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# Morphological characteristics and shoot regeneration potential of the fasciated rhizome of *Cymbidium kanran* Makino

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**Abstract**

Stable fasciation appeared in *in vitro* rhizome cultures of *Cymbidium kanran* Makino. Fasciated rhizomes had ridge-like flat rhizomes with elliptical epidermal cells, stomates and rhizoids. Most axillary buds of fasciated rhizomes were inactive resulting in rare branching. Fasciated rhizomes did not respond to cytokinins which were effective in the shoot conversion of non-fasciated normal rhizomes.

Fasciation is a widely occurring mutation in higher plants. Genetics, physiology and morphology of fasciations in legume plants have been intensively studied by Knight (1993), LaMotte *et al.* (1988), Leffel *et al.* (1993) and Tang *et al.* (1997). *In vitro* micro-propagation (Barotti *et al.* 1995; Jemmali *et al.* 1994; Stimart and Harbage 1989; Varga *et al.* 1988) and infection of micro-organisms (Wang and Jin, 1992) also cause fasciation. In the Orchidaceae, *in vitro* fasciated plants have not yet been reported. Several temperate terrestrial *Cymbidium* species native to Asia, including *C. kanran* Makino, produce rhizomes in *in vitro* culture, while other tropical epiphytic *Cymbidium* species produce protocorms after axenic seed germination. Rhizomes can be multiplied successfully *in vitro* (Hasegawa 1987; Paek and Kozai, 1998). Partially fasciated *C. kanran* rhizomes appeared in our cultures. Fasciated parts were isolated and subcultured. This fasciation is stable whereas most fasciations that appear *in vitro* are epigenic (Jemmali *et al.* 1994; Stimart and Harbage 1989; Varga *et al.* 1988).

The aims of this work are to describe the developmental morphology and to compare the potential for shoot conversion or induction of fasciated and non-fasciated normal rhizomes in *C. kanran*.

Both fasciated and normal rhizomes were maintained on a medium containing 3 g $l^{-1}$ Hyponex (complete soluble fertilizer, N-P-K=6.5-6-19, Hyponex Co. Ltd. Tokyo), 3 g $l^{-1}$ Bact pepton, 1 mg $l^{-1}$ 1-naphtylacetic acid (NAA), 30 g $l^{-1}$ sucrose and 2 g $l^{-1}$ gellan gum (H medium) (Hasegawa 1987). Stock cultures were incubated under 35 $\mu$mol $m^{-2}s^{-1}$ of photosynthetic photon flux density (PPFD) provided by fluorescent lamps for plant growth (FL40SPG, National) for 16 h per day at 25 ℃.

*Morphological observations* - Fasciated rhizomes had ridge-like structures, while normal rhizomes had a cylindrical shape. Both normal and fasciated rhizomes showed geotropism, resulting in drooping branches in the normal rhizome and a crest shape under the medium in the fasciated rhizome (**Fig. 1**). Top active growing parts of rhizome were collected from both fasciated and normal rhizomes and fixed with FAA (formalin : acetic acid : 70% ethanol, 5 : 5 : 90). Specimens were dehydrated in an ethanol-acetone series, critical point dried, coated with Pt and observed with a scanning electron microscope (S-2150, Hitachi).

Fasciated rhizomes produced scaly leaves along with a linear apical meristem (**Fig. 2-A**) as observed in fasciated soy bean (Tang *et al.* 1997). Epidermal cells, stomates and rhizoids in the fasciated rhizome were similarly orientated towards

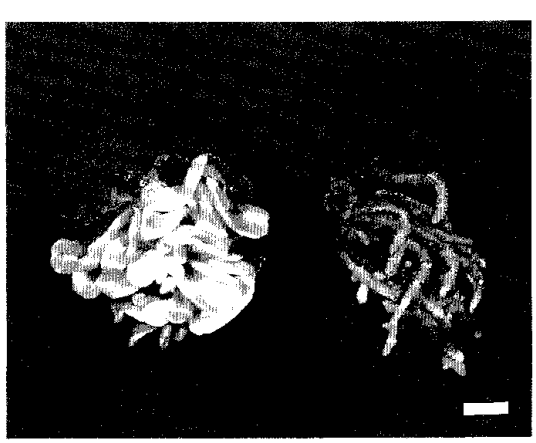

**Fig. 1.** Fasciated(left) and normal(right) rhizomes of *C. kanran*. bar = 1 cm

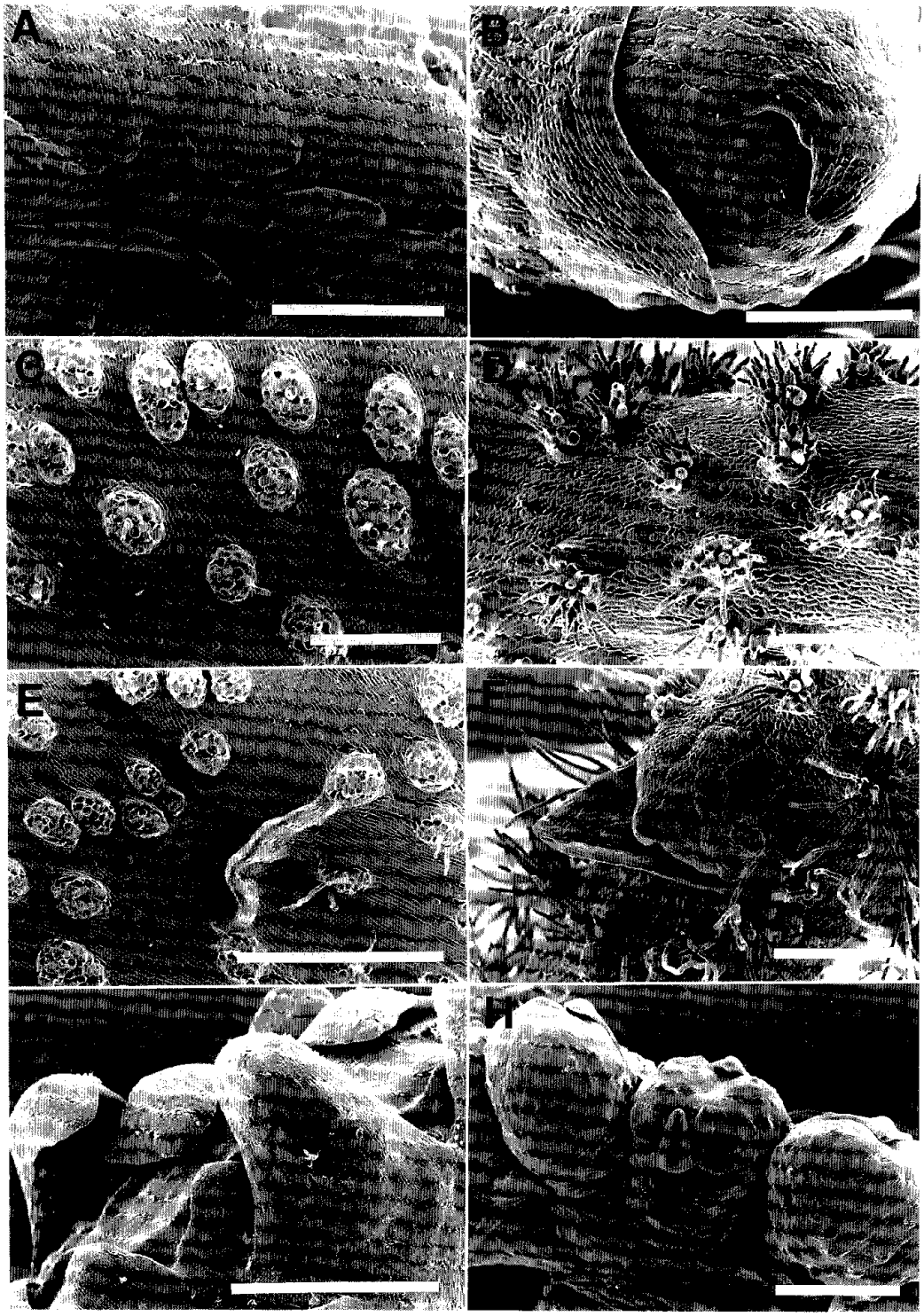


**Fig. 2.** Scannning electron micrographs of fasciated and normal rhizomes of *C. kanran*.
A: shoot apex of faciated rhizome, x80, bar = 500 $\mu$m,
B: shoot apex of normal rhizome, x80, bar = 500 $\mu$m,
C: surface structure of fasciated rhizome, x60, bar = 500 $\mu$m,
D: surface structure of normal rhizome, x60, bar = 500 $\mu$m,
E: axis of fasciated rhizome, x50, bar = 1mm,
F: axillary bud of normal rhizome x60, bar = 500 $\mu$m,
G: fasciated rhizome explant exposed to TDZ, x50, bar = 1 mm,
H: fasciated rhizome explant exposed to zeatin, x30, bar = 1 mm.

the base (**Fig. 2-C**) and those shape were elliptical. On the other hand, normal rhizomes had blunt shoot tips producing scaly leaves with a longer plastochron (**Fig. 2-B**). Round-shaped epidermal cells, stomates and rhizoids were randomly orientated on the normal rhizome (**Fig. 2-D**). Normal rhizomes

**Table 1.** Effects of cytokinins on shoot induction from zhizome of *Cymbidium kanran*.

| Cytokinin ($mg\ l^{-1}$) | | Normal rhizome | | | Fasciated rhizome | | |
|---|---|---|---|---|---|---|---|
| | | Branching (%) | shoot (%) | No. shoots* | Branching (%) | Shoot (%) | Development type |
| TDZ | 2.0 | 62.5 | 67.5 | 1.33 ± 0.12 | 0 | 0 | S** |
| | 0.2 | 50.0 | 62.5 | 1.00 ± 0.00 | 0 | 0 | S |
| | 0.02 | 35.0 | 47.5 | 1.00 ± 0.00 | 0 | 0 | S |
| Zeatin | 2.0 | 45.0 | 40.0 | 1.00 ± 0.00 | 0 | 0 | R |
| | 0.2 | 70.0 | 5.0 | 1.00 ± 0.00 | 0 | 0 | R |
| | 0.02 | 60.0 | 0.0 | – | 0 | 0 | R |
| Cont. | 0 | 22.5 | 0.0 | – | 0 | 0 | – |

*Average number of shoots converted from rhizome explant ± SE.

**S = produced many scaly leaves (see Fig. 2-G), R = produced some new fasciated rhizomes (see Fig. 2-H).

often branched on the medium mentioned above. Axillary buds swelled and developed into lateral rhizomes (**Fig. 2-F**). In contrast, axillary buds of fasciated rhizomes were inactive and branching was rare (**Fig. 2-E**), probably due to strong apical dominance produced by the large linear apical meristem.

*Shoot conversion* - Normal rhizomes converted to shoots at a low frequency when cultures were maintained on H medium during a long period without subculture (Hasegawa, 1985). Some cytokinins are effective to stimulate the conversion of rhizomes to shoots in some terrestrial *Cymbidium* species *in vitro* (Hasegawa *et al.* 1987; Shimazaki 1995; Ueda and Torikata, 1969a).

Both normal (top part of 5 mm in length) and fasciated rhizomes (3x3 mm including meristem) were placed on Murashige and Skoog medium containing 0.02, 0.2 or 2.0 $mg\ l^{-1}$ thidiazuron (TDZ) or Zeatin, 30 $g\ l^{-1}$ sucrose and 2 $g\ l^{-1}$ gellan gum. Twenty explants were placed on 25 m*l* medium in a 9 cm petri dish. Each treatment had two replications. The cultures were incubated under dim light (less than 5 $\mu mol\ m^{-2}\ s^{-1}$ of PPFD) at 25 ℃ for 60 days.

Higher cytokinin levels produced higher frequencies of shoot conversion in normal rhizomes of *C. kanran* (**Table 1**). TDZ was more effective than zeatine in inducing shoot conversion. However, no shoot conversion was observed in fasciated rhizomes. Cytokinins stimulated branching in normal rhizomes but had no effect in fasciated rhizomes. Fasciated rhizomes explants exposed to different cytokinins showed a different developmental morphology. The explants exposed to TDZ produced many scaly leaves with a shorter plastochron (**Fig. 2-G**), while the explants exposed to zeatine produced a small amount of new fasciated rhizomes with slow growth (**Fig. 2-H**). A short plastochron was the early signal of shoot conversion in *C. goeringii* (Ueda and Torikata 1969b). Other explants from fasciated rhizomes were exposed to higher levels of TDZ (2,5,10 $mg\ l^{-1}$) for 90 days under dark conditions and then transferred to H medium. Again no shoot conversion was observed. All explants transferred to H medium produced again fasciated rhizomes (data not shown).

In the case of fasciated soybeans, normal developmental events, i.e. shoot elongation, leaf expansion, flower initiation, flowering and seed set were observed besides broad and flat stem morphology (LaMotte *et al.* 1988; Leffel *et al.*, 1993). Loss of shoot conversion ability in the fasciated rhizomes of *C. kanran* might be due to genetic changes. The polyploidy level of fasciated and non-fasciated rhizomes was compared by using a flow cytometer (Polyploidy analyzer PA, Partec). Fasciated rhizomes were not polyploid (data not shown). The other most probable explanation for the loss of shoot conversion ability is that large linear apical meristems require different phytohormonal conditions from normal rhizome requirments to induce shoot conversion. Further trials are required to get a fasciated plant of *C. kanran*.

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