Organogenesis of leafy spurge from hypocotyl segments

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Leafy spurge cell suspension cultures have been regenerated into plants (Davis et al. 1988). Successful regeneration depended on the accession used to originate the cultures. Numerous attempts were made to routinely and consistently manipulate the cultures to determine critical pathways in organogenesis that might be regulated by application of specific chemicals at certain stages in the life cycle of the plants. The results from cell cultures were often variable and inconsistent. Therefore, alternate systems were tested for these studies. In preliminary experiments, hypocotyl segments were more useful than cell suspension cultures for regenerating leafy spurge plants.

The conclusions outlined in this abstract may be tenuous, partly because of inconsistent experimental results between duplicate experiments and partly because of the long time periods required for data analysis: typical experiments lasted 60 days.

Roots and shoots formed on the hypocotyls were observed microscopically in unopened Petri dishes four times before harvest, usually at 60 days. In some experiments hypocotyls were pulse treated with chemicals and transferred at various time periods to fresh control media.

Root segments of germinated seedlings were compared to hypocotyl segments for their ability to regenerate leafy spurge plants. The root segments were capable of organ formation, but they were difficult to work with due to their small size and the limited amount of material compared to hypocotyl segments. In general, root segments produced greater numbers of new roots than hypocotyl segments, whereas hypocotyls formed more shoots than root segments.

The basic salt and vitamin formulations of B5 (Gamborg et al. 1968) and MS (Murashige and Skoog 1962) media were the standard media chosen for the early experiments. A modified B5 medium was also tested and contained a reduced/oxidized nitrogen ratio that was adjusted to the same ratio as for MS medium; all other components remained the same as the original B5 formulation. The B5 formulation was chosen for later experiments because the other media tested had no advantage compared to it.

Hypocotyl segments of dark-grown seedlings formed roots and shoots on media lacking exogenous growth regulators. Segments less than 0.5 mm long produced few organs, while those 1.5 cm long produced an average of 2 shoots/segment and 0.66
roots/segment. One cm long segments were used for the experiments described in this report. Shoots formed on about 40% of the hypocotyl segments within 10 days, while 10 to 20 days were required before roots were visible. The numbers of hypocotyls forming roots were consistently less than those forming shoots.

The results to date indicate that:

1. The auxins indoleacetic acid and indolebutyric acid stimulated root formation when added exogenously in concentrations of 0.17 mg/L for 2 to 5 days followed by transfer to the same medium without auxin. Naphthalene acetic acid gave variable results, whereas 2,4-dichlorophenoxyacetic acid inhibited roots at higher concentrations. Shoot formation was inhibited by auxins at higher concentrations (>0.1 mg/L) but in some experiments were slightly stimulated at lower concentrations (<0.1 mg/L).

2. Cytokinins at concentrations of 0.6 to 6 mg/L stimulated shoot formation by 40 to 200%, but effects on root formation varied.

3. Light had little or no effect on shoot formation, but it inhibited root formation (even at low fluences of 0.4 to 9.0 2x11xE/m2/s). Light quality had no consistent effect on the formation of either organ.

4. There is no evidence that ethylene plays a role in organ formation in leafy spurge hypocotyls in the described system. Hypocotyls treated with the ethylene inhibitors AgNO3 and aminovinylglycine or with 1-aminocyclopropane-1-carboxylic acid (a precursor of ethylene in plants) did not differ from the controls.

5. Gibberellic acid (0.1 to 2.0 mg/L) inhibited shoot formation by 50% in one experiment. Because few roots were present on control or treated plantlets, gibberellic acid effects on rooting could not be detected.

6. Abscisic acid gave inconsistent results, although fewer shoots were formed at 1 to 3 mg/L if the hypocotyls were exposed to ABA continuously. Lower concentrations of abscisic acid had no effect.

The search is continuing to discover the key media components and environmental factors that influence organogenesis. The concentration of endogenous growth regulators will be quantified to determine more precisely the relationships of those compounds on root and shoot formation in leafy spurge.

References

