agricultural & Environmental Sciences at the University of California, Davis, which produces, tests, maintains, and distributes disease-tested propagating material for use by California nurseries (For additional information, see the FPMS website at http://fpms.ucdavis.edu). At this time, FPMS is responsible for grape (*Vitis*), strawberry (*Fragaria*), fruit tree, nut tree, rose (*Rosa*), and sweet potato (*Ipomoea batatas*) clean-stock programs. Since its inception, scientists working with FPMS have pioneered techniques in grapevine virus disease detection and elimination (Nyland and Goheen,1969; Alley and Golino, 2000). These are the two cornerstones of the clean stock program: the ability to determine whether target virus(es) or other pathogens are present and the ability to eliminate virus from diseased stock.

Micro-shoot tip culture is the method of choice to eliminate virus(es) and other pathogens from many plant species (Baker, 1962; Langhans, 1977; Murashige, 1974). The micro-shoot tip culture technique has the advantage of regenerating a single plant from a single, minuscule (approximately 0.5 mm) shoot. The combination of low hormone levels combined with a minimum time in culture reduces the chance of mutation and regeneration of an off-type plant. At the same time, many pathogens, including viruses, are eliminated by this technique. Micro-shoot tip culture is used as the preferred method of virus elimination for our sweet potato, strawberry, and grape programs. We are also working to optimize procedures for rose shoot tip culture.

In this paper, we will provide the details of our current procedures for shoot tip therapy and briefly review the background of each crop program. Our therapy programs for sweet potatoes, strawberries, and grapes have been in place for many years. The rose effort is new and not yet fully successful.

GENERAL PROTOCOLS FOR SHOOT TIP CULTURE

Each plant species is harvested as described in the following individual crop sections. Tissue is surface sterilized by submersion in 10% commercial bleach plus 1 drop (~0.1 ml) of dishwashing liquid for 10 min. Tissue is removed under aseptic