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Organogenesis of *Euphorbia esula* L. from hypocotyls

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Abstract:

Studies of organogenesis from leafy spurge were undertaken to identify tissues or organs that would consistently produce roots and shoots for use in bioassays for herbicides and to determine those factors that influence the regenerative potential.

Regeneration of leafy spurge occurred from all parts of germinated seedlings without the use of exogenous growth regulators on B5 medium (Gamborg *et al*., 1968) containing 2% sucrose. Isolated whole roots, hypocotyls, and the shoots of germinated seedlings (13 to 19 days old) produced both roots and shoots, with the intact hypocotyl producing five times as many shoots as roots. Segments of hypocotyls also formed shoots more readily than roots, but the reverse was true for the isolated root segments. Short hypocotyl segments (< 7 mm) produced relatively few organs, whereas segments up to 15 mm long formed both organs readily. Treatment with IAA increased root formation greatly, with a threshold value of about 0.04 mg/L (0.23 μM). Shoot formation was inhibited at 0.2 mg/L (1.1 μM) IAA. Low concentrations of cytokinins had little effect on shoot formation, but inhibited root formation at the higher concentrations used (up to 0.9 μM)

Root formation on hypocotyl segments in the presence of exogenous auxin was extensive in B5 medium with the salts and vitamins diluted to 0.1X normal concentration (constant 2% sucrose). At 0.01X B5, roots appeared on nearly 20% of the hypocotyls and less than 10% formed shoots. Omission of phosphate from the B5 medium did not affect root or shoot formation; presumably because of inadvertent phosphate (0.29 mM) already present in the agar used to solidify the medium, as well as reserves in the isolated hypocotyl segments (0.29 ug/mg fresh weight).

Introduction

The control of leafy spurge is likely to be a result of a combination of biocontrol methods and the application of specific herbicides that interfere with plant development. Such growth regulators must either translocate long distances from the point of application to other parts of the plant that are capable of regeneration, or induce the plant to synthesize messengers that in turn will initiate the biosynthesis of endogenous inhibitors in other organogenic parts of the plant. Therefore, it is important to learn the potential regenerative capacity of the plant and to understand the factors that promote or inhibit organ formation.

In previous work in this laboratory using leafy spurge cell suspensions (Davis *et al*., 1988), it was discovered that regeneration of plants from cultures obtained from one specific accession was possible by removing all exogenous growth regulators from the culture medium. Cultures initiated from other accessions were not capable of organogenesis, and even the regenerable accession was unpredictable in its response to various manipulations in vitro. A more consistent system for regenerations studies has been established by using isolated hypocotyl segments.

Materials and methods

Field-collected seeds were used because leafy spurge will not produce seeds in a greenhouse or growth chamber. Seeds were sterilized 2 minutes in 70% (v/v) ethanol followed by 20 minutes in 60% (v/v) bleach (5.25% Ca hypochlorite). Isolated plant parts from dark grown sterile seedlings (13 to 19 days old) were placed onto B5 medium with 2% (w/v) sucrose and 0.7% (w/v) agar in 6 cm plastic petri dishes wrapped with parafilm. Experiments were conducted in darkness (dishes wrapped with aluminum foil) at 26ºC. Replicates contained eight hypocotyl segments per dish with five dishes per treatment in each experiment. Visible roots and shoots were recorded with time up to 60 days (earlier experiments) or 28 to 30 days (later experiments). Data were expressed as percentages of the hypocotyl (or root) segments that produced at least one root or shoot visible under a dissecting microscope. Phosphorous was determined spectrophotometrically at 820 nm on ashed samples treated with acidified ascorbic acid-ammonium molybdate reagent (Chen *et al*., 1956).

Results

All parts of germinating seedlings produced both roots and shoots without exogenous growth regulators when they were tested as unsegmented parts (i.e., both roots and shoots contained apical meristems). Unsegmented hypocotyls produced numerous shoots (average of 12.1) but only a few roots (average of 2.2). The other two parts of the seedlings (unsegmented roots and cotyledons plus apex) formed about two of each organ per plant part.

One cm segments of roots and hypocotyls (neither of which contained apical meristems) were organogenic without the need for exogenous growth regulators. Both roots and shoots were produced to varying degrees. Hypocotyl segments produced shoots more readily than roots segments whereas; the reverse was true for isolated root segments. Typically, about 60 to 90% (or sometimes greater) of the hypocotyl segments formed shoots and 20 to 40 percent formed roots. Root segments typically formed visible adventitious roots in about 30 to 40% of the segments while 20 to 30% formed shoots.

Hypocotyl segments shorter than about 7 mm produced few roots or shoots and the orientation of the hypocotyl had little influence. One cm segments from the basal, middle or cotyledonary end of the hypocotyls behaved similarly in that approximately the same percentage of all three segments produced both roots and shoots at about the same rate.

Formation of visible roots was stimulated by the addition of IAA to the culture medium, with a threshold concentration of about 0.04 mg/L (0.23 μM) (i.e., 0.04 to 0.2 mg/L IAA induced nearly equal numbers of hypocotyl segments to form roots). The threshold concentration had no effect on shoots, but IAA at 0.2 mg/L (1.1 μM) inhibited shoot formation.

Visible root formation was inhibited by cytokinins at concentrations of 0.1 mg/L or greater. Shoot formation appeared to be slightly stimulated, but the results were significant ($p < 0.05$) only with the highest concentration of zeatin riboside (0.2 mg/L, 0.56 μM).

Root formation on hypocotyl segments was similar in B5 medium containing 0.1 times the normal salt and vitamin concentration as full strength B5 medium. Further dilutions of the salts resulted in formation of fewer roots, but did not totally suppress them. Shoot formation was reduced by a greater amount, but even at 0.01X salt concentration a few shoots were formed. In other experiments organogenesis occurred in the absence of B5 salts and vitamins with 2% sucrose as the only nutrient. In one experiment 48% of hypocotyls produced roots and 3% formed shoots, in the dark.

Reduction of phosphate in the medium had only minor effects on organogenesis. Elimination of P04 from the medium had no effect on root formation, which remained close to 100% and 30% in the presence and absence of IAA, respectively. Shoot formation was lowest when phosphate was eliminated in the presence of IAA, but in the absence of IAA no effect was observed on shoot formation. However, analysis of the agar used to solidify the medium revealed the presence of approximately 0.29 mM phosphorous. This value is 26% of that contained in full strength B5 medium. The hypocotyl segments also contained an average of 0.2 ug of phosphorous per mg fresh weight. There appears to be ample phosphorous for good root and shoot development when all other factors are optimum.

Discussion

Leafy spurge is an extremely versatile perennial weed capable of regeneration at a fairly high level from a variety of tissues and organs. Apical dominance was evident when unsegmented hypocotyls were isolated and found to develop about five times as

many adventitious shoots as roots. Hypocotyl segments one cm or greater in length formed both shoots and roots when grown on agar medium containing dilute salts and vitamins or sucrose alone. Hypocotyl segments less than 7 mm in length may have had insufficient reserves of nutrients or growth regulators to develop roots or shoots to any great extent. Shoot formation was more sensitive to the reduced nutrient concentrations than were the roots, presumably because shoots are more complex than roots. Roots formed readily on hypocotyl segments cultured at 0.1X the normal B5 salts and vitamins, provided 2% sucrose was available; whereas shoots required higher salt concentration for full expression. The phosphate contained in hypocotyl tissues and added inadvertently with the agar to solidify the medium was sufficient for both root and shoot formation.

The response of leafy spurge hypocotyls to auxin treatment appears to follow the traditional classical pattern in that auxin stimulates root formation and inhibits shoot initiation. Likewise, the response of the hypocotyl segments to exogenous cytokinins was somewhat predictable. Because shoot formation was already quite high, only slight stimulation occurred at higher concentrations of cytokinins. Visible root formation was inhibited by cytokinins, possibly due to reduced growth as much as to reduced root initiation, since the root is, initiated internally and the data reported here is for visible roots.

The formation of organs by leafy spurge tissue may be regulated by the ratio of the auxins to cytokinins as indicated by Skoog and Miller (1957) working with tobacco callus. Since both of these growth regulators occur naturally in higher plants the endogenous levels of both of these classes of compounds must first be established. In experiments with externally applied growth regulators the different solubilities as well as different steric configurations of the cytokinins and their respective relative activities within the tissues must be considered. If the relative rates and interactions of the processes influenced by each of the growth regulators are the sole controlling mechanisms, it will be difficult to design specific herbicides for leafy spurge, or possibly any other, perennial weed because of the lack of sufficient specificity.

This report covers only some of the very basic physiology of leafy spurge hypocotyls. Other studies are being conducted in our laboratory to establish unique features of leafy spurge that can be utilized to ultimately control the weed. Whether that program is successful or not, the hypocotyl system has potential for use as a screening system for herbicides that interfere with organogenesis.

Literature cited

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Technical abstract

Studies of leafy spurge organogenesis were done to establish a bioassay for herbicides and determine factors that alter regeneration. Regeneration occurred from all parts of germinated seedlings on B5 medium without exogenous growth regulators. Intact hypocotyls produced five times many shoots as roots. Isolated hypocotyl segments formed shoots more readily than roots, but the reverse was true for the isolated root segments. Hypocotyl segments < 7 mm long produced few organs, but segments 8-15 mm long formed both organs readily**.** IAA increased root formation greatly, with a threshold value of about 0.04 mg/L (0.23 μM). Shoot formation was inhibited at 0.2 mg/L (1.1 μM) IAA. Cytokinins (up 0.2 mg/L) had little effect on shoot formation, but inhibited root formation. Hypocotyl segments formed roots readily (in the presence of IAA) when the salts and vitamins were diluted to 0.1X normal concentration (constant 2% sucrose). At 0.01X B5, roots appeared on nearly 20% of the hypocotyls but less than 10% formed shoots. Omission of phosphate from the medium did not affect organ formation; presumably due to mobilization of sufficient phosphate reserves from the isolated hypocotyl segments and inadvertent phosphate contained in the agar used to solidify the medium.