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High multiplication frequency and genetic stability analysis of *Ceropegia panchganiensis*, a threatened ornamental plant of Western Ghats: Conservation implications

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ABSTRACT

The aim of the present study was to develop a protocol for *in vitro* propagation and genetic stability analysis of *Ceropegia panchganiensis* Blatter and McCann, a threatened ornamental plant of Western Ghats, India. Axillary shoots were generated from nodal explants on MS medium supplemented with different concentrations of plant growth regulators. The best frequencies of shoot multiplication (100 ± 0.0%), maximum numbers of shoots (13.2 ± 0.5) and longest shoot lengths (10.1 ± 0.8 cm) were obtained on MS media containing BAP (13.31 μM) and NAA (2.69 μM). Optimum callus induction response (95 ± 2.3%) was observed on MS medium supplemented with 2,4-D (9.05 μM). Half-strength MS medium supplemented with BAP (4.44 μM) in combination with sucrose (175 mM) produced an average of 8.2 ± 0.4 flower buds with 80 ± 2.9% flowering response. Cultures treated with BAP (17.74 μM) along with sucrose (175 mM) showed the highest percentage (85%) of microtuber formation. Microshoots rooted best (96 ± 1.9%) in ½MS medium supplemented with IBA (7.36 μM), produced an average of 9.3 ± 0.9 roots/shoot with 3.6 ± 0.5 cm root length. The rooted plantlets survived best (85%) in a mixture of sterile soil, sand and coco peat (1:2:1). The molecular status/genetic stability of micropropagated plants was assessed by random amplified polymorphic DNA (RAPD) and inter-simple sequence repeats (ISSR) markers. A total of 1149 bands were generated with 10 RAPD and 10 ISSR primers in a mother plant and 10 micropropagated plants, out of which 1134 (98.69%) bands were monomorphic and rest 15 (1.31%) were polymorphic. The present study has established the rapid micropropagation protocol for the first time and will be of great use in conservation of *C. panchganiensis* with low risk of genetic instability.

1. Introduction

The genus *Ceropegia* L. (Apocynaceae, APG III, 2009) with more than 220 species is distributed in tropical and sub tropical regions of the Old World (Bruyns, 2003). Ceropegias possess great diversity in flower design, corolla size, shape and coloring patterns, corona structure and mechanisms for illumination of essential organs. The variety of structures and hairs, the coloration and the pollination strategies make this a fascinating group of plants. It is an interesting group of plants which has attracted much attention from botanists, horticulturalists, gardeners and succulent enthusiasts (Yadav and Kamble, 2006). Many *Ceropegia* species have been domesticated as ornamental house plants and some of these are commercially available in Europe and United States (Hodgkiss, 2004; Reynolds, 2006).

Ceropegia panchganiensis Blatter and McCann is a threatened and potential ornamental plant species of Western Ghats, India. It is commonly known as Kharpudi and Khartundi. The hall mark features of *C. panchganiensis* are ornamental flowers and its tuberous roots (Fig. 1a). Natural populations of *C. panchganiensis* have been reported from the Western Ghats of India with fragmented distribution (Yadav and Kamble, 2006). However, well established horticultural practices have been developed for this plant and it is cultivated for its beautiful flowers (unpublished).

This plant has a well-established ecological role because numerous butterflies rear on this plant and complete their life cycles. Some of them are host specific and disappearance of this plant may also lead to their disappearance from the region (Yadav and Kamble, 2006). Natural propagation of this plant is through seeds and tubers. However, increasing exploitation of tubers along with widespread

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; BAP, 6-benzylaminopurine; IAA, indole-3-acetic acid; IBA, indole-3-butyric acid; ISSR, inter simple sequence repeats; KN, kinetin; MS, Murashige and Skoog medium; NAA, α-naphthalene acetic acid; PGRs, plant growth regulators; RAPD, random amplified polymorphic DNA; TDZ, thidiazuron.

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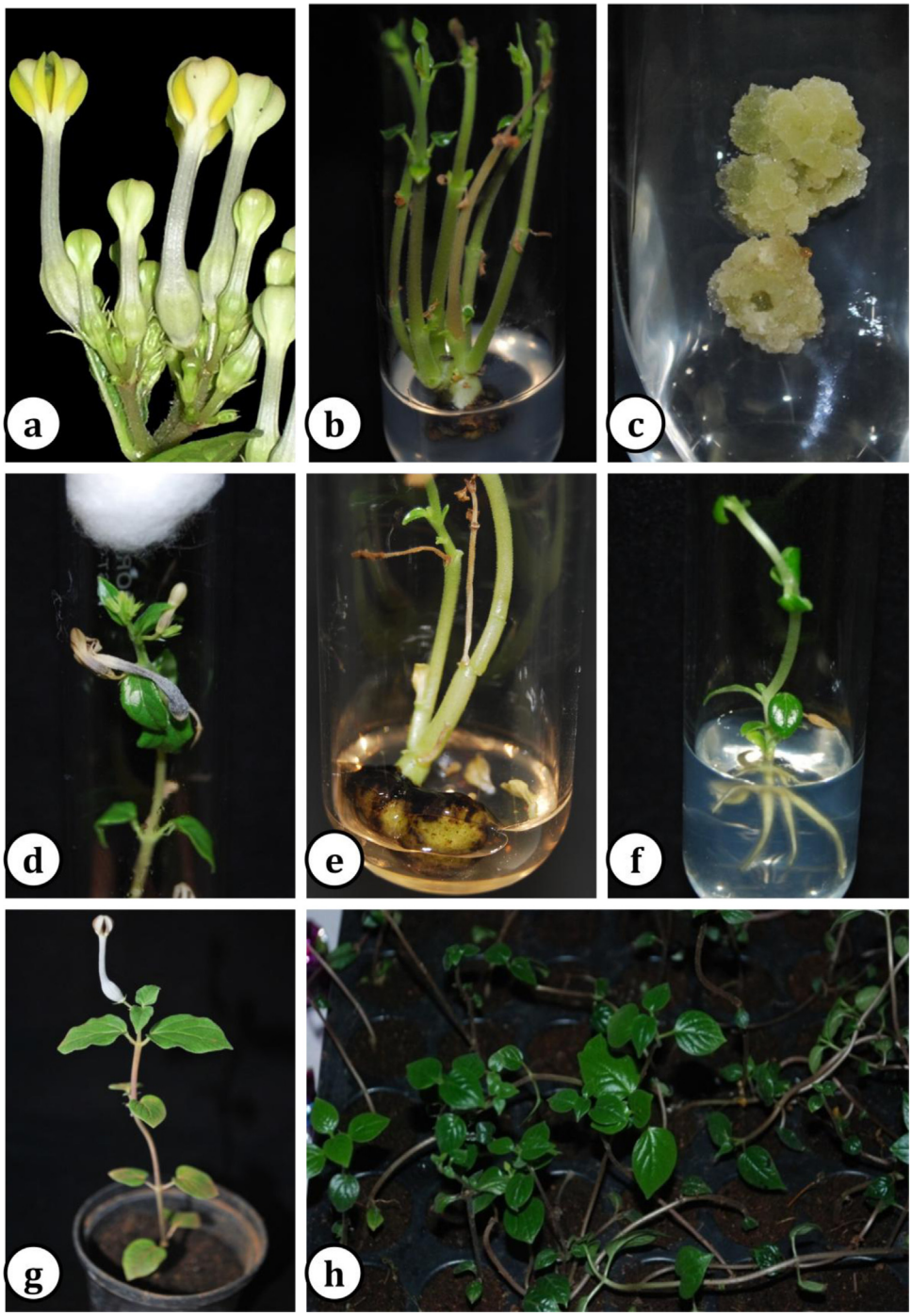

Fig. 1. *Ceropegia panchganiensis*; (a) flowers; (b) shoot multiplication on MS + BAP (13.31 μM) + NAA (2.69 μM); (c) callus proliferation on MS + 2,4-D (9.05 μM); (d) *in vitro* flowering on ½MS + BAP (4.44 μM) + sucrose (175 mM); (e) *in vitro* tuber formation on MS + BAP (17.76 μM) + sucrose (175 mM); (f) *in vitro* rooting on ½MS + IBA (7.36 μM); and (g–h) hardened plants.

habitat destruction, the plant is on the verge of extinction. Besides, the natural propagation of this species is hampered due to absence of legitimate pollinators, poor seed setting, low seed germination and poor rooting ability of the vegetative cuttings.

Rapid plant regeneration using biotechnological tools *viz*. plant tissue culture would minimize the damage to remnant populations and compensate for the poor regeneration capacity of natural populations of *C. panchganiensis*. Several workers have proposed micropropagation protocols for different *Ceropegia* species (Patil, 1998; Beena et al., 2003; Karuppusamy et al., 2009; Nikam and Savant, 2009; Chandore et al., 2010; Chavan et al., 2011a,b). However, no such studies have been conducted with *C. panchganiensis*. The efficient regeneration protocol reported here provides an important method of micropropagation of this plant. Moreover, genetic stability of micropropagated plants was assessed by using RAPD and ISSR markers. Furthermore, this protocol may be used for mass multiplication and conservation of this potential ornamental plant for its further improvement. This is the first illustration of micropropagation in *C. panchganiensis* and utility of molecular markers for assessing genetic stability in genus *Ceropegia*.