Direct Adventitious Shoot Formation on Seedling Radicles in Seed Cultures of Strawberry

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Mature seeds of strawberry (*Fragaria* x *ananassa*) were placed on Murashige and Skoog medium supplemented with 2.22 μM 6-benzyladenine. After four weeks of culture, and without an intervening callus phase, approximately 36% of the resulting seedling radicles had formed numerous adventitious buds near their tips. A few buds on each radicle developed into shoots, while others formed disorganized calli. Consequently, the seedlings exhibited shoot apices at both ends of the axis of polarity. Our overall results suggest that a considerable level of plasticity in organ determination occurs even in higher plants, and that exogenous growth regulators can cause a root primordium in the radicle to be converted to a shoot primordium.

Keywords: *Fragaria* x *ananassa* Duch, organogenesis, polarity

A variety of plants ordinarily form shoots on roots (Raju et al., 1966). However, a few of these species, such as field bindweed (*Convolvulus arvensis*; see Bonnet and Torrey, 1966), develop buds from the pericycle in a location identical to the point of normal lateral root formation. Because root and shoot meristems are very stable entities, the root meristems in higher plants are rarely converted directly into shoot meristems, or vice versa (Peterson, 1975). Nevertheless, cultured root tip segments of *Catasetum* (Orchidaceae) and other allied taxa are capable of forming protocorm-like bodies, thereby providing an authentic example of direct conversion from root apex to bud (Kerbauy, 1984; Kraus and Monteiro, 1989; Kerbauy and Estelita, 1996). This root bud formation in *C. fimbriatum* can be promoted by both exogenous cytokinins (Colli and Kerbauy, 1993) and ethylene (Kerbauy and Colli, 1997); auxin inhibits this process (Colli and Kerbauy, 1993). However, regardless of the presence of growth substances in the culture medium, direct root-to-shoot conversion has not been possible in the attached roots of *C. fimbriatum* (Colli and Kerbauy, 1993). Here, we report that the radicles of intact strawberry seedlings directly form adventitious shoots in a culture medium supplemented with 6-benzyladenine (BA).

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MATERIALS AND METHODS

Plant Material

Mature seeds (achenes) of strawberry (*Fragaria* x *ananassa* Duch. cv. Sweet Charlie) were isolated from mature fruits and scarified with conc. H_2SO_4 for 10 min. After being rinsed with sterilized distilled water three times, they were placed on culture media.

Culturing Media and Conditions

The basal medium used throughout these experiments consisted of half-strength Murashige and Skoog (MS) (1962) inorganic salts, 100 mg L^{-1} myo-inositol, 0.4 mg L^{-1} thiamine·HCl, 3% (w/v) sucrose, and 8 g L^{-1} Phytagel. The pH of all media was adjusted to 5.8 before autoclaving at 121°C for 15 min. Afterward, 25 mL was dispensed into each plastic Petri dish (100 × 15 mm). All cultures were maintained at 27°C under light (approximately 3 W m^{-2} from cool-white fluorescent lamps, with a 16-h photoperiod).

Induction of Adventitious Shoots

To induce adventitious shoot buds, the surface-sterilized seeds were placed on an MS medium supplemented with 0.0, 2.22, or 4.44 μM 6-benzyladenine (BA). Each treatment comprised five seeds per dish, with four replicates. A seed was considered ger-

minated when the radicle was visible. The numbers of seedlings producing adventitious shoots and adventitious shoots produced per seedling were determined after four weeks of culture.

RESULTS AND DISCUSSION

Seed germination was accelerated as the concentration of BA in the medium increased (Fig. 1). However, the germination frequency reached approximately 80%, regardless of the BA level, after two weeks of culture. After three weeks, seedlings on media containing any amount of BA showed enlarged radicles and had started to form adventitious buds in the vicinity of the radicle tips, without having undergone an intervening callus phase (Fig. 2A). Seedlings on the medium without BA developed normally, i.e., the distal end of the radicle tip covered by the root cap did not give rise to an adventitious bud. The frequency of adventitious bud formation on the radicles reached approximately 36% at 2.22 μM BA (Fig. 3). Adventitious buds numbered 30 to 50 per radicle.

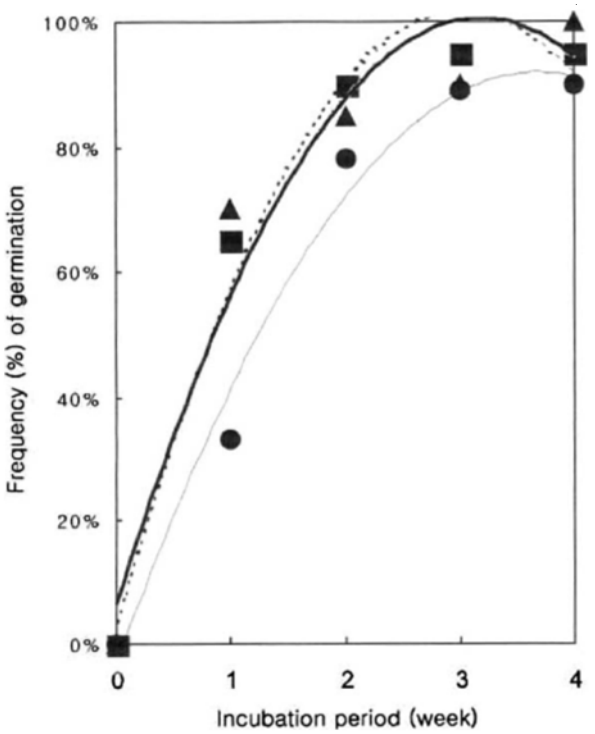

Figure 1. Frequency of strawberry seed germination. Seeds were placed on half-strength MS medium supplemented with 0.0 (●), 2.2 (■), or 4.4 μM BA (▲). The best fit models for 0.0, 2.2, and 4.4 μM BA, respectively, were $y=-7x^2+51.6x-3.3$, $r^2=0.98142$ ($P<0.05$), $y=-10.71x^2+64.86x+3.57$, $r^2=0.9813$ ($P<0.05$), and $y=-9.29x^2+59.14x+6.43$, $r^2=0.94192$ ($P<0.05$).

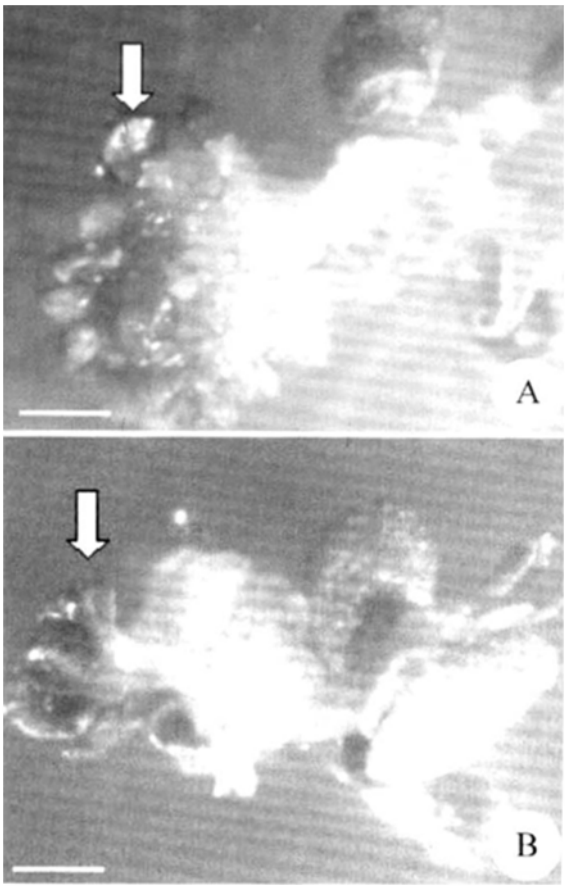

Figure 2. Adventitious shoot formation on strawberry seedling radicle. **A.** Numerous adventitious shoot buds (arrow) were formed, without an intervening callus phase, near the radicle tip. **B.** A few buds on each radicle developed into shoots (arrow). This seedling had shoot apices at both ends of the axis of polarity. Bars indicate 1 mm.

Within the same Petri dish, seedlings that formed adventitious buds on the radicles were less developed than those without buds. In addition, seedlings with

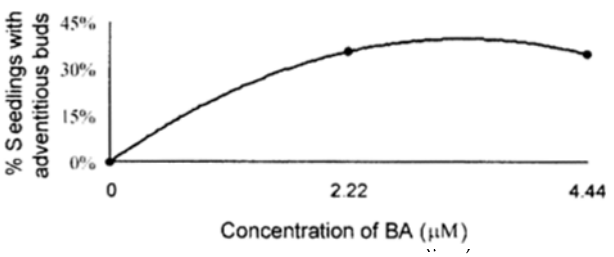

Figure 3. Frequency of adventitious bud formation on strawberry seedling radicles. Seeds were placed on half-strength MS medium supplemented with 0.0, 2.2, or 4.4 μM BA. Data were collected after four weeks of culture. The best fit model: $y=-3.7x^2+24.5x$, $r^2=0.999$ ($P<0.05$).

radicle-derived buds were positioned nearly horizontally on the medium, while the radicles of those without buds penetrated vertically into the medium, developing into normal roots. Based on these results, we believe the radicles that form adventitious buds lost their gravitropism. As the culturing period proceeded, only a few buds on each radicle developed into shoots, whereas the others formed disorganized calli. Consequently, those altered seedlings had shoot apices at both ends of the axis of polarity (Fig. 2B).

Whether or not growth regulators are added to culture medium, direct root-to-shoot conversion has not been reported for the attached roots of *C. fimbriatum* (Colli and Kerbauy, 1993). In contrast, we have demonstrated here that strawberry seedling radicles, which then develop into roots, are capable of directly forming adventitious shoots when cultured on media supplemented with BA. Based on the results of this study, as well as those from research with *Catasetum* (Kerbauy, 1984; Kraus and Monteiro, 1989; Colli and Kerbauy, 1993; Kerbauy and Estelita, 1996; Kerbauy and Colli, 1997; Peres and Kerbauy, 1999), we suggest that this direct root-to-shoot conversion, as observed in intact strawberry seedlings, reveals a considerable amount of plasticity in organ determination, even in higher plants. This plasticity may possibly be explained by the same sets of genes acting in both root and shoot to regulate cell fate and patterning (Benfey, 1999). However, research is still required on whether an undifferentiated primordium exists that can develop into either a bud or a root.

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