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Physiological and microscopic characteristics of a regenerating leafy spurge culture

DAVID G. DAVIS, ROSA L. STOLZENBERG, and PRUDENCE A. OLSON

USDA-ARS, Metabolism and Radiation Research Laboratory, State University Station, Fargo, ND 58105.

Aseptic tissue culture systems were used to determine environmental and physiological conditions for root and shoot formation of leafy spurge cells. Comparisons were made among three accessions: 1978A1 from Austria (presumably *Euphorbia esula*), 1979ND1 from North Dakota (possibly *E.* × *Psuedovirgata*) and 1978OR1 from Oregon (taxonomic classification unknown). The three accessions differ phenotypically: most conspicuous is the fact that 19780R1 has larger leaves than the other two accessions. Also, the Oregon accession has a slightly different latex composition than the other two. Shoots of accession 1978A1 grew from crown buds and produced flowers within two weeks in a greenhouse in North Dakota in May 1986; accession 1979ND1 required more than three weeks, and accession 1978OR1 four weeks to produce flowers.

All three accessions produced callus from stem sections on either B5 or MS media with either 2,4-D alone or with 2,4-D, NAA and kinetin at 0.4, 0.4 and 0.2 mg/L, respectively. Accession 19780R1 produced a more compact callus than the other two accessions. Organogenesis occurred in all accessions maintained (after the-initial callus formation) for three subcultures on B5 + 1 mg/L 2,4-D (B5 + 1) agar medium, followed by two subcultures in liquid MS with no growth regulators. Accession 1978A1 produced roots and a few shoots. Accession 1978OR1 produced extremely variable growth in suspension, but regenerated one 25 mm root and three shoots in one flask. Accession 1979ND1 produced numerous abnormal roots and root initials from callus induced with 2,4-D as the only growth regulator, and several more normal-looking roots and shoots from callus induced with all three growth regulators. This latter accession has been regenerated from suspension cultures maintained on B5 + 1 medium in our laboratory for the past three years, and is the accession used almost exclusively for the studies presented below. Therefore, all three accessions retained the capacity for organogenesis in culture, although to very different degrees, and the medium used to initiate the callus appears to be important.

Cell suspensions of accession 1979ND1 produced from freshly isolated callus cultures formed more roots than did suspensions that have been maintained for several months or years. Both large clumps and suspensions of small clumps produced roots: from several hundred to several thousand roots per flask. In most flasks, greater than 90% of the clumps in each flask formed roots. Cells grown in B5 + 1 medium up to 14 (and in

one experiment 20) days before transfer to 2,4-D-free media produced roots; therefore, the time from inoculation into fresh B5 + 1 medium before transfer into root inducing media did not appear to be critical.

Microscopic observations of the culture are being made to detect unusual cell types that may serve as targets for ultimate control of growth of the plant. Tracheary elements have been observed in most cultures. Non-regenerating cultures stained with toluidine blue in borax contained cells with a granular appearance. These cells may be identical to those located conspicuously near the base of roots in regenerating cultures. We are attempting to determine the relationship of these cells to other cells containing electron dense material (observed with an electron microscope) and cells stained with $FeS0_4$ (observed with a light microscope) that may be tannin-containing cells. Other cells were observed to form spherical droplets that eventually coalesced into non-spherical structures after staining with toluidine blue. Some granular cells, stained with toluidine blue, were located in similar regions of cell clumps and organs as were cells of unstained material that contained anthocyanins. However, these two cell types were not the same cells as indicated in our preliminary studies. Laticifers were observed within organized tissues near the base of the root.