Note

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Plant Regeneration from *Wahlenbergia marginata* Hairy Root Cultures

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Wahlenbergia marginata A. DC. is a small campanulaceous weed distributed in the countries of east to south-east Asia, India and the South Pacific Islands. In China, the plant has traditionally been used for treatment of cough, hemoptysis and gastralgia. Recently, hairy roots have been induced by infection with *Agrobacterium rhizogenes* ATCC 15834 and MAFF 03-01724 strains [1]. Secondary metabolites, *i.e.* polyacetylenes such as lobetyol, lobetyolin and lobetyolinin, produced in the hairy roots have been identified [1]. In this experiment, we succeeded in direct shoot regeneration from the hairy root tissues. The morphological traits and polyacetylene contents of the regenerants are described.

One clone of the hairy roots (Wm-A), which has been induced by *A. rhizogenes* ATCC 15834 [1], was used for this experiment. When Wm-A was cultured in hormone-free Murashige-Skoog (MS) [2], 1/2 MS and Woody Plant (WP) [3] media under light condition (*ca.* 3000 lux, continuous light) for 4 weeks, adventitious shoots directly regenerated from almost every culture, both in solid and liquid culture conditions (**Fig. 1**).

The regenerated shoots which appeared both in solid and liquid media were cut off and transferred to hormone-free MS, 1/2 MS and WP solid medium (solidified with 2.5 g/*l* Gelrite). The regenerants proliferated well in all media tested. The plantlets, after acclimatization by submerging the root portion in water in a beaker indoors for 3 days, were easily transplanted to soil in a pot.

The *in vitro* plantlets showed some interesting morphological traits, *i. e.* 1) formation of many leaves (**Fig. 2-a**), 2) flowering in the short period of 1-2 months, and 3) variation of the petal number from 3 to 5 (**Fig. 3**). Among 24 regenerants, the clones with 3, 4 and 5 petals were numbers 1, 8 and 15, respectively. Flowers with different petal number appeared even in a single clone of the regenerants in the subculturing (the exact frequency of the occurrence of different petal number in a clone is not identified). In the regenerants, priority of genes, which work to yield the flowers with 5 petals, might be decreased. To clarify this possibility, more detailed investigation is required.

Lobetyolin, the predominant secondary metabolite in this species [1], was also determined by HPLC [4]. Lobetyolin contents in the regenerants cultured *in vitro* in hormone-free WP solid medium [shoot portion: 0.01%, as dry weight (dw)] and *in vivo* ones cultivated in a pot (shoot portion: 0.06%, root portion: 0.05%, as dw) were almost identical to that of the parent plants *in vivo* (shoot portion: 0.01% as dw).

These regenerants might be suitable for studies on genes and enzymes which are related to the expression or regulation of the morphological traits of this plant. In the future, *Agrobacterium*-mediated gene transformation might become a valuable method for obtaining useful transgenic clones of *W. marginata*.

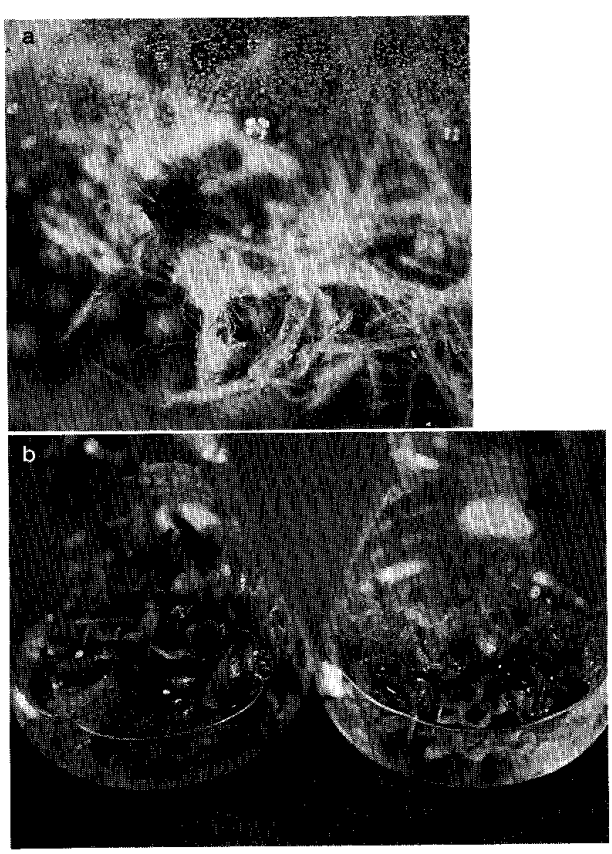

Fig. 1 Adventitious shoot regeneration in Wm-A; a: in WP solid medium, b: in MS (left) and WP (right) liquid media.

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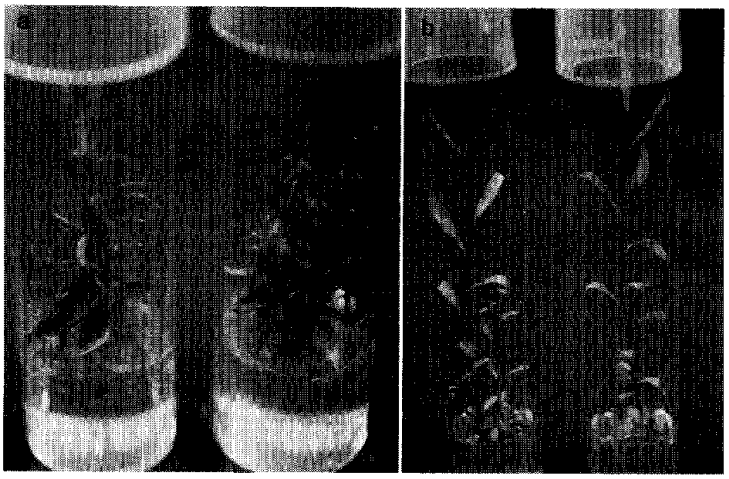

Fig. 2 *W. marginata* shoots cultured in hormone-free WP solid medium; a: regenerants from the hairy roots, b: non-transformed shoots.

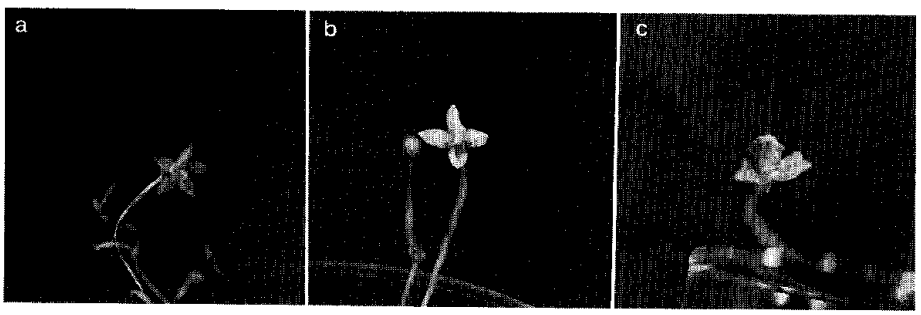

Fig. 3 Flower of *W. marginata* regenerant; a: with 5 petals, b: with 4 petals, c: with 3 petals.

Acknowledgements

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