Reprinted with permission from: Leafy Spurge Symposium. Riverton, WY. July 9-10, 1986. p. 27.

Published by: Great Plains Agricultural Council: Leafy Spurge Symposium.

Relationship of nitrogen source and molybdenum content of media to regeneration of leafy spurge cell cultures

K. J. EVENSON, D. S. GALITZ and D. G. DAVIS

Botany Department, North Dakota State University and USDA, ARS, Metabolism and Radiation Research Laboratory, State University Station, Fargo, NO 58105

Cell suspension cultures of leafy spurge (*Euphorbia esula* L.) (1979ND1) were used to determine if specific biochemical processes could be correlated with root regeneration when cultures were subjected to various nitrogen regimes. Nitrate reductase (NR) levels in whole plants fluctuate in response to environmental conditions such as pH, plant age, light and temperature, and with the presence of various nutrient elements including nitrate, ammonium, molybdenum, potassium, sulfur and sucrose. If developmental changes in activities of nitrogen assimilating enzymes, such as NR can be monitored and correlated with leafy spurge regeneration, a better understanding of the mechanism of root formation in leafy spurge might lead to specific biological or chemical control measures.

In preliminary experiments, root formation in leafy spurge cultures was low or absent in high NO_3^- B5 media (27 mM $N0_3^-$, 2 mM NH_4^+) containing 3% sucrose. At the same time, greater root numbers and longer roots were found in high NH_4^+ B5 media (23 mM NH_4^+, 4 mM NO_3^-) containing 3% sucrose. Consequently, NR activity may also differ between rootforming and non-rootforming cultures. Because molybdenum (Mo) induces the formation of the protein moiety of NR, removal of Mo from the media should decrease root formation if NR is critical to root regeneration.

Low Mo status cells were obtained by subculturing cells in B5 medium containing 1 mg/L 2,4-D without Mo for three passages. Low Mo cells and control cells (grown with 1 µM Mo) were then transferred to either high NH_4^+ or high NO_3^- B5 medium but without 2,4-D. Root regeneration occurred almost exclusively in the control cells grown with high NH_4^+. *In vivo* NR activity and soluble protein concentration differed significantly between cells grown in high NH_4^+ or high NO_3^- media during the first two weeks of growth. In all cultures of low Mo status, NR activity was reduced. Fresh weights were greater for cultures grown in high NO_3^- media compared to high NH_4^+ media during the first two weeks of growth. NO_3^- was utilized completely in control cells grown in high NO_3^-, but NO_3^- remained in both media when cultures were Mo-deficient.

Root numbers induced in the above experiments were low (less than 30 roots per flask) possibly because the cultures had been maintained longer than one year (cells had been subcultured 10 times as callus and 40 times as suspensions in B5+1 mg/L 2,4-D before the first experiment). When recently isolated cultures (subcultured once as callus and nine times as cell suspensions in B5 + 1) were grown in the same high NO_3^- or high NH_4^+ media, the results were opposite those seen in the older cultures, i.e. higher root numbers (~3000/flask) were found in the high NO_3^- media compared to the high NH_4^+ media (~1000/flask). Maximum NR activity was higher in the older cultures (rootforming and non-rootforming) however, the same patterns of NR activity were seen in highly regenerative younger cultures.