

Non-requirement of Vernalization for Flowering in Ri-transformed Chicory is Transmissible to Untransformed Scions by Grafting

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Agrobacterium rhizogenes transforms many dicotyledonous plant cells to form hairy roots by inserting a part of its Ri-plasmid, the T-DNA, into the host plant cell (see review¹⁾). Ri-transformed plants regenerated from hairy root display a class of characteristics different from the mother plant, so-called the hairy root syndrome which includes characteristics such as dwarfism and wrinkled leaves²⁾. One remarkable trait of the Ri-transformant is the altered flowering behavior. For example Ri-transformants showed early flowering in a day-neutral tobacco species³⁾. In carrot, a cold-requiring species, the biennial flowering behavior was changed to annual in the Ri-transformants²⁾. A biennial plant chicory (*Cichorium intybus* L. var. Witloof), which requires vernalization and long-days to flower⁴⁾, became a non-chilling requirement plant in the Ri-transformant⁵⁾.

To study the nature of this non-requirement of vernalization for flowering in the Ri-transformed chicory, we made grafts between the untransformed scions and the Ri-transformed stocks to investigate whether there is a flower-inducing factor in the flowering Ri-transformant.

Transgenic plants of Witloof chicory cv. Vilmorin No. 5 (Vilmorin, France) with the T-DNA of an agropine type Ri plasmid pRiA4b were established as described elsewhere⁶⁾. Three-month-old untransformed chicory seedlings were used as scions. Both Ri-transformed and untransformed plants were maintained in 900 ml culture bottles containing 200 ml of Murashige and Skoog's (MS) medium⁷⁾ solidified with 0.2 g/l gellan gum under 16 hr light (20 klx) and 8 hr dark cycles in a CU-356 growth chamber (TOMY Seiko Co., Japan) at 25°C.

Excess leaves and all roots of the untransformed seedlings were removed and the scion with several leaves was stuck into a cleft (ca. 1 cm depth) made on a segment of flower stalk (ca. 3-4 cm long) of Ri-transformant (**Fig. 1-A**). The graft junction was then fastened with a cotton thread (**Fig. 1-A**). For the control to determine the mechanical effect on bolting and/or flowering, the scion was grafted onto an untransformed plant grown under the same conditions as the scion. The scion made as described above was stuck into a cleft made on the cut surface of the rootstock, which had been decapitated at a position just below the outermost leaf, and the graft junction was also fastened with a cotton thread. All the grafts were grown in 900 ml culture bottles containing 200 ml of MS solid medium under the long-day condition described above for one month and then scored for bolting, the phenomenon preceding to flowering in chicory. After the experiment, some of the grafts were sectioned to confirm the graft union.

The number of grafts made and those with bolting at the end of the experiment were shown in **Table 1**. The rootstocks were taken from several different lines of Ri-transformants. None of the controls bolted (**Fig. 1-C**), indicating the mechanical stress could not be a cause of erratic bolting. On the other hand, bolting was observed in most of the scions grafted to the Ri-transformant (**Fig.**

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Table 1. Bolting of grafts under the long-day condition.

Graft portion		No. made	No. bolted* ¹
Scion	Stock		
Untransformed	Untransformed	24	0
Untransformed	Transformed	10	8

*¹ scored 1 month after grafting.

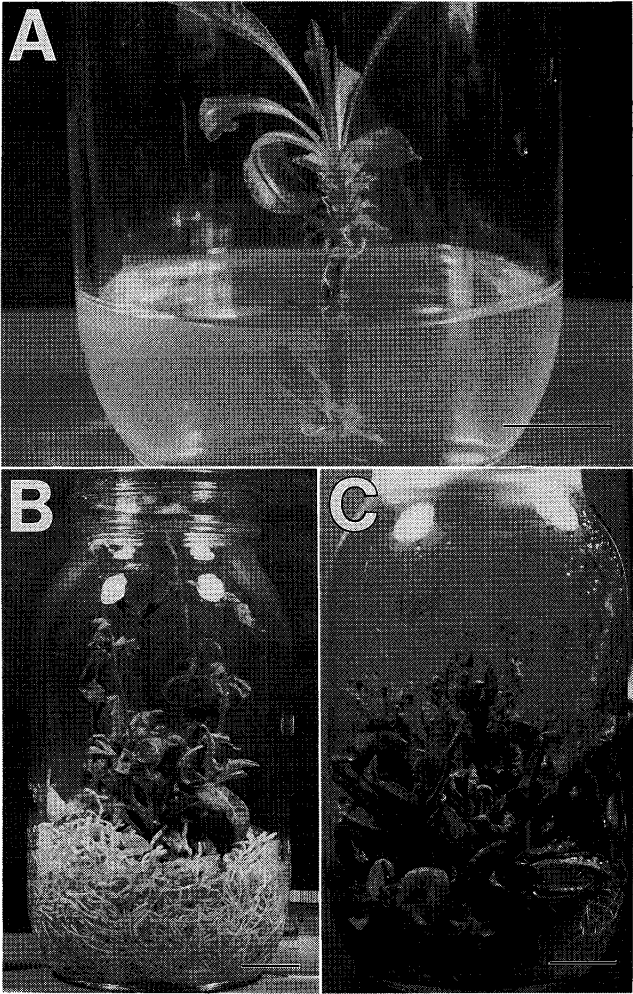


Fig. 1 Grafts of chicory cultured under the long-day condition. A: an untransformed scion grafted on an Ri-transformed stock, photographed just after grafting. B: the graft shown in panel A, photographed 4 months after grafting. Note flower stalks appeared on the untransformed scion and well-developed roots on the Ri-transformed stock. C: A control graft photographed 4 months after grafting. No stalk on the scion nor the stock could be seen. Scale bar=2 cm.

1-B). Flowering was observed in most of the stalks(data not shown). At least, in one of the two grafts on the Ri-transformant that did not flower, the scion had failed to attach to the rootstock and formed adventitious roots that reached to the medium.

Although only a limited number of grafts have been made so far, the result was remarkable and the difference was apparent. This suggests that the Ri-transformant flowering under long-day condition possibly contains some diffusible factor(s) which could induce flowering on chicory under

noninductive conditions (*e. g.* no vernalization). Untransformed plants of which flowering has been induced under the inductive condition may also contain the factor(s). In our very preliminarily grafting experiment with flower stalks of untransformed chicory as the rootstock, we have observed bolting in the untransformed scion (data not shown), supporting the speculation. Furthermore, Harada reported that shoots which were regenerated from flower stalk segments of untransformed chicory bolted to flower⁸⁾. On the other hand, when shoots were regenerated from leaf segments (*ca.* 1 cm square) of flowering Ri-transformant or flowering untransformed plant, most regenerants of Ri-transformant bolted to flower but those of untransformed plant never bolted (the result will be presented elsewhere⁶⁾). All these, in addition to the result presented here, seem to imply that the Ri-transformant contains or can continue to produce a stimulus to induce flowering, not only in a flower stalk but even in a leaf segment. To prove this, however, we need extensive and more detailed experiments.

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