Short Communication

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Low frequency of Somaclonal Variations in Floral Bud Culture-derived Plants of *Vandofinetia* (Orchidaceae)

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There are many reports on somaclonal variation in tissue culture-derived plants [1-6]. However, the cause of somaclonal variation is not always clear [7] and may differ among plant species.

Occurrence of somaclonal variations has also been reported in orchids. Variegated *Cattleya* plants were regenerated through micropropagation of normal plants [8]. Vajrabhaya [9] observed 5 tetraploids with large flowers among 205 plants derived from a clone of *Dendrobium*. Also, *in vitro* propagated triploid *Dendrobium* became octaploids [9]. Some of the new variant varieties of *Mokara* are becoming popular after receiving awards at the Singapore Orchid Shows [10]. In our previous studies, we have established a suitable method for the induction and micropropagation of PLBs in some monopodial orchids such as *Vandofinetia* Nara 'Yumika Pink' and other 4 monopodial orchids [11]. In this report, we checked the variations among the plants of 'Yumika Pink' obtained by this micropropagation method at the flowering stage.

More than 10 thousand plantlets of *Vandofinetia* Nara 'Yumika Pink' micropropagated by the method reported previously [11] were potted in sphagnum and acclimated in greenhouses by the conventional method. The temperature in the greenhouses was maintained at *ca.* 18~25°C. They were irrigated almost every day in summer, at 2~4 day intervals in spring and fall, and at about 5~7 day intervals in winter. Five thousand-fold diluted solution of a liquid fertilizer, BOB-PETERS (Asahi Industries Co., Ltd., Tokyo, Japan; N: P: K=20: 20: 20) was applied once per 2~3 time of irrigations during cultivation. About 2 years after acclimation, about 1500 plants flowered. For these plants, the number of phenotypic variants was counted. Moreover, some characteristics such as plant height, number of leaves, number of flowers and thickness of the leaf were investigated for 30 normal plants and 30 dwarf variants. Statistical analysis (t-test; $P=0.05$) was made for these characteristics.

The plantlets derived from tissue culture grew vigorously after transfer to sphagnum pots. The first flower was observed 21 months after acclimation. The blooming season was irregular from spring to early winter in the greenhouses. Blooming frequency was 1~3 times/year. In most cases, the number of flowers ranged from 6 to 8. Among the tissue culture-derived plants, only 3.8% of the plants showed abnormal phenotypes (**Table 1**), which were classified into two types, A and B (**Fig. 1**).

The plants of type-A showed dwarfness with thick leaves and grew a little slower than the normal one. However, they produced normal flowers in shape although the blooming season was 2~3 months later than that of normal plants. Moreover, most of the type-A plants became normal in the 4 characteristics listed in **Table 2** 2~4 years after acclimation. Therefore, it is possible that type-A variation was not genetic but physiological in origin. This type of variation may be caused by the following reasons hypothesized by Pierik [12]. The first possible reason is that plantlets just after acclimation may have an abnormal appearance as a result of the physiological disease called vitrification which may be caused by the repeated subculture of PLBs in liquid medium. The second possible reason is that plantlets can undergo changes such as in the number of morphology of stomata and/or in the composition of the cuticular wax layer due to the relatively high humidity. The third possible reason is that virus-free plantlets may differ completely in their external appearance from plantlets still possessing the virus if the mother plants possessed viruses such as CyMV and/or ORSV. Further studies will be needed to clarify the reasons for this variation.

Another variation (type-B) was observed in only one plant which showed variegation along the midrib of the leaves. As the variegation was observed in every leaf, the plant was thought to be a stable periclinal chimera containing mutated corpus tissues with chlorophyll deficiency. This plant grew much more slowly than both type-A and normal plants and no

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flower has yet been observed.

The remaining 8500 plants grew normally in shape and phenotypic variation as did the 1500 flowering plants but flowering was not yet observed. The slower growth observed in these 8500 plants would mainly be due to the difference in size of the acclimated plantlets. It is a characteristic of this variety, that even if plants have grown large enough to flower, the flowering time is irregular. This phenomenon is also common in other *Vandofinetia* hybrids and may occur even if the plantlets were not obtained through tissue culture. Therefore, the 1500 plants investigated must be enough for evaluating somaclonal variations. Also, since we recognized 3.8% as having somaclonal variations, more somaclonal variations would not be obtained in the future.

It is well known that micropropagation sometimes induces somaclonal variations which cause serious problems in commercial micropropagation of plants. In orchids, less than 10% somaclonal variation is generally acceptable for the commercial production of micropropagated plants [13]. Therefore, the relatively low occurrence of somaclonal variations (3.8%) obtained in our present study suggests that the micropropagation method developed for the wide range of monopodial orchids in our previous study [11] could be utilized economically and safely in their commercial production.

Table 1.
Variant types obtained in micropropagated plants of *Vandofinetia* Nara 'Yumika Pink'. (just before flowering)

Variant type	Number of plants (%)
Normal	1443 (96.2)
Abnormal-A*[1]	56 (3.7)
Abnormal-B*[2]	1 (0.1)
Total	1500 (100.0)

*[1] Dwarf plants with thick leaves.
*[2] A plant with variegated leaves.

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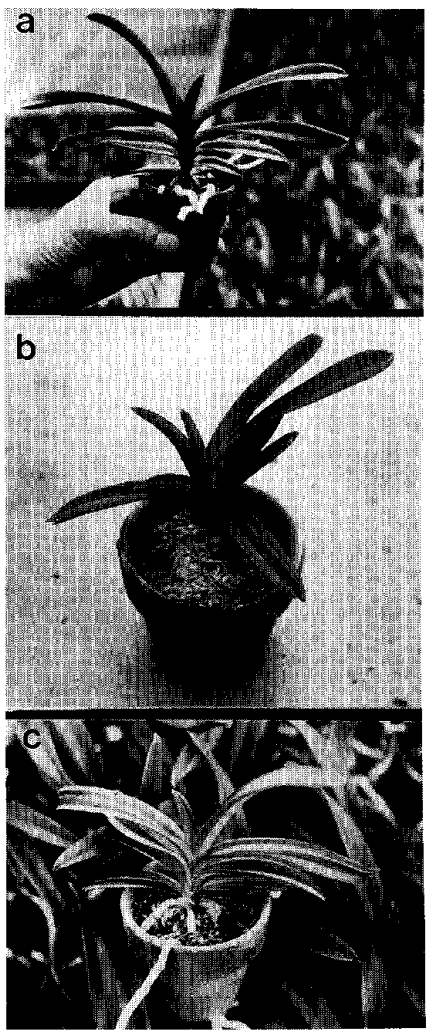

Fig. 1 Comparison of a normal plant and two types of abnormal plants in *Vandofinetia* Nara 'Yumika Pink' just before flowering.
(a) a normal plant (b) an abnormal dwarf plant with thick leaves (c) an abnormal plant with variegated leaves

Table 2.
Characters of normal plants and dwarf variants of *Vandofinetia* Nara 'Yumika Pink'. (at flowering stage)

Type of plant	Number of plants investigated	Plant height (cm)	Number of leaves	Number of flowers	Thickness of leaf*[1] (mm)
Normal	30	12.3	12.5	7.2	1.9
Abnormal-A*[2]	30	9.7†	9.0†	6.5NS	2.7†

*[1] Thickness of 3rd leaf from bottom was measured.
*[2] Dwarf plants with thick leaves.
Statistical analysis (t-test; $P=0.05$) was made between the data of Normal and Abnormal-A plants.
† Significantly different within the column by t-test ($P=0.05$).
NS; Non-significant within the column by t-test ($P=0.05$).

cultivation of tissue culture-derived plants. The authors also wish to thank Dr. M. Mii, Prof. of Plant Cell Technology, Faculty of Horticulture, Chiba Univ. for his revision of this manuscript.

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