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Micropropagation of *Heliconia psittacorum* var. St. Vincent Red

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Abstract : Shoot tips of *Heliconia psittacorum* var. St. Vincent Red were successfully established in the MS medium supplemented with BA 5.00 mg l^{-1}. Addition of BA 2.00 mg l^{-1} to the MS medium gave better results with respect to multiple shoot proliferation. Supplementation of NAA 0.50 mg l^{-1} to the basal medium gave the highest rooting response. The rooted plantlets were successfully planted out in sand medium.

Key words : *Heliconia psittacorum*, St. Vincent Red, Micropropagation, Shoot tips

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Heliconias are among the most popular garden plants, both for the ease with which they are grown and the sheer magnificence of the blooms. The variety St. Vincent Red belongs to *Heliconia psittacorum* group with small erect inflorescence. The brilliant colours of their blooms have made them exceptionally popular as cut flowers and also as landscape plants. These plants are usually propagated by rhizomes or suckers. This method of propagation has got high risk of bacterial disease transmission and moreover, inorder to meet the increasing demands of the planting material, a reliable and faster multiplication method is necessary. Hence, the present study was undertaken to standardize *in vitro* propagation techniques in this variety using shoot tip explants.

RESEARCH METHODS

The present experiment was conducted in the Department of Pomology and Floriculture and the Department of Plant Biotechnology, College of Agriculture, Vellayani, Thiruvananthapuram during 2006-2008. The explants (shoot tips) were collected from young actively growing plants and were washed thoroughly in running tap water to remove all the dirt and soil particles adhering to them. They were reduced to a length of about 2 cm using surgical blade, retaining the apical dome (1 cm). Thereafter, they were kept immersed in water with a few drops of wetting agent, labolene for half an hour. It was immediately followed by rinsing in distilled water to remove traces of labolene. Further sterilization procedures were carried out inside laminar air flow chamber, where the shoot tips were subjected to surface sterilization using mercuric chloride 0.10 per cent for 10 minutes followed by dipping in mercuric chloride 0.05 per cent for 5 minutes after trimming. Thereafter, they were transferred carefully to sterile blotting paper placed over sterile Petri plate to remove excess water and were then inoculated into the culture establishment medium using sterile forceps.

The basal medium used for *in vitro* culture was MS (Murashige and Skoog) medium. For culture establishment, cytokinins like BA (1.00-10.00 mg l^{-1}), kinetin (5.00 and 10.00 mg l^{-1}) and 2 ip (5.00 and 10.00 mg l^{-1}) alone or in combination with auxins namely NAA (0.50 mg l^{-1}) and IAA (0.05 mg l^{-1}) and gibberellic acid (2.00 mg l^{-1}) were tried (Table A). Activated charcoal @

Table A : Treatments tried to study the effect of plant growth regulators on culture establishment in heliconia (Medium – MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1} + AC 0.05 %)

Treatment No.	Plant growth regulators (mg l^{-1})
HCE_1	BA 1.00
HCE_2	BA 2.00
HCE_3	BA 3.50
HCE_4	BA 5.00
HCE_5	BA 5.00 + IAA 0.05
HCE_6	BA 5.00 + IAA 0.05 + GA_3 2.00
HCE_7	BA 5.00 + NAA 0.50
HCE_8	BA 5.00 + NAA 0.50 + GA_3 2.00
HCE_9	BA 5.00 + IAA 0.05 + NAA 0.50 + GA_3 2.00
HCE_{10}	BA 10.00
HCE_{11}	BA 10.00 + IAA 0.05
HCE_{12}	BA 10.00 + IAA 0.05 + GA_3 2.00
HCE_{13}	BA 10.00 + NAA 0.50
HCE_{14}	BA 10.00 + NAA 0.50 + GA_3 2.00
HCE_{15}	BA 10.00 + IAA 0.05 + NAA 0.50 + GA_3 2.00
HCE_{16}	Kinetin 5.00
HCE_{17}	Kinetin 5.00 + IAA 0.05
HCE_{18}	Kinetin 5.00 + IAA 0.05 + GA_3 2.00
HCE_{19}	Kinetin 5.00 + NAA 0.50
HCE_{20}	Kinetin 5.00 + NAA 0.50 + GA_3 2.00
HCE_{21}	Kinetin 5.00 + IAA 0.05 + NAA 0.50 + GA_3 2.00
HCE_{22}	Kinetin 10.00
HCE_{23}	Kinetin 10.00 + IAA 0.050
HCE_{24}	Kinetin 10.00 + IAA 0.05 + GA_3 2.00
HCE_{25}	Kinetin 10.00 + NAA 0.50
HCE_{26}	Kinetin 10.00 + NAA 0.50 + GA_3 2.00
HCE_{27}	Kinetin 10.00 + IAA 0.05 + NAA 0.50 + GA_3 2.00
HCE_{28}	2 ip 5.00
HCE_{29}	2 ip 5.00 + IAA 0.05
HCE_{30}	2 ip 5.00 + IAA 0.05 + GA_3 2.00
HCE_{31}	2 ip 5.00 + NAA 0.50
HCE_{32}	2 ip 5.00 + NAA 0.50 + GA_3 2.00
HCE_{33}	2 ip 5.00 + IAA 0.05 + NAA 0.50 + GA_3 2.00
HCE_{34}	2 ip 10.00
HCE_{35}	2 ip 10.00 + IAA 0.05
HCE_{36}	2 ip 10.00 + IAA 0.05 + GA_3 2.00
HCE_{37}	2 ip 10.00 + NAA 0.50
HCE_{38}	2 ip 10.00 + NAA 0.50 + GA_3 2.00
HCE_{39}	2 ip 10.00 + IAA 0.05 + NAA 0.50 + GA_3 2.00

0.05 per cent was added to the culture establishment medium. Four replications were kept for each treatment. For shoot proliferation *via* enhanced release of axillary buds, cytokinins like BA or kinetin were added to the basal medium either alone or in combination with auxins like IAA or NAA (Table B). Each treatment was replicated

Table B : Treatments tried to study the effect of plant growth regulators on *in vitro* shoot proliferation in heliconia (Medium – MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1})

Treatment No.	Plant growth regulators (mg l^{-1})
HSP_1	BA 1.00
HSP_2	BA 2.00
HSP_3	BA 2.00 + IAA 0.20
HSP_5	BA 2.00 + NAA 0.20
HSP_7	BA 3.50
HSP_{12}	BA 5.00
HSP_{18}	Kinetin 1.00
HSP_{19}	Kinetin 2.00
HSP_{20}	Kinetin 2.00 + IAA 0.20
HSP_{22}	Kinetin 2.00 + NAA 0.20
HSP_{24}	Kinetin 3.50
HSP_{29}	Kinetin 5.00

three times. Individual shoots measuring 3-5 cm in length having at least 2-3 leaves were transferred to rooting medium. All the cultures were incubated at 26 ± 1^0C and 60 per cent relative humidity under 10-12 hours light and dark cycle. The observations were recorded for a period of four weeks. The shoots were then subjected to *in vitro* rooting treatments (Table C). The experimental design was completely randomized and the effects of treatments were tested by analysis of variance technique.

Table C : Treatments tried to study the effect of plant growth regulators on *in vitro* rooting in heliconia (Medium - MS + inositol 100 mg l^{-1} + sucrose 30.00 g l^{-1} + agar 6.30 g l^{-1})

Treatment No.	Plant growth regulators (mg l^{-1})
HR_1	IAA 0.05
HR_2	IAA 0.10
HR_3	IAA 0.50
HR_4	NAA 0.05
HR_5	NAA 0.10
HR_6	NAA 0.50
HR_7	IBA 0.05
HR_8	IBA 0.10
HR_9	IBA 0.50

RESEARCH FINDINGS AND DISCUSSION

In order to assess the effect of plant growth regulators on *in vitro* culture establishment, thirty nine treatments were tried. The results are presented in Table 1. Significant difference was noticed among these treatments. The average minimum days for shoot initiation ranged from 5.00 to 16.50 days. Earliest shoot initiation (5.00 days) was observed in the treatment HCE_4 (BA

Table 1 : Effect of plant growth regulators on *in vitro* culture establishment in heliconia

Treatments No.	St. Vincent Red		
	Response (%)	Minimum days for shoot initiation	Survival (%)
HCE_1	50.00	12.50	0.00
HCE_2	50.00	9.50	0.00
HCE_3	50.00	8.00	33.33
HCE_4	75.00	5.00	33.33
HCE_5	75.00	9.00	33.33
HCE_6	50.00	10.00	0.00
HCE_7	50.00	8.50	0.00
HCE_8	50.00	10.50	0.00
HCE_9	50.00	14.00	0.00
HCE_{10}	75.00	6.67	33.33
HCE_{11}	75.00	8.67	33.33
HCE_{12}	0.00	-	-
HCE_{13}	50.00	9.50	0.00
HCE_{14}	50.00	12.50	0.00
HCE_{15}	50.00	11.50	0.00
HCE_{16}	75.00	7.67	33.33
HCE_{17}	75.00	9.33	33.33
HCE_{18}	50.00	10.50	0.00
HCE_{19}	75.00	7.67	0.00
HCE_{20}	50.00	12.50	0.00
HCE_{21}	50.00	13.00	0.00
HCE_{22}	75.00	7.00	33.33
HCE_{23}	0.00	-	-
HCE_{24}	50.00	11.50	0.00
HCE_{25}	75.00	8.67	33.33
HCE_{26}	50.00	14.00	0.00
HCE_{27}	50.00	13.50	0.00
HCE_{28}	75.00	8.67	33.33
HCE_{29}	50.00	11.50	0.00
HCE_{30}	0.00	-	-
HCE_{31}	50.00	12.00	0.00
HCE_{32}	50.00	14.00	0.00
HCE_{33}	50.00	14.50	0.00
HCE_{34}	50.00	9.50	0.00
HCE_{35}	50.00	12.00	0.00
HCE_{36}	50.00	14.50	0.00
HCE_{37}	50.00	12.00	0.00
HCE_{38}	50.00	15.00	0.00
HCE_{39}	50.00	16.50	0.00
Control	50.00	16.50	0.00
F		18.881**	
SE		0.580	
		0.648	
		0.710	
#CD (0.05)		1.648	
		1.843	
		2.019	

5.00 mg l^{-1}), which was significantly superior to all other treatments. Talukdar *et al*. (2002) reported almost similar finding in *H. psittacorum*. They observed highest regeneration percentage on treatment with BAP 6.00 mg l^{-1}. In the present study, the best treatment was immediately followed by the treatments HCE_{10} (BA 10.00 mg l^{-1}) and HCE_{22} (Kinetin 10.00 mg l^{-1}) which recorded shoot initiation in 6.67 and 7.00 days, respectively.

The survival percentage was drastically reduced when the concentration of BA was lowered in the establishment medium. The control recorded zero per cent survival after four weeks of culture. Observation of similar kind was made earlier by Bora and Paswan (2003) in *H. psittacorum*, where the buds turned brown and subsequently died when the BA level was below 2.00 mg l^{-1}. The report of Trigiano and Gray (2004) finds relevance in this context. In tissue culture, small explants are transferred into the culture media. Often such explants are too small to make the plant growth regulators needed for a developmental or growth response. Hence, by supplying the medium with exogenous plant growth regulators, it is possible to stimulate the response that is desired from the plant tissue, including overwhelming the effect of endogenous plant growth regulators present in the explant.

During the course of the study, it was observed that addition of GA_3 to the culture establishment medium generally delayed shoot initiation in all the three heliconia varieties. In banana, Krishnamoorthy (1981) and Bhaskar (1991) had reported similar kind of observation.

Shoot proliferation response was not at all observed in plain MS medium (control). The nutrients in the basal medium may not be sufficient to initiate shoot multiplication. When cytokinins and auxins were added to the basal medium, it resulted in varied response (Table 2). The response to various treatments ranged from 0.00 to 100.00 per cent. The average number of days for axillary bud initiation ranged from 8.67 in HSP_2 (BA 2.00 mg l^{-1}) to 18.50 in HSP_{22} (Kinetin 2.00 mg l^{-1} + NAA 0.20 mg l^{-1}). HSP_2 was significantly superior to others and recorded the lowest number of days for axillary bud initiation (8.67) and the highest number of axillary shoots (3.33).

The treatment HSP_2 (BA 2.00 mg l^{-1}) recorded cent per cent response and the shoots in this medium exhibited cent per cent survival also. The present finding is in agreement with the reports on multiple shoot proliferation in *Alpinia purpurata* (Moron, 1987) and *Zantedeschia albomaculata* cv. BLACK MAGIC Koech *et al*. (2005).

The treatment BA 2.00 mg l^{-1} was immediately followed by a combination of BA 2.00 mg l^{-1} and IAA

Table 2 : Effect of plant growth regulators on *in vitro* shoot proliferation in heliconia var. St. Vincent Red Culture period–4 weeks

Treatment No.	St. Vincent Red			
	Response (%)	Minimum days for axillary bud initiation	Total number of axillary shoots	Survival (%)
HSP_1	66.67	11.00	1.50	50.00
HSP_2	100.00	8.67	3.33	100.00
HSP_3	66.67	12.50	2.00	50.00
HSP_5	66.67	14.50	1.50	50.00
HSP_7	66.67	14.50	1.00	0.00
HSP_{12}	0.00	-	-	-
HSP_{18}	0.00	-	-	-
HSP_{19}	66.67	16.50	1.00	0.00
HSP_{20}	66.67	14.50	1.50	50.00
HSP_{22}	66.67	18.50	1.00	0.00
HSP_{24}	66.67	17.00	1.00	0.00
HSP_{29}	0.00	-	-	-
Control	0.00	-	-	-
F		14.371**	3.676*	
S.E.±		0.808	0.417	
		0.885	0.456	
		-	-	
#CD (P=0.05)		2.546	1.313	
		2.789	1.438	
		-	-	

Table 3 : Effect of plant growth regulators on *in vitro* rooting in heliconia var. St. Vincent Red Culture period – 4 weeks

Treatment No.	Rooting response (%)	Minimum days for root initiation	Total number of roots	Length of the longest root (cm)	Remarks
HR_1	0.00	-	-	-	-
HR_2	0.00	-	-	-	-
HR_3	0.00	-	-	-	-
HR_4	0.00	-	-	-	-
HR_5	66.67	22.00	1.00	1.00	Medium thick, short roots
HR_6	66.67	15.00	6.00	1.50	Medium thick, short roots
HR_7	0.00	-	-	-	-
HR_8	0.00	-	-	-	-
HR_9	66.67	18.00	1.00	1.00	Medium thick, short roots
Control	33.33	22.00	1.00	4.00	Thin, long roots

Thin - <1.00 mm Medium thick -1.00 -1.50 mm

0.20 mg l^{-1} with respect to the number of axillary shoots. This finding is supported by the report of Talukdar *et al*. (2002) in *H. psittacorum* where, treatment with 2.00 mg l^{-1} BA + 0.25 mg l^{-1} IAA recorded the highest shoot proliferation percentage within 4-5 weeks of culture.

The established cultures were subcultured in shoot proliferation media and the individual shoots measuring 3-5 cm in length having at least 2-3 leaves were transferred to rooting medium. Rooting response was comparatively lower in plain MS medium (16.67 per cent). In this variety, rooting was observed only with the higher concentrations of NAA (0.10 mg l^{-1} and 0.50 mg l^{-1}) and IBA (0.50 mg l^{-1}). Only 66.67 per cent of the shoots responded to these rooting treatments. The lowest minimum number of days (15) and the highest number of roots (6.00) were recorded with NAA 0.50 mg l^{-1} (Table 3). The effectiveness of NAA 0.50 mg l^{-1} on *in vitro* rooting was earlier stated by Bora and Paswan (2003) and Jun *et al*. (2004) in heliconia and Jagadev *et al*. (2008) in ginger. The roots were short and had a maximum of only 1.50 cm length in this treatment. The plantlets exhibited 90.00 per cent survival in sand after a period of two months.

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