ground of beans of Meso-American origin, the behavior of this trait should also be studied when incorporated into larger-seeded beans of Andean origin. This information will help to determine if the dwarf outcrossing trait may be useful for population improvement of all or for only certain bean seed types.

Literature Cited

Barrons, K.C. 1939. Natural crossing in beans at different degrees of isolation. Proc. Amer. Soc. Hort. Sci. 36:637–640.

Bliss, F.A. 1980. Common bean, p. 273–284. In: W. Fehr and H.H. Hadley (eds.). Hybridization of crop plants. Amer. Soc. Agron., Crop Sci. Soc. of Amer. Madison, Wis.

Gepts, P. and F.A. Bliss. 1985. F_1 hybrid weakness in the common bean. J. Hered. 76:447–450.

Jain, S.K. 1979. Estimation of outcrossing rates: Some alternative procedures. Crop Sci. 19:23–26.

Lamprecht, H. 1936. Zur genetik von *Phaseolus vulgaris*: XII. Uber die vererbung der blutenund stammfarbe. Hereditas 21:129–166.

Mackie, W.W. and F.L. Smith. 1935. Evidence of field hybridization in beans. J. Amer. Soc. Agron. 27:903–909.

Martin, G.B. and M.W. Adams. 1985. The role of outcrossing in the generation of variability in Malawian bean landraces. Annu. Rpt. Bean Improv. Coop. 28:49–50.

Miranda, C.S. 1974. Cruzamiento natural en frijol. Campo 49(986):34–40.

Nagata, R.T. and M.J. Bassett. 1984. Characterization and inheritance of gamma ray induced mutations in common bean. J. Amer. Soc. Hort. Sci. 109(4):513–516.

Nagata, R.T. and M.J. Bassett. 1985. A dwarf outcrossing mutant in common bean. Crop Sci. 25:949–954.

Pereira Filho, I.A. and C. Cavariani. 1984. Taxa de hibridacão natural do feijoeiro comun en Patos de Minas, Minas Gerais. Pesquisa Agropecuaria Brasileiro 19(9):1181–1183.

Rachie, K.O. and C.O. Gardner. 1975. Increasing efficiency in breeding partially outcrossing grain legumes. Intl. Workshop on Grain Legumes ICRISAT, Hyderabad, India. p. 285–300.

Schull, G.H. 1908. A new mendelian ratio and several types of latency. Amer. Nat. 42:433–451.

Smith, F.L. and C.B. Madsen. 1948. Seed color inheritance in beans. J. Hered. 39:190–194.

Stoetzer, H.A.I. 1984. Natural cross-pollination in bean in Ethiopia. Annu. Rpt. Bean Improv. Coop. 27:99–100.

Tucker, C.L. and J. Harding. 1975. Outcrossing in common bean *Phaseolus vulgaris* L. J. Amer. Soc. Hort. Sci. 199(3):283–285.

Vieira, C. 1960. Sobre a hibridacão natural em *Phaseolus vulgaris* L. Revista Ceres 11(63):103–107.

Wyatt, J.E. 1984. An indehiscent anther mutant in common bean. J. Amer. Soc. Hort Sci. 109(4):484–487.

HortScience 24(4):671–673. 1989.

Origin of Somatic Embryos in Celery Tissue Culture

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Abstract. **Somatic embryos were induced from the primary callus of celery (*Apium graveolens* L. cv. Utah Improved #15) arising from leaf blade explants placed on Murashige and Skoog (MS) salts and vitamins medium supplemented with 9 μM 2,4-D for 4 weeks and then subcultured to 2,4-D-free MS medium. The histological origin of somatic embryos was from single cells along the surface of callus clumps. Embryos proceeded in a standard developmental pattern through the globular-, heart-, and torpedo-shaped stages. Secondary somatic embryos occurred on the cotyledons and hypocotyls of primary embryos. Chemical names used: 2,4-dichlorophenoxyacetic acid (2,4-D).**

Somatic embryogenesis in celery was first reported by Williams and Collin (1976a) and has become a model system for the study of this phenomenon. Histological studies were carried out by Al-abta and Collin (1978) and Zee and Wu (1979, 1980), who traced the origin of celery somatic embryos to globular structures embedded in the callus. The objective of this study was to histologically determine the cellular origin of somatic embryos in primary callus derived from leaf blade explants.

Callus induction. Explants were obtained from the leaves of greenhouse-grown seedlings of 'Utah Improved #15' celery (Takii Seed Co., Tokyo, Japan) at the four-leaf stage. Explants were surface-sterilized by soaking the tissues for 10 min in 1.0% sodium hypochlorite and rinsing three times with sterile distilled water. Leaf blades were cut into 9- to 25-mm² and petioles into 5-mm portions. Explants were placed (either adaxial or abaxial side up) in 60 × 15 mm petri dishes (seven explants per dish) containing 10 ml of MS salts and vitamins medium (Murashige and Skoog, 1962) supplemented with 9 μM 2,4-D and 87.6 mM sucrose. All media were solidified with 3 g Gelrite/liter and the pH was adjusted to 5.8 before autoclaving. Cultures were kept under 1 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR in a culture room for 4 weeks at 25C.

Production of somatic embryos. Primary callus arising on the explants was transferred to MS medium containing 87.6 mM sucrose without 2,4-D to produce somatic embryos. Cultures on semisolid medium were kept at 25C under low-intensity illumination (45 $\mu mol \cdot s^{-1} \cdot m^{-2}$ PAR) from cool-white fluorescent lamps on a 16-hr photoperiod.

Induction of somatic embryos and their subsequent maturation on MS medium was influenced by 2,4-D concentration and explant source. Leaf blade and petiole explants were grown on MS + 2,4-D and then transferred to MS without 2,4-D for 1 month (Table 1). Petiole explants produced more callus at 2.7 and 4.5 μM 2,4-D concentrations than leaf explants, but the callus was nonembryogenic and appeared white, transluscent, and

Table 1. Effect of 2,4-D concentration and explant source on callus induction and somatic embryo production in celery.[z]

2,4-D (μM)	Leaf blade explant		Petiole explant	
	Callus fresh wt[y] (mg)	No. somatic embryos[x]	Callus fresh wt[y] (mg)	No. somatic embryos[x]
0	0	---	0	---
0.9	11.4 ± 4.2	0	14.3 ± 4.0	0
2.7	20.0 ± 6.7	2.0 ± 0.9	33.9 ± 6.1	0
4.5	28.5 ± 6.2	4.5 ± 1.0	39.6 ± 6.0	2.4 ± 1.3
9.0	39.2 ± 7.1	8.6 ± 3.0	23.5 ± 5.8	0
13.5	27.0 ± 7.0	2.5 ± 1.0	16.0 ± 5.2	0

[z]Data are means of 30 observations.
[y]Four weeks on MS + 2,4-D medium.
[x]Four weeks on MS medium without 2,4-D for somatic embryo formation.

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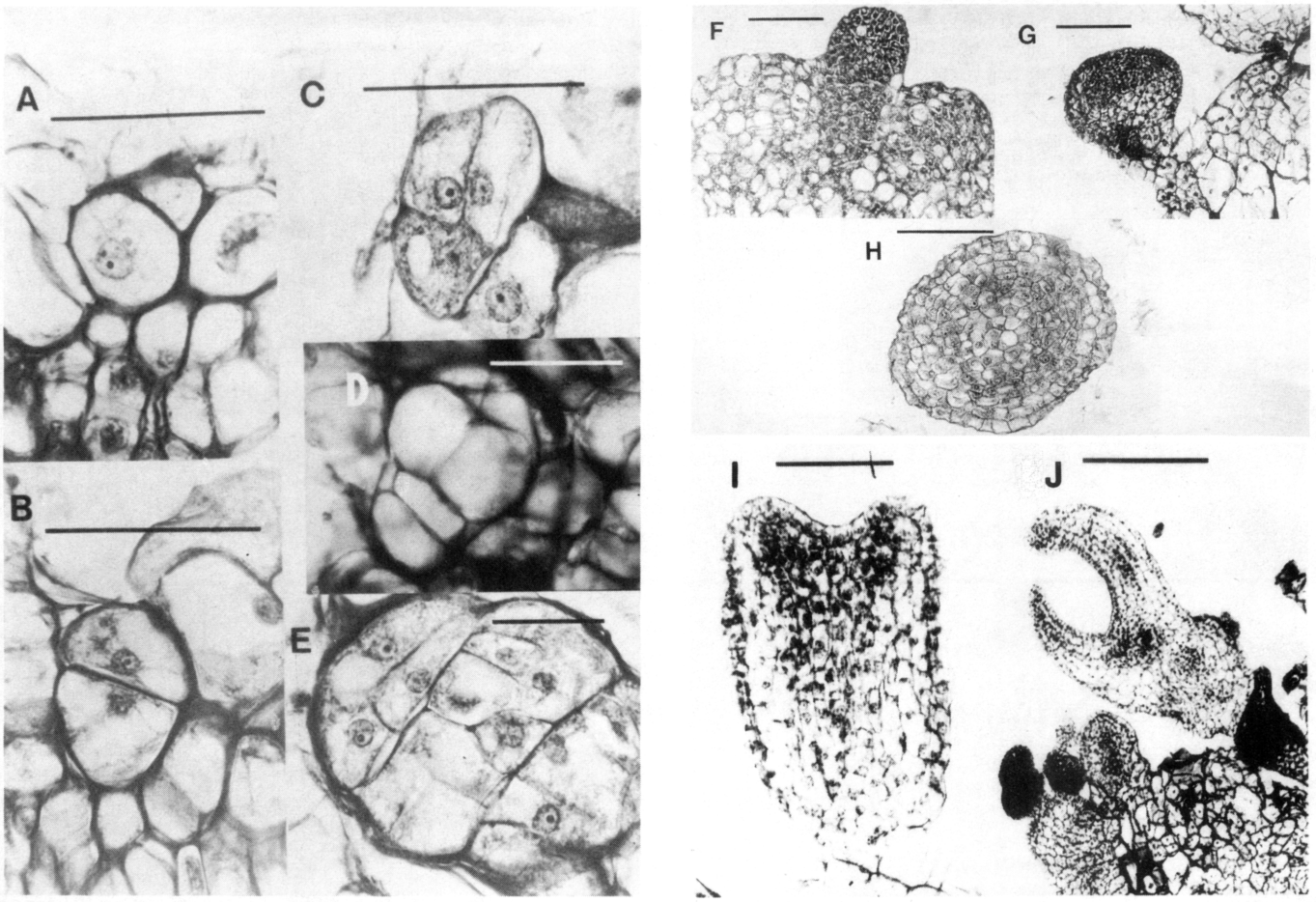

Fig. 1. Origin of celery somatic embryos in leaf blade-derived callus cultures. (**A**) Embryogenic single cell. Bar = 50 μm. (**B**) Dyad after transverse division of single cell. Basal cell gives rise to suspensor. Bar = 50 μm. (**C**) Three-celled stage after a longitudinal division of terminal cell in dyad. Bar = 50 μm. (**D**) Six-celled stage after the division of terminal cells and basal cell (stained by toluidine blue O). (**E**) Multicelled globular embryo in callus. Bar = 25 μm. (**F**) Early globular embryo protruding from callus. Bar = 100 μm. (**G**) Late globular embryo on callus surface. Bar = 100 μm. (**H**) Transverse section of globular embryo showing epidermal layer development. Bar = 100 μm. (**I**) Heart-shaped stage. Darkly stained cells from base to cotyledonary region give rise to vascular strands. Bar = 100 μm. (**J**) Torpedo stage. Bar = 200 μm.

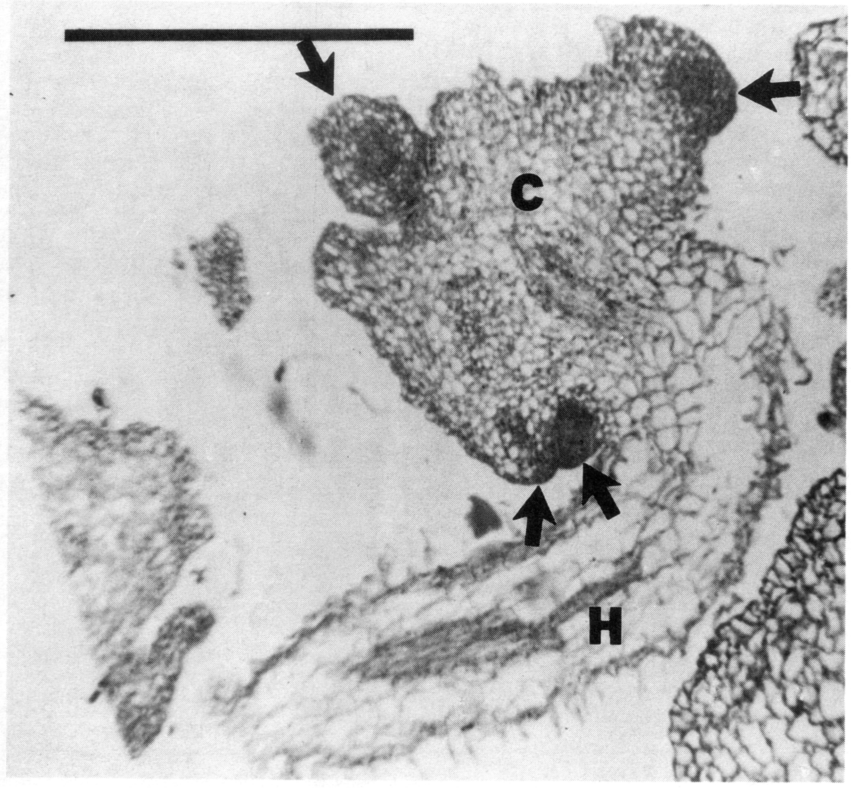

Fig. 2. Secondary somatic embryos arising from the cotyledon. C = cotyledon; H = hypocotyl; arrows point to secondary somatic embryos. Bar = 100 μm.

watery. Embryos produced from petiole callus were few and were only observed at 4.5 μM of 2,4-D. Callus from leaf blade explants was compact and light yellow; maximum callus (fresh weight) was produced at 9 μM 2,4-D. The highest frequency of somatic embryos also was produced from leaf blade callus induced with 9 μM 2,4-D.

Previous studies on somatic embryogenesis of celery (Williams and Collin, 1976a, 1976b) indicated that the petiole was the optimum explant for induction of somatic embryos. In this study, we found leaf-blade tissue to be superior to leaf-petiole. Leaf explants produced typical embryogenic-competent callus characterized as compact, friable, and light yellow. Callus induced on MS containing a low concentration of 2,4-D showed poor production of embryos, while concentrations >9 μM inhibited embryo production.

In the present study, exposure of explants to 9 μM 2,4-D for 4 weeks was optimal for induction of somatic embryos, but continual exposure to 2,4-D suppressed embryo development. This confirms that 2,4-D stimulates embryo initiation from cultured cells in celery but inhibits subsequent embryo development.

Histology. Callus and tissue samples were fixed in FAA [5 formalin : 5 glacial acetic acid : 90 ethanol (by volume)], dehydrated

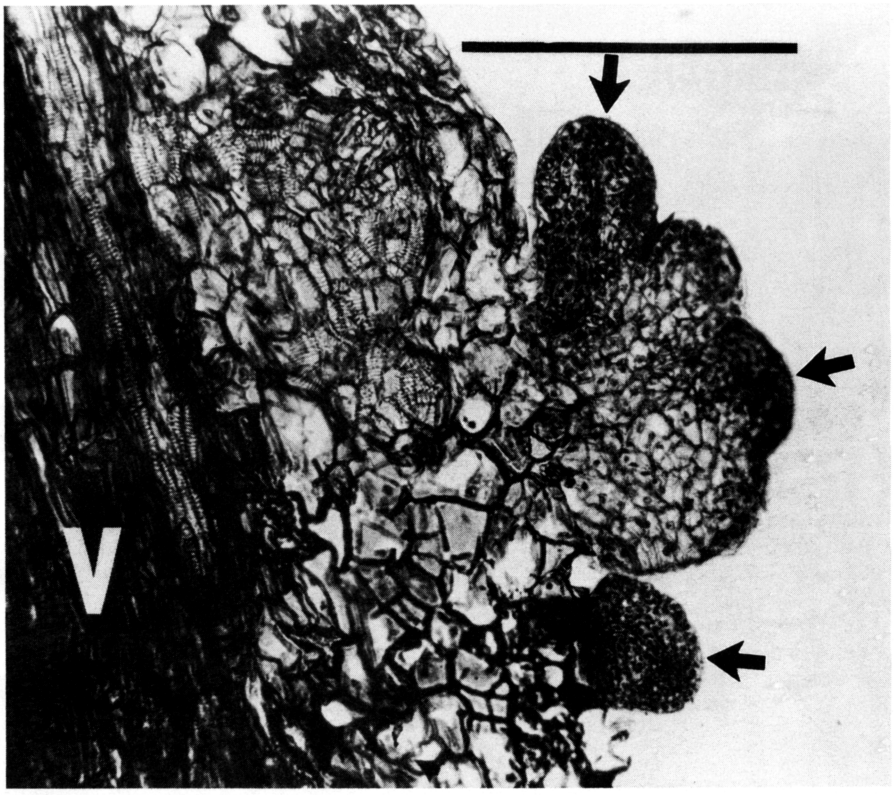

Fig. 3. Secondary somatic embryos arising from the hypocotyl of a celery somatic embryo. V = vascular strand of the hypocotyl; arrows point to somatic embryos. Bar = 50 μm.

using an ethanol-tertiary butanol series, and embedded in paraffin (Paraplast). Embedded tissues were sectioned at 8 μm and mounted on glass microscope slides. Paraffin was removed in a xylene-ethanol series; tissues were stained with safranin and fast green (Sass, 1958).

Histological observations of embryogenic-competent callus indicated that competent cells were isodiametric and easily distinguished from nonembryogenic cells by their darkly stained nuclei and nucleoli (Fig. 1A). A transverse division of an embryogenic single cell (usually parallel to the callus surface) formed a two-celled dyad (Fig. 1B). A three-celled proembryo formed after a longitudinal division of the terminal cell (Fig. 1C). Transverse divisions of the basal cell and each of the two terminal cells transformed the three-celled proembryo into a six-celled proembryo (Fig. 1D). Subsequent division of terminal cells formed a multicelled globular embryo (Fig. 1E). The suspensor appeared to degenerate and was not observed in mature somatic embryos. Globular embryos protruded from the callus surface during development (Fig. 1F). A prominent epidermal layer, formed on the globular embryo (Fig. 1H), was soon followed by vascular strand development at the heart-shaped stage (Fig. 1G). Darkly stained cells extending from the base of the embryo to the cotyledonary regions represent the initiation of vascular strands (Fig. 1I). Heart-shaped embryos developed into torpedo-shaped embryos showing well-developed cotyledons (Fig. 1J). Secondary embryos occurred with high frequency on the cotyledons (Fig. 2) and the hypocotyl (Fig. 3), which inhibited growth of the apical meristem and limited the conversion of primary somatic embryos into normal plantlets.

Al-abta and Collin (1978) and Zee and Wu (1979, 1980) have previously traced celery somatic embryogenesis to the multicelled globular stage, but, in this study, we further traced the origin to a single cell. Single cell origin of somatic embryos had been previously observed in carrot (McWilliam et al., 1974) and jojoba (Wang and Janick, 1986).

The cytoplasm of the initial embryogenic cells of celery were darkly stained by fast green, indicating a condensed cytoplasm with abundant starch. Nuclei and nucleoli of the embryogenic cells were stained deep-red from safranin, indicating a large volume of DNA. These cytological characteristics are consistent with previous reports in other species (Haccius, 1978; Halperin and Jensen, 1967; Konar et al., 1972). Vasil and Hildebrandt (1966) have reported the development of somatic embryos from floating single cells in liquid medium. In the present study, abnormal divisions occurred from floating single cells of celery, but their further development into somatic embryos was not observed.

Literature Cited

Al-abta, S. and H.A. Collin. 1978. Cell differentiation in embryoids and plantlets of celery tissue cultures. New Phytol. 80:517–521.

Haccius, B. 1978. Question of unicellular origin of non-zygotic embryos in callus cultures. Phytomorphology 28:74–81.

Halperin, W. and W.A. Jensen. 1967. Ultrastructural changes during growth and embryogenesis in carrot cells. J. Ultrastruct. Res. 18:428–443.

Konar, R.N., E. Thomas, and H.E. Street. 1972. Origin and structure of embryoids arising from epidermal cells of the stem of *Ranunculus sceleratus* L. J. Cell Sci. 11:77–93.

McWilliam, A.A., S.M. Smith, and H.E. Street. 1974. The origin and development of embryoids in suspension cultures of carrot (*Daucus carota*). Ann. Bot. 38:243–250.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 15:473–497.

Sass, J.E. 1958. Botanical microtechnique. 3rd ed. Iowa State Univ. Press, Ames.

Vasil, I.K. and A.C. Hildebrandt. 1966. Variations of morphological behavior in plant tissue cultures: I. *Cichorium endivia*. Amer. J. Bot. 53:860–869.

Wang, Y.-C. and J. Janick. 1986. Somatic embryogenesis in jojoba. J. Amer. Soc. Hort. Sci. 111:281–287.

Williams, L. and H.A. Collin. 1976a. Embryogenesis and plantlet formation in tissue cultures of celery. Ann. Bot. 40:325–332.

Williams, L. and H.A. Collin. 1976b. Growth and cytology of celery plants derived from tissue cultures. Ann. Bot. 40:333–338.

Zee, S.-Y. and S.C. Wu. 1979. Embryogenesis in the petiole explant of Chinese celery. Z. Pflanzenphysiol. 93:325–335.

Zee, S.-Y. and S.W. Wu. 1980. Somatic embryogenesis in the leaf explants of Chinese celery. Austral. J. Bot. 28:429–436.