apparently there is an inhibitory effect due to apical dominance.

Rooting. Rooting of shoots was accomplished on a 1/3 strength MS inorganic salt medium containing (per liter) 0.4 mg thiamine·HCl, 100 mg i-inositol, 30 g sucrose, and 1.5 g activated charcoal; 61% of the cultures formed roots on this medium. Culturing the shoot tips on full strength MS inorganic salts plus 3 mg/liter NAA or indolebutyric acid (IBA) for one week prior to transfer to 1/3 MS inorganic salts resulted in 58% rooting. Plants were established in a sphagnum peatmoss–perlite mix after a 3-week hardening-off period under high humidity.

Table 2. Comparison of inorganic salt formulation and growth promoters (16 mg/liter $Na\text{-}H_2PO_4\cdot H_2O$ and 80 mg/liter adenine sulfate) on shoot development of *Nandina domestica* 'Purpurea' (both media contained 0.1 mg/liter NAA, 1.0 mg/liter BA, and 1.5 g/liter activated charcoal).

Salt formulation	Growth promoters	No. of cultures	Shoot development (%)
MS	–	99	54 (44–64)[z]
	+	140	49 (41–57)
B5	–	64	80 (70–90)
	+	38	32 (17–47)

[z]95% confidence limits in parenthesis.

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Regeneration of Plants from Cell Suspensions of *Lactuca saligna*, *Lactuca sativa*, and *Lactuca serriola*

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Abstract. **'Black Seeded Simpson', 'Buttercrunch', and 'New York 12' lettuce (*Lactuca sativa* L.), Plant Introductions (PI) 261653 of *Lactuca saligna* L., and Acc. No. 500–4 of *L. serriola* L. regenerated plants from cell suspensions originated from leaf callus. Liquid cultures of these *Lactuca* spp. in either B5 or Murashige and Skoog (MS) basic medium amended with alpha-naphthaleneacetic acid (NAA) and 6-benzylamino purine (BA) developed roots, shoots, and complete plants when transferred to agar plates of B5 with several concentrations of BA under 16 hr of fluorescent illumination. Shoots were induced to produce a root system when cultured in B5 agar medium amended with NAA. Variation in response between cultivars and between species was observed, with *L. serriola* and 'Black Seeded Simpson' responding best to treatments.**

Two close relatives of lettuce (*Lactuca sativa*)—*L. saligna* and *L. serriola*—are potential sources of disease and insect resistance for commercial cultivars. Resistance to turnip mosaic virus, cucumber mosaic virus, and the cabbage looper (*Trichoplusia ni*) has been reported in PI 261653 of *L. saligna* (12, 15). Other representatives of this species may be sources of resistance to new races of the downy mildew pathogen (*Bremia lactucae*) that have caused a rapid breakdown of the resistance in lettuce that derived from *L. serriola* (11). Conversely, *L. serriola* may offer resistance where *L. saligna* fails, as in lettuce drop caused by *Sclerotinia minor* (1) and to strain CMV-LsS of cucumber mosaic virus (R. Provvidenti, personal communication), and also to *T. ni* (7).

The use of cell and tissue-culture methods and plant regeneration of *Lactuca* has been limited in the past to cultivars of *L. sativa* (2, 3, 5, 8, 13, 14). This report discusses the response of 'Black Seeded Simpson', 'Buttercrunch', and 'New York 12' lettuce (*L. sativa*), *L. saligna* PI 261653, and *L. serriola* Acc. 500–4 to culture *in vitro* as callus cell suspensions and the subsequent regeneration of plants. The purpose of these studies was to find efficient methods of *in vitro* culture of *Lactuca* spp. and subsequently to evaluate possible somaclonal variations for their usefulness in the development of disease resistant lines.

Commercial seed sources were used for the lettuce cultivars. Seeds of *L. saligna* and *L. serriola* were obtained from R. Provvidenti of the New York State Agricultural Experiment Station. To induce callus for cell suspensions, young expanded leaves were harvested from one- to 2-month-old seedlings maintained in a greenhouse at 24–30°C under sunlight conditions supplemented in winter by 2 additional hours of fluorescent lighting in the morning. The leaves were surface-sterilized by immersion in 40% ethyl alcohol for a few seconds and 10 min in 20% Clorox (1% NaOCl), and then washed 3 times in sterile distilled water. The leaves were cut aseptically into 2 cm^2 pieces, plated onto 0.8% Difco Bacto agar media in 100 × 15 mm disposable Petri dishes, and incubated in the dark for one month in the laboratory at 24–27°C. Callus that developed from the leaf pieces was transferred monthly to fresh agar plates for increase and maintenance. Callus cell suspensions were prepared by transferring 0.5 g of friable callus from the plates into 250-ml Erlenmeyer flasks with 50 ml of liquid medium and shaken at 40–60 rpm on Junior Orbit shakers. Transfers to fresh liquid medium were made weekly, separating the suspension roughly according to size in 2 categories: very fine suspensions with single cells to a few cell aggregates; and large aggregates that passed easily through large-bore pipettes. Twenty-five ml of the cell suspensions were added to 25 ml of fresh liquid medium and again placed on the shakers. After 4 weekly transfers, the cell suspensions were pipetted directly onto dishes with agar medium or centrifuged for 10 min at 100 g, washed twice in liquid basic medium, and plated at 3 ml per dish. The cultures were incubated in the dark, or with either 16 hr or 24 hr of fluorescent light (2.5 klx) for periods of up to 2 months. Regenerated shoots were transferred to root-inducing medium and one week later to 20-ml glass vials with Cornell mix (4). The plants were usually ready for transfer to the greenhouse one week later for further observation.

Soft, white-to-cream color, friable callus developed from the leaf pieces of most test plants in either B5 (6) or MS (10) basic media supplemented with 0.1 mg/liter of Picloram (4-amino-3,5,6 trichloropicolinic acid) or 1–2 mg/liter of NAA and 0.5–1.0 mg/liter kinetin, or BA. *L. saligna* calli were usually slower to develop and more compact than the rest, but satisfactory cell suspensions were also prepared from them. The callus pieces could be separated easily into small cell

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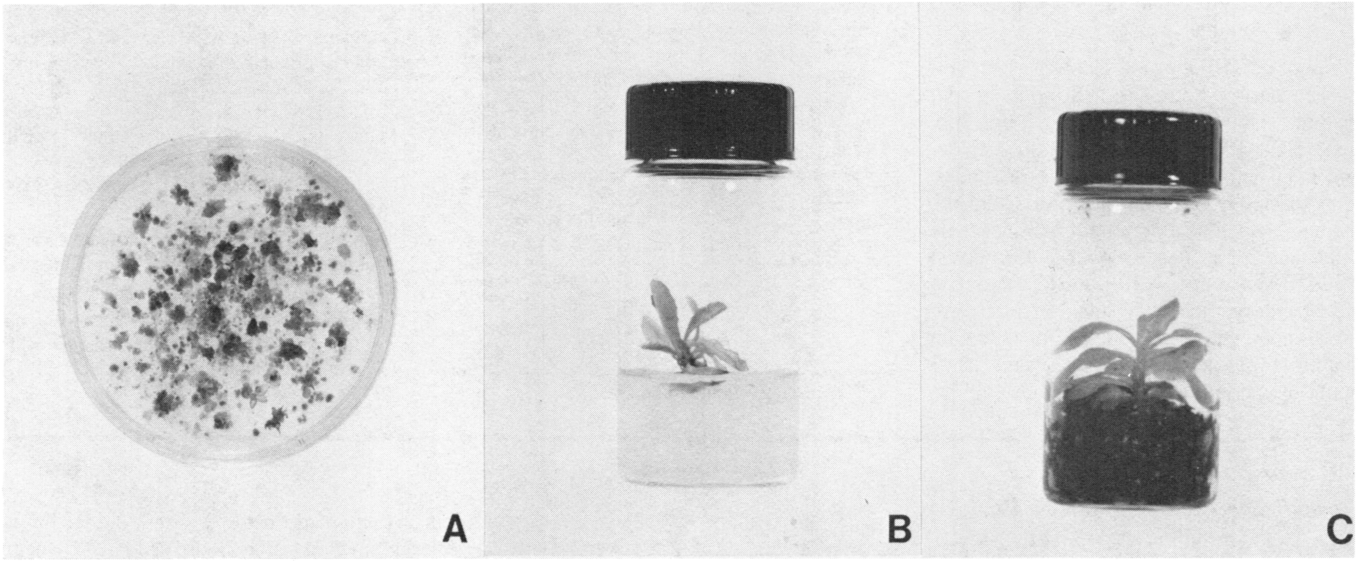

Fig. 1. Shoot development on agar plate by *L. serriola* (A), root induction on agar medium of *L. saligna* plantlet (B), and established rooted plant of *L. sativa* cv. Black Seeded Simpson in Cornell mix (C).

Table 1. Response of *Lactuca* cell suspensions when transferred to agar plates with different concentrations of BA.

Liquid culture medium (B5)		Agar culture medium (B5)	Response[z]				
				L. sativa			
NAA (mg/liter)	BA (mg/liter)	BA (mg/liter)	*L. saligna* PI 261653	Black Seeded Simpson	Buttercrunch	NY 12	*L. serriola* Acc. 500–4
1	0.5	0	R	R,S	R	R	R,S
		1	S	R,S	S	S	S
		2	S	S	S	S	S
		3	S	P,S	S	S	P,S
2	0.5	0	R	R	R	R	R,S
		1	R	R,S	S	S	R,S
		2	R,S	S	S	S	S
		3	S	S	S	S	P,S

[z]R = roots; S = shoots; P = complete plants with roots and shoots.

clumps; subsequent weekly transfers of the fine and large cell aggregates into fresh liquid medium resulted in good cell division and growth. Microscopic observation of the cell suspensions showed that the finer portion contained 1–3% single cells and the rest aggregates of from 5 to 20 cells. The heavier portion contained cell aggregates with a larger number of cells.

Green to red bud formation and further differentiation into plantlets occurred in the 16- and 24-hr illumination treatments when the agar medium was composed of either MS or B5 with 1–3 mg/liter kinetin or BA with no auxin added. Regeneration of shoots occurred with 30 days, usually from the larger callus aggregates (5–10 mm diameter) where multiple shoots often formed and from small (1 mm diameter) calli where single shoots developed. Numbers of plantlets produced per plate varied among species: *L. serriola* performed best with an average of 18 shoots per plate, 'Black Seeded Simpson' with 13, 'Buttercrunch' and 'New York 12' each with 8, and *L. saligna* with 5. Roots developed from shoots when they were transferred to agar medium B5 supplemented with 1–2 mg/liter NAA, especially if the transfer was made when the shoots were at least one cm long. Transfer to Cornell mix in glass vials resulted in good root development even in shoots exposed to the root regenerating medium but transferred to vials before root development was apparent. Transfer to Cornell mix without previous treatment in the root regenerating medium did not usually result in root formation. Fig. 1 illustrates shoot development and growth of regenerants for *L. serriola, L. saligna,* and *L. sativa*.

Washing the cell suspensions with the basic medium before plating resulted in some increase in shoot formation, but the response was not consistent. It did, however, inhibit the development of roots only when the suspension was plated on B5 or MS devoid of auxins and cytokinins. Plants with shoots and roots developed in plates where the liquid medium contained 1–2 mg/liter of NAA and the plate medium had 3 m g/liter of BA (Table 1).

In these studies, *L. serriola* Acc. 500–4 and 'Black Seeded Simpson' responded best to *in vitro* culture from cell suspensions and *L. saligna* PI 261653 less so. Further modifications in growing conditions may lead to improvement in the regeneration of complete plants in these and other *Lactuca* species. While the regenerated plants resulting from these studies did not differ superficially from the seedlings used for callus formation, a more detailed study of their possible genetic variations is underway. Further manipulations *in vitro* with these species to induce genetic variations may result in useful somaclonal lines as has been the case in other crop species (9). Useful induced changes in uncultivated species with potential interest for their use in plant breeding may prove to be a beneficial approach to germplasm enhancement.

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Increased Embryo Viability of Early Ripening Peaches in Response to Daminozide, Maleic Hydrazide, and Thiourea

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Additional index words. *Prunus persica,* embryo culture, seed germination, butanedioic acid mono-(2,2-dimethylhydrazide)

Abstract. **Trees and fruit of 6 early ripening cultivars of peach and nectarine [*Prunus persica* (L.) Batsch] were sprayed about 4 weeks prior to commercial harvest with either butanedioic acid mono-(2,2-dimethylhydrazide) (daminozide), maleic hydrazide, or thiourea. The date of 50% drop of overripe fruit was retarded one to 4 days. Chemical treatments increased germination of *in vitro* embryo culture of 'Fla. 3–1' and 'Flordaking' up to 27–32%. Seed germination from cracked pits of 'Fla. 7–3N', 'Fla. 7–4N', 'Earligrande', and 'Fla. M6–6N' generally increased up to 50%. The delayed fruit drop did not appear to account for all of the increase in germination. The results support an enhancement of embryo development in addition to that attributed to delayed fruit maturity.**

Early ripening resulting from a short fruit development period (FDP) is a major goal in most peach and nectarine improvement programs, but breeding is hampered by the inability to germinate seed from maternal parents with a short FDP. The earlier ripening cultivars generally have proportionally less embryo development and lower percentage viability (1). Embryos from fruit ripening less than 85 days from bloom generally need specialized culture for proper germination and growth, but even with embryo (1) or ovule (2) culture, the percentage of germination is proportional to FDP (7).

Peach embryo development generally occurs after stage I of fruit development (6). Methods to speed up embryo development or delay fruit development may be a means of increasing percentage of germination. For example, thiourea and maleic hydrazide were found to depress fruit development in banana and sporadically stimulate seed formation (3). Chemicals that would increase percentage of seed germination in peach would permit using shorter FDP maternal parents in breeding to obtain a larger population of seedlings with early ripening.

Fruit from single limbs in 2–3 trees of 'Flordaking', 'Fla. 3–1', and 'Earligrande' peaches and 'Fla. M6–6N', 'Fla. 7–3N', and 'Fla. 7–4N' nectarines were used to evaluate the effect of daminozide, maleic hydrazide, and thiourea on fruit ripening and seed formation in 1981 and 1982. Single and double applications of daminozide (2000 ppm), maleic hydrazide (1000 ppm), and thiourea (2000 ppm) were made prior to the final fruit swell (about 25–30 days before first commercial picking) in 1981 and 1982. Fruit and trees were sprayed using Tween 20 (0.5%) as a wetting agent.

When 50% of the fruit dropped, the rest were harvested. Pits from 20 to 30 fruits for each treatment were extracted and cracked and the seed were refrigerated at 5°C for 4 weeks in sterilized and humidified perlite medium. Seed were removed from cold treatment and placed at room temperature under 15 hr/day fluorescent light for 4 weeks. Germination (the emergence >2 cm of both the radical and plumule) was then determined.

The earliest-ripening cultivars—'Flordaking' and 'Fla. 3–1'—having a FDP <70 days did not germinate in 1981, whereas seed of other cultivars having a FDP ≥70 days did germinate. In 1982, embryos of 'Flordaking' and 'Fla. 3–1' were excised and cultured on modified Knops medium (4). They were left at room temperature for 20 days after planting to allow embryo maturation. The embryos were then moved to a cold dark room where the temperature was maintained at 4–7°C for 4 weeks before germination. 'Fla. 3–1' (50 days FDP) had small embryos (4.3 to 5.7 mm length) surrounded by large amounts of endosperm at 50% fruit drop. These embryos doubled in length within 2 weeks in culture before they were placed in cold treatment. Cotyledons of 'Flordaking' (65 day FDP) were full-size, softened, and slightly opaque and no endosperm was visible. Later-ripening cultivars (≥70 days FDP) had full-size, soft, and white embryos.

The 1981 and 1982 germination data for seeds not embryo-cultured resulting from daminozide, maleic hydrazide, and thiourea sprays on the 4 cultivars were similar and thus combined in Table 1.

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