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In vitro culture establishment techniques from field-grown *Heliconia* plants

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ABSTRACT

An experiment was conducted to establish *in vitro* cultures from field grown plants of *Heliconia*. The most responsive explant was shoot tip. For surface sterilization of shoot tip explants, double sterilization with 0.10 per cent mercuric chloride for 10 minutes followed by dipping in 0.05 per cent mercuric chloride for 5 minutes after trimming, gave the best results. Media supplementation with 0.05 per cent activated charcoal resulted in earlier shoot initiation and better survival percentage. Among the different injury treatments tried, longitudinal cutting of the shoot tip with apical dome into two halves yielded the highest number of axillary buds.

Key words : Heliconia, Culture establishment, Shoot tip explant, Surface sterilization, Physical injury treatments, Activated charcoal

Heliconias (*Heliconia* spp.) are attractive tropical plants with banana-like leaves and beautiful, long lasting inflorescences. They possess subterraneous rhizomes, commonly used for propagation from which new buds are developed. This system of vegetative propagation, produces a reduced number of plants and is also prone to serious risks of bacterial disease dissemination. Techniques to mass multiply the planting material through micropropagation serve as a means to bring down the cost of cultivation of elite varieties. However, special precautions need to be taken when explants are derived from field grown materials. Since the shoot tips emerge from below the ground level, they accumulate lot of soil and dirt. The presence of endophytic microorganisms such as *Ralstonia* sp. presents an obstacle even for tissue culture. Hence, thorough and effective surface sterilization is inevitable.

Success of *in vitro* propagation primarily depends on the proper selection of explants. Shiau *et al*. (1999) used terminal shoot tip explants for *in vitro* propagation of *H. psittacorum* cv. RHIZOMATOSA. When shoot buds were used as explants, Talukdar *et al*. (2002) obtained shooting and rooting in *H. psittacorum*. Babu (2005) tried shoot apex, rhizome bits, leaf segments and root segments for culture establishment in *H. psittacorum* cv. DEEP ORANGE and shoot apex was found to be most effective.

The technique of splitting banana shoot tips longitudinally through their apex in order to induce multiple shoot formation was first described by De Guzman *et al*. (1980). Dividing the apical dome into two halves and culturing each half separately was found to be the best one in enhancing the release of axillary buds. By longitudinally splitting the shoot through the apex, individual shoots of banana were induced to form multiple shoot clusters by several workers like Cronauer and Krikorian (1984) and Bhaskar (1991).

In *H. psittacorum* cv. DEEP ORANGE, the highest survival percentage (45.00) was recorded with absolute alcohol one minute wash + treatment with mercuric chloride (0.10 per cent) for 10 minutes, followed by the treatment with 0.05 per cent mercuric chloride for 10 minutes after trimming of the explants (Babu, 2005). In gladiolus, Misra and Singh (1999) recommended treatment with 0.10 per cent mercuric chloride for ten minutes for surface sterilization. In *Polianthes tuberosa*, Krishnamurthy *et al*. (2001) reported the effectiveness of 0.10 per cent mercuric chloride.

Media browning and explant blackening were severe during the culture establishment phase of *Heliconia*. Hosni (2001) which suggested the application of 1.00 per cent activated charcoal to the culture medium for controlling oxidative browning in *Strelitzia reginae*. The decreased oxidation level of banana (cv. GRAND NAIN) shoots in the presence of 0.30 per cent activated charcoal, reported by Costa *et al*. (2006)

In this study an attempt has been made to initiate *in vitro* culture from field-grown *Heliconia* plants belonging to three varieties belonging to three distinct groups *viz*., St. Vincent Red (*Heliconia psittacorum*), Golden Torch Adrian (*Heliconia psittacorum* x *Heliconia spathocircinata*) and Sexy Pink (*Heliconia chartacea*).

MATERIALS AND METHODS

Two types of explants *viz*., shoot tips and rhizome bits were tried for *in vitro* establishment. Shoot tips were collected from young actively growing plants. The selected plants were dug out, detopped and the shoot tips were reduced to a length of about 2 cm using surgical