micronized nicarbazin was prepared for SEM by adhering the specimen to an aluminum stub, which was then carbon-coated and gold-coated. An identical sample of nicarbazin was immersed in water for 5 hours, vortexed, filtered, air-dried, and prepared for SEM in the same manner as the unwetted material. The water-treated nicarbazin (Fig. 2b) had much finer crystals of DNC than the dry nicarbazin (Fig. 2a). In a test run, DNC was observed to be unaffected by water immersion; the DNC used was factory-processed material that is wet-milled in methanol before being used in complex formation. The median diameters of dry and wetted DNC crystals were 0.51 and 0.53 μm, respectively, indicating no significant change, whereas the median diameter of DNC crystals produced from nicarbazin after immersion was 0.11 μm. In the size determinations, observations were made of 25 particles from each sample. Measurements were taken in a transverse plane at the anterior and posterior extremities, and diameters were calculated with the Zeiss MOP Videoplan. The four- to fivefold reduction in particle diameter, comparing factory-processed with nicarbazin-derived DNC, is not far from the reduction estimated as necessary to explain tenfold complex superiority.

Earlier recognition of a role for complexes in improved dissolution is evident in Higuchi and Ikeda's (*16*) study of a digoxin-hydroquinone molecular compound. However, since complexation and solution are related phenomena, recognition of such a role is also implied by biopharmaceutical interest in obtaining better absorption of material when solutions of poorly soluble drugs are administered (*17*). Generally, the emphasis has been on consistent performance with formulations of drugs soluble in the 1 mg per 100 ml range, rather than on enhanced activity. In no case has an improvement in efficacy been reported that is comparable to that found with DNC complexes, but here drug solubility is 1000 times less.

Our DNC observations suggest that the disintegration in water of crystals of hydrogen-bonded complexes formed from water-insoluble and water-soluble compounds will yield more finely divided crystals of the water-insoluble component. The same situation probably prevails on wetting of easily hydrolyzable salts of water-insoluble compounds. We suspect that another consequence of the replacement of the strong intermolecular bonding in highly insoluble compounds by complex or salt bonding is greater effectiveness in grinding operations. Other "particle size problems," as difficulties relating to the utilization of insoluble materials are often described, may yield to an approach involving the formation and dissolution of complexes.

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A Morphogenetically Competent Soybean Suspension Culture

Abstract. *A morphogenetically competent suspension culture was derived from embryonic axes of* Glycine max *cv. Mitchell. The cultural history included visual selection for nonfriable, embryo-like structures, recurrent selection in a regime of 2,4-dichlorophenoxyacetic acid exposure and withdrawal, and the replacement of the nitrogen in a Murashige and Skoog salts–based medium with 20 millimolar ammonium citrate. The embryoids produced by this suspension are capable of completing plantlet development. The suspension can be maintained by serial subculture.*

There are two routes of plant production from cell cultures: somatic embryogenesis and shoot organogenesis. For any particular culture, the choice of route is generally made on an empirical, operational basis (*1*) and, in most cases, if regeneration can occur at all, it occurs in response to only one of the two kinds of manipulations. Removal or reduction of high levels of auxin, usually 2,4-dichlorophenoxyacetic acid (2,4-D), leads to the formation of embryoids from certain cultures of certain species, while in other species exposure to a defined balance of auxin and cytokinin leads to the formation of shoots. Such manipulations, however, do not lead to plantlet production from cell lines of many species. We believe the physiological or epigenetic state of those cell lines is such that the cells are not competent for the induction of the processes underlying meristem organization. The molecular nature of the phenomenon, morphogenetic competence, remains obscure (*1*).

Wernicke *et al.* (*2*) maintain that in sorghum, and perhaps in other species, embryogenic cultures proliferate as small masses of suppressed primordia. They and others feel that high levels of 2,4-D suppress differentiation but allow continuance of a morphogenetically competent or determined state. For example, embryogenic suspension cultures of *Schizachyrium* require levels of 2,4-D in excess of the level giving optimal growth rates (minimal doubling times); lesser levels result in spontaneous plantlet formation in the suspensions (*3*).

Despite recent successes in achieving shoot or plantlet formation from explants, callus, or suspension cultures of various legumes, published reports on the genus *Glycine* are limited to shoot production from hypocotyl slices, multiplication of cotyledonary buds, and an incomplete somatic embryogenesis (*4–6*). The embryogenesis from suspension culture proceeds as far as late torpedo stage (*6*). Histological examination showed, however, that the embryoids were aberrant and lacked a well-organized shoot apical meristem. Such structures are termed "neomorphs" by Krikorian and Kann (*7*). Additional rounds of 2,4-D induction and transfer to em-

bryogenic conditions were successful in developing neomorphs of day lily into a highly morphogenetic culture (*7*) and in developing a highly embryogenic line of carrot (*8*). A similar scheme of recurrent selection was used to develop a highly rhizogenic culture of barley (*9*).

This report describes the selection and development of a morphogenetically competent culture of soybean, *Glycine max*, and considers the implications of this culture on the general nature of embryogenic competence in grain legumes and other plants.

We initiated callus from young embryos of soybean. The embryos were removed aseptically from 2.5- to 3.0-cm pods on plants of *Glycine max* cv. Mitchell grown in our greenhouses in Palo Alto under a natural November photoperiod. Embryo axes were cut into pieces of 1 to 2 mm and cultured as detailed in Table 1. The hard, nonfriable tissue was selected for transfer to new medium; the scheme of recurrent selection resulted in a tissue line that gave rise to hard, green, glossy, abnormal embryoids.

Somatic embryogenesis from competent cultures of alfalfa requires a minimum of 12.5 m*M* ammonium; at concentrations of 50 m*M* or more, somatic embryoids are produced even by cells exposed to rhizogenic hormone combinations (*10*). This interaction of hormone combination and level of reduced nitrogen led us to replace the two nitrogen salts in Murashige and Skoog (MS) salts (*11*) with 20 m*M* ammonium citrate (N-amended medium). Subsequent transfer from this N-amended medium produced one exceptional piece of tissue that was covered with small embryoids. Half of these were placed in N-amended liquid 2,4-D medium shaken at 125 rev/min in light and the other half were placed on a medium containing indole-3-butyric acid (IBA) (0.005 mg/liter) and 6-benzylaminopurine (BA) (0.2 mg/liter), a medium Cheng *et al.* (*5*) found to support the elongation of buds proliferated from soybean cotyledonary nodes. Embryoids placed on this latter medium gave rise to plantlets, each with several elongating internodes (Fig. 1).

Embryoids transferred to N-amended liquid 2,4-D medium proliferated and gave rise to a nodular suspension culture that could be maintained by serial subculture (Fig. 2). This culture retained the ability to form embryoids when transferred to the medium of Cheng *et al.* (*5*). Histological examination showed that the embryoids are bipolar, as the gross morphology suggests (Fig. 3). Radicle development is extremely rare on Cheng's medium. Unbalanced growth of embryoids leading to shoot but not root development is not unusual for somatic embryogenic systems (*12*); other cultural conditions may exist that allow balanced growth of the embryoids (*13*). At present, shoots produced in vitro will have to be rooted in a subsequent step. Shoots transferred to basal medium plus indole-3-acetic acid (IAA) (0.1 mg/liter) did root.

Table 1. Cultural history of the morphogenetic soybean suspension. Abbreviations not previously identified: 2-ip, 2-isopentenyladenine; s, medium solidified with Difco Bacto-Agar (9.0 g/liter); and l, liquid medium (50 ml) shaken at 125 rev/min in light.

Date	Medium*
Embryonic axes explanted to	
14 November 1981	2,4-D (5 mg/liter) (s)
2 December 1981	2,4-D (5 mg/liter) (s)
11 January 1982	IAA (2 mg/liter) + 2-ip (0.2 mg/liter) (s)
1 April 1982	2,4-D (5 mg/liter) (s)
10 May 1982	2,4-D (5 mg/liter)† (s)
17 June 1982	2,4-D (5 mg/liter) (s)
One piece with numerous small embryoids	
16 August 1982‡	2,4-D (5 mg/liter) (l)†
22 September 1982	2,4-D (5 mg/liter) (l)†
12 October 1982	2,4-D (5 mg/liter) (l)†
5 November 1982	2,4-D (5 mg/liter) (l)†
17 November 1982	2,4-D (5 mg/liter) (l)†
9 December 1982	2,4-D (5 mg/liter) (l)†
Continued to present	

*Basal medium contained MS salts, nicotinic acid, pyridoxine, thiamine, and inositol at 0.5, 0.5, 10.0, and 100 mg/liter, respectively, and sucrose at 20 g/liter (*p*H adjusted to 6.0 prior to autoclaving). †Nitrogen in MS salts replaced by 20 m*M* dibasic ammonium citrate. ‡Tested for extent of regeneration potential by transfer to IBA (0.005 mg/liter) + BA (0.2 mg/liter) (*5*).

Further selection on the suspension produced a more finely divided but still embryogenic culture. Delaying the subculture of the suspension by a few days resulted in large amounts of free cells in addition to the nodular aggregates of cells. These cells, poured off from the nodular part of the suspension and diluted appropriately with fresh medium, proliferated as a cell suspension. Transfer of such cell suspensions to Cheng's medium resulted in occasional embryoids. We believe that these arose from small nodular contaminants and that the cells in the suspension did not retain the morphogenetic competence of the parent culture.

Somatic embryogenesis in our soybean culture occurs after the coordinate removal of 2,4-D and a change from 40 m*M* ammonium to 20 m*M* ammonium and 40 m*M* nitrate. Change in auxin level alone is not enough to trigger somatic embryogenesis. Transfer of morphogenetic suspension from N-amended 2,4-D medium to N-amended IBA (0.005 mg/liter) and BA (0.2 mg/liter) medium did not result in embryoid formation and development.

Change in nitrogen source is essential.

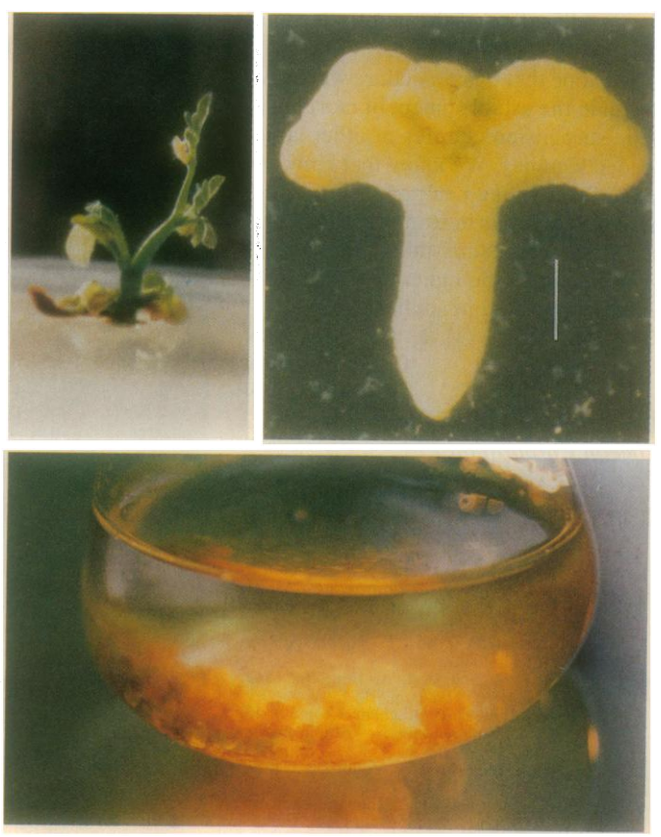

Fig. 1 (top left). Plantlet from embryogenic suspension, showing trifoliate leaves and several elongating internodes. Plantlets are transferred to rooting medium at this stage. Fig. 2 (bottom). Embryogenic suspension. This nodular suspension is maintained by serial subculture in N-amended 2,4-D medium. Fig. 3 (top right). Young somatic embryoid. Two weeks after transfer to Cheng's medium, the culture contains many well-formed embryoids. These are often found in clusters attached by the tips of their radicles. Embryoids undergo precocious shoot development after this stage. Scale bar, 1 mm.

A subline of the morphogenetic culture was established in 2,4-D medium with standard MS salts (*12*). Transfers to Cheng's MS-based medium (*5*) after 1 week produced a few abnormal embryoids. After 4 weeks in standard MS salts, transfers to Cheng's medium resulted in glossy green structures but not a single embryoid. N-amended salts maintain morphogenetic competence; standard MS salts allow embryoid formation and development.

From our results, the coordinate change in auxin level and nitrogen source is the key to triggering complete somatic embryogenesis in competent cultures of soybean. Although greater than 90 percent of the aggregates in our suspension can become green and "leafy" after transfer, not all give rise to well-formed embryoids. It may be that further reduction of the ammonium ion or other changes in the composition of the medium could lead to greater efficiency in embryoid production.

Change in auxin level alone can induce incomplete somatic embryogenesis from cell suspensions of a range of soybean cultivars and related species (*6*). This indicates that morphogenetic competence can be achieved in almost any soybean cell line. Progress to date on this cultivar of soybean suggests that a morphogenetic cell suspension can be established from our culture by a combination of further selection and further modification of the culture medium (*7*).

We hope that this procedure will prove useful in the establishment of morphogenetic suspension cultures of other large-seeded legumes. We know that embryos taken from young pea pods will make a hard glossy callus in response to the first step of the procedure; this callus appears very similar to the material we successfully manipulated in soybean.

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Nocturnal Aerial Predation of Fireflies by Light-Seeking Fireflies

Abstract. *Female* Photuris *fireflies guided by their prey's luminescence attack flying fireflies at night. They sometimes use this hunting tactic together with prey attraction by mating-signal mimicry. Such predation could have been a major factor in the evolution of signaling behavior of American fireflies. Nocturnal aerial predation by an insect and attack guidance on energy emitted by airborne prey have not previously been reported.*

Male fireflies seek mates by emitting luminescent signals as they fly about after dark (*1*). Although predation on searching males by bats and nocturnal birds has been reported (*2*), there has been no indication that males might be subject to heavy predation. Through three experiments with "airborne," light-emitting decoys that simulated male fireflies, we have found that female fireflies of several *Photuris* species are light-seeking, aerial predators (that is, "sidewinder" hawkers). These predators are the only known nocturnal, aerial hunters among the insects, and the only hunters to use the energy emissions of airborne prey for attack guidance (*3*).

Fig. 1. Apparatus for flying sticky light-emitting diodes.

We flew light-emitting diodes (LED's) (*4*) covered with insect-sticking paste (*5*) from the tips of three 2.3-m fish poles that were slowly rotated (that is, swung from a hub; 120° apart) by a battery-powered motor (*6*) (Fig. 1). The decoys "flew" 1.3 ± 0.3 m above the ground and moved 0.24 or 0.48 m/sec. One LED glowed, one flashed (about 0.2-second duration) at 1-second intervals and one was unlit. A total of 33 *Photuris* females belonging to four species struck the glowing decoy, but the flashing and unlit decoys were not attacked (24 nights; nine sites; total running time, 34.7 hours). During two evenings (for 148 minutes) at one site, 21 attacks were made by females of one species, and in one instance three struck the same glowing LED within 2 seconds.

In the second experiment we trolled along a wooded roadside with a flashing, sticky decoy hanging on fine black wire from the tip of a 2-m fish pole (*7*). The decoy was held against the skylight to observe attacks. When an attacker was first seen, usually about 20 cm from the decoy, we made the decoy "hover" and do one of three things: stop flashing, continue flashing as before, or continue flashing and begin glowing dimly (*8*) in a manner simulating a firefly with a malfunctioning light organ, as sometimes seen in nature. (i) When the decoy stopped flashing incipient attackers flew away ($N = 11$). (ii) When the decoy continued flashing, incipient attackers attacked ($N = 10$) (Fig. 2), landed (flew into, then perched?) on the wire or pole up to 0.3 m from the decoy ($N = 13$), or flew away ($N = 7$). The ten that attacked took an average of 14.0 seconds to strike (*9*). (iii) When the decoy flashed and glowed incipient attackers struck the de-