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Micropropagation of Ornamental *Alocasia*

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In vitro multiplication of *Alocasia micholitziana* 'Green Velvet', *Alocasia* × *amazonica* and *Alocasia cuculata* was achieved from axillary buds excised from the corms of greenhouse-grown plants. Shoots were developed directly within 6–8 weeks on the MS medium supplemented with 10 mg l⁻¹ 2-iP for *A. micholitziana* 'Green Velvet' or 10 mg l⁻¹ BA for *A. × amazonica* and *A. cuculata*. Considering the quality of the shoots, high frequency of shoot regeneration was achieved on similar medium in subsequent subcultures for *A. micholitziana* 'Green Velvet' (5.0 shoots/culture). Five mg l⁻¹ BA gave the best shoot regeneration in *A. × amazonica* and *A. cuculata* (4.6 and 5.0 shoots/culture, respectively). Shoots of the regenerated plants rooted abundantly on the hormone free MS within 4 weeks. Acclimatization was successful with approximately 100% of the *in vitro* plantlets which survived in a greenhouse. The regenerated plants seemed morphologically to be similar to the respective mother plants. The established procedure provided a basic technique to carry out the future *in vitro* experiments in *Alocasia*.

INTRODUCTION

Belonging to Araceae family which contains about 120 genera, 2,000 species, *Alocasia* is one of the largest and most morphologically diverse genus consisting of over than 60 species. They are found mainly in tropical forests of South and Southeast Asia. Plants of this herbaceous monocot are terrestrials or lithophytes, normally evergreen, mainly rhizomatous, sometimes tuber-bearing perennials. The extravagant and exotic features of the foliage make *Alocasia* suitable for cultivation as valuable ornamental potted plants.

Traditional methods of vegetative propagation of *Alocasia* such as corm division provide an extremely slow rate of multiplication. Thus, there has been a distinct need to investigate the potential of *in vitro* propagation systems for this plant, as has been

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successfully achieved with other ornamental foliage species in Araceae family (Geier, 1986; Hutchinson *et al.*, 1994; Lin *et al.*, 2000). The development of a rapid mass propagation system through tissue culture would not only be a powerful tool for commercial propagation but also be an important step that will open new avenues for the further breeding research in *Alocasia* in which modern technologies of molecular genetics would be applied (Broertjes and Van Harten, 1978; Ibrahim *et al.*, 1988). In this study, the feasibility of establishing a protocol for the micropropagation of *Alocasia* that will be used in further mutation breeding studies was investigated.

MATERIALS AND METHODS

Plant materials

Three species/cultivars of *Alocasia* were chosen for the study including *A. micholitziana* 'Green Velvet', *A. ×amazonica* and *A. cuculata*.

Plants of these species/cultivars were purchased from commercial growers in Japan and kept in a greenhouse of Laboratory of Horticultural Science, Kyushu University for further *in vitro* research.

Surface sterilization

Corms of the plants were collected. They were brushed carefully to remove soils with detergent and washed under tap water. After removing the outermost whorl of the petiole, the corms were rinsed with distilled water. Two thirds of the corm was peeled off from the base and the remaining portion was washed again with distilled water. Then it was surface sterilized by stirring in NaOCl solution (active chlorine 1%) containing 0.01% Tween 20 for 20 minutes. The sterilized corms were washed three times with sterile distilled water. The petiole bases were removed and apical shoots and axillary buds of the corms were excised aseptically. They were cultured on initial medium for shoot proliferation.

Culture medium

The basal medium was Murashige and Skoog medium (MS; Murashige and Skoog, 1962) supplemented with 30 g l⁻¹ sucrose and 8 g l⁻¹ agar. Shoot regeneration for *A. micholitziana* 'Green Velvet' was induced with 10 mg l⁻¹ 2-iP added to the basal medium and for *A. ×amazonica* and *A. cuculata* was with 10 mg l⁻¹ BA. For shoot multiplication, 2-iP or BA at various concentrations (0–10 mg l⁻¹) was tested. The pH was adjusted to 5.7 prior to being autoclaved for 15 min at 121 °C.

Culture conditions

All cultures were maintained at 25 ± 2 °C in continuous light condition (44.73 μmol sec⁻¹m⁻²) and subcultured monthly.

Rooting and plantlet regeneration

Shoots that developed on multiplication media were transferred to hormone free MS medium for rooting. Complete plantlets (shoots with roots) were transplanted to a mixture of 1:3 (v:v) vermiculite and soil in plastic pots and placed in the greenhouse for

acclimatization.

RESULTS AND DISCUSSION

Shoot regeneration

The regeneration of new clean shoots from primary explants is a prerequisite for any micropropagation programme. In this experiment, the pre-existing buds started to develop earliest and new shoot development was observed within eight weeks of their cultures. Explants showing contamination were discarded. Only visibly clean shoots were retained for further propagation.

Effect of cytokinin on the shoot multiplication

The transfer of primary regenerated shoots onto hormone-free MS medium did not lead to shoot multiplication but they elongated and rooted abundantly.

Shoot multiplication with the reference to the multiplication rate and the shoot quality was significantly affected by genotypes, the types and concentrations of cytokinins applied (Table 1, Fig. 1). Among three species/cultivars, the shoot formation of *A. × amazonica* responded poorly on the media containing 2-iP and the best shoot multiplication was recorded on the MS medium supplemented with 5 mg l⁻¹ BA.

In case of *A. micholitziana* 'Green Velvet', although no statistical differences in shoot multiplication rate were observed between the MS medium containing 5 mg l⁻¹ BA and 10 mg l⁻¹ 2-iP (on which the highest rate of shoot multiplication were induced), the quality of the shoot was obviously affected by BA. The frequency of abnormal shoot increased with increasing concentrations of BA in the medium. The best medium for the shoot multiplication of *A. micholitziana* 'Green Velvet' in terms of the number of shoots per culture and shoot quality, therefore, appeared to be MS supplemented with 10 mg l⁻¹ 2-iP.

For *A. cuculata*, the highest shoot multiplication was obtained on MS added with 5 mg l⁻¹ BA. The medium with 2-iP at 5 mg l⁻¹ showed a slightly lower rate of multiplication but the growth of shoots also appeared to be better than that of the shoots induced on the medium with BA.

Table 1. Effect of cytokinins on shoot multiplication rate in *Alocasia*

Plant growth regulators	Concentration (mg l ⁻¹)	No. of explants examined	Shoot multiplication rate (mean±SE)		
			<i>A.</i> 'Green Velvet'	<i>A. × amazonica</i>	<i>A. cuculata</i>
Control	0	27	1.1±0.1a*	1.0±0.0a*	1.0±0.0a*
BA	1	27	4.0±0.2c	3.3±0.2c	4.3±0.2c*
	5	27	5.0±0.2d	4.6±0.3d	5.0±0.2d
	10	27	3.8±0.2c	4.7±0.2d	4.3±0.2c
2-iP	1	27	1.6±0.1a*	1.1±0.1a*	1.3±0.1a*
	5	27	2.8±0.2b	1.3±0.1a*	4.4±0.2c
	10	27	5.0±0.3d	2.6±0.2b	3.2±0.2b

* The treatments that showed root formation. Mean separation by Duncan's multiple range test ($P < 0.05$).

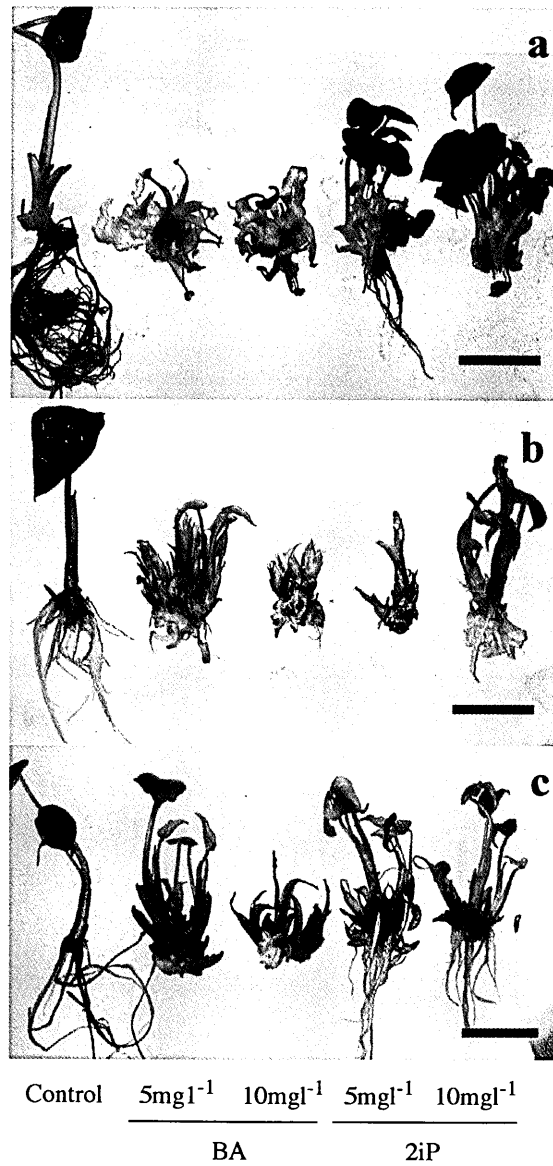


Fig. 1. Organogenetic response of *A. micholitziana* 'Green Velvet' (a), *A. × amazonica* (b) and *A. cuculata* (c) on MS medium supplemented with various concentrations of BA or 2-iP. Scale bar=2 cm.

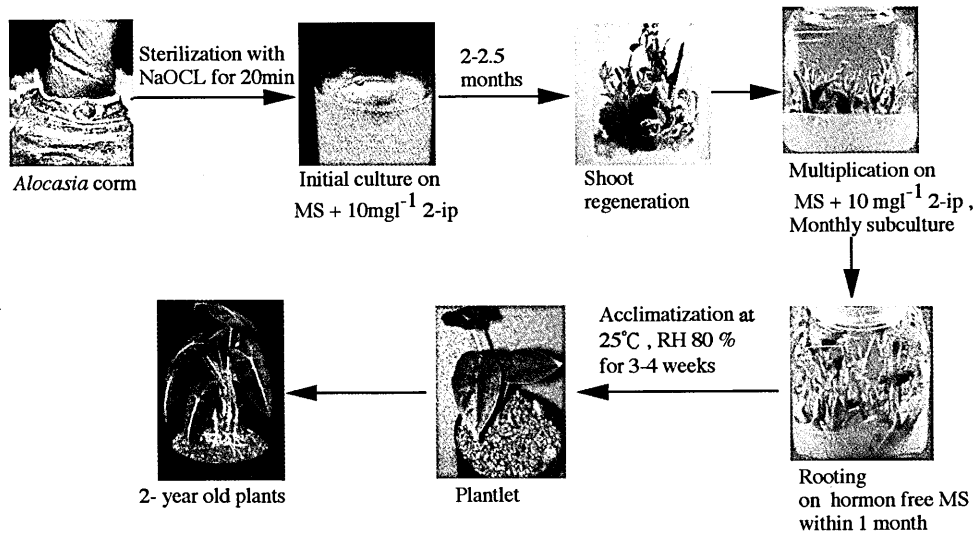


Fig. 2. Micropropagation procedure for *A. micholitziana* 'Green Velvet'.

Rooting and acclimatization

Rooting was simply induced by transferring the shoots onto MS medium without adding any kind of auxin. The acclimatization can be successfully achieved in all *Alocasia* with survival rate up to 100%.

Procedure for *Alocasia* multiplication

A general protocol for the *in vitro* propagation of *Alocasia* would consist of the following steps (Fig. 2); adventitious buds or shoot tips from the corms of the original plants can be used as explants. The explants can then be cultured on MS + 10 mg l⁻¹ 2-iP (in case of *A. micholitziana* 'Green Velvet') or 10 mg l⁻¹ BA (in case of *A. ×amazonica* and *A. cuculata*) for primary shoot regeneration. Regenerated shoots then can be transferred onto MS medium supplemented with 10 mg l⁻¹ 2-iP for *A. micholitziana* 'Green Velvet' or 5 mg l⁻¹ BA for *A. ×amazonica* and *A. cuculata* for shoot multiplication. Root formation of isolated shoots can take place on MS medium without growth regulators. Initially high moisture conditions are needed for the acclimatization of *in vitro* plants before transfer to greenhouse.

It has been proved *in vitro* culture to be an efficient method for mass propagation of three *Alocasia* species/cultivars investigated. The established protocol provides a basic technique to carry out the future *in vitro* experiments in *Alocasia*.

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