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ORGANOGENESIS AND SHOOT-TIP MULTIPLICATION FROM TISSUE CULTURES OF ATRIPLEX SPECIES

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ABSTRACT: Roots and shoots were successfully grown from callus cultures of Atriplex canescens and A. griffithsii in some experiments. Attempts to replicate organogenesis in callus or liquid cultures from these species and in A. acanthocarpa, A. confertifolia, and A. polycarpa were unsuccessful, indicating that specific genetic or cultural requirements were necessary for induction. Shoot-tips were multiplied rapidly after two months in culture from diploid (2x), tetraploid (4x), and hexaploid (6x), plants and from three different collections of 4x populations. Shoot-tip multiplication is a promising method of mass propagation of specific genotypes of Atriplex species.

INTRODUCTION

Atriplex species native to the more arid regions of western North America are a valuable component of rangeland for forage, cover, and revegetation of disturbed sites. They may potentially replace undesirable species or be used in marginal agricultural areas to provide forage with minimal or no irrigation. These native plants possess high genetic variability (McArthur and others 1983) and a remarkable ability to survive under extreme conditions. Therefore, they may be used as model systems to study stress tolerance and other survival mechanisms. If survival mechanisms can be identified and the corresponding genes isolated and transferred--using recombinant gene transfer technology--to other economically important plant species, then these other native plants would potentially be a resource of unique genes for stress tolerance and other useful traits.

Plant regeneration and in vitro propagation methods have been developed for a number of agriculturally important plants including agronomic plants, ornamentals, and trees (Conger 1980). By modifying methods, increasing numbers of plants are successfully being cultured. Norma Trolinda (personal communication) successfully regenerated plants from a collection of A. canescens, native to the Lubbock, Texas area, but was unsuccessful in regenerating plants from other A. canescens collections. Wochok and Sluis (1980) reported a method of shoot tip multiplication in A. canescens, with some spontaneous rooting. Apparently they were not successful in transferring rooted shoots to soil.

In the current research program, plant tissue culture methods were applied to Atriplex species to develop new approaches to shrub research. Cell cultures are used to study cellular responses to environmental stress and to develop unique selection methods. Plant regeneration capability would allow the recovery of valuable genes identified from in vitro experiments and allow their expression in whole plants. Shoot multiplication is a method of mass propagating specific genotypes for genetic studies, plant improvement, and other research approaches.

MATERIALS AND METHODS

In preliminary studies, embryos from two species, A. canescens and A. griffithsii, were removed from fruits and sterilized 7 min in 95% ETOH, then 20 min in 40% commercial bleach (2% sodium hypochlorite), and rinsed 30 min in sterile distilled water. They were germinated on 1/10 concentration BDS medium, which was modified from the B5 medium of Gamborg and others (1968), by Dunstan and Short (1977) with no growth regulators (table 1). Hypocotyls 1/2 to 1 cm long from A. griffithsii were plated on L2 medium of Phillips and Collins (1978) and labeled L2-C-init medium (table 2) to initiate callus. Callus was subcultured at approximately 1 cm in diameter, on the same initiation medium, and then subcultured monthly on L2-C-prlf to increase the quantity of the tissue. After 2 subcultures, the callus was transferred to L2-C-reg medium to induce regeneration. A similar sequence was used to culture A. canescens on the BDS series of media.

Callus cultures from five species, A. acanthocarpa, A. canescens, A. confertifolia, A. griffithsii, and A. polycarpa, were generated in the above manner and cultured on the L2 media series to determine their regeneration capacity. Liquid cultures were developed by culturing callus pieces in liquid L2-C-prlf medium on a rotary shaker under 16 h light, rotating at approximately 60 rpm.

Shoot-tip cultures were initiated from three cytotypes, diploid (2x), tetraploid (4x), and hexaploid (6x), plants described by Dunford (1984); and from 4x plants from three locations, one in west Texas, a high elevation site in New Mexico, and an arid site in southeastern Arizona.

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Table 1--Nutrient concentrations of culture media

	Final concentration (mM)	
	BDS	L2
Major salts		
$CaCl_2$	0.00	4.10
KNO_3	23.20	20.77
$KH_2PO_4.H_2O$	0.00	2.39
$NH_4H_2PO_4$	2.00	0.00
$Na_4H_2PO_4.H_2O$	1.25	0.62
$(NH_4)SO_4$	1.01	0.00
$MgSO_4.7H_2O$	1.00	1.76
NH_4NO_3	4.00	12.50
$FeSO_4.7H_2O.EDTA$	0.09	0.09
	Final concentration (µM)	
	BDS	L2
Minor salts		
H_3BO_3	48.00	80.85
KI	2.20	6.02
$MnSO_4.H_2O$	29.60	88.75
$ZnSO_4,7H_2O$	3.50	17.40
$CuSO_4.5H_2O$	0.08	0.40
$Na_2MoO_4.2H_2O$	0.50	1.66
$CoCl_2.6H_2O$	0.05	0.42
	Final concentration (mM)	
	BDS	L2
Vitamins		
Inositol	0.55	1.39
Thiamine.HCL	0.03	0.006
Pyridoxine.HCL	0.005	0.024
Nicotinic Acid	0.008	0.000
Other Additives		
Sucrose	30.0 g per liter	
Agar	6.0 g per liter	
pH adjusted to 5.6		

For multiplication, growing points approximately 1 to 2 mm were excised from the growing tip of germinating seedlings and cultured base down in BDS-G-Sm (table 2). Lateral shoots from mature plants were also collected, sterilized 20 minutes in 40% commercial bleach and rinsed in sterile distilled water. Then, 3 to 5 mm shoot-tips were excised and plated on BDS-G-Sm medium in 100 mm Petri dishes.

To induce rooting, individual shoots approximately 1 cm long, were excised from the shoot multiplication cultures and placed base down on the BDS-G-Rm medium and cultured in the growth chamber. All tissue cultures were incubated at 25 °C in a growth chamber with a 12 h light period.

RESULTS AND DISCUSSION

A total of 22 shoots developed from callus of A. canescens and 32 shoots from callus of A. griffithsii in the initial experiment, as a response to decreasing picloram from 0.1 to 0.025 mg per litter and increasing 6, bensyladenine (6BA) to as high as 4.0 mg per liter in the regeneration medium. Because of the difficulty of maintaining A. canescens callus on BDS medium, all species were grown in L2 medium in subsequent experiments. Callus formed readily from both species and grew well in subcultures. Cell suspension cultures were readily initiated in liquid media from the callus cultures. Subsequent attempts to produce shoots on previously successful or modified media were not successful, indicating a genetic requirement or a specific nutritional requirement for organogenesis to occur.

Table 2--Growth regulator concentration of media

Media	Pic.(mg/l)	6,BA(mg/l)	GA	IAA
BDS-C-init	0.75	1.5	0	0
BDS-C-prlf	0.25	1.5	0	0
BDS-C-reg	0.00	0-4.0	0	0
BDS-G-Sm	0.00	0.5	4.0	0
BDS-G-Rm	0.00	0.0	0.1	0.5
L2-C-init	0.20	0.1	0	0
L2-C-prlf	0.10	1.5	0	0
L2-C-reg	0.025	1.0-2.0	0	0

Shoot multiplication experiments were generally successful independent of the cytotype or the collection used. This technique promises to be a useful method of replicating plants of a specific genotype. Tetraploid plants were the most responsive to shoot-tip multiplication, with several collections having shoots increasing 4 times every 10 days. This rate allows replicating large numbers of plants from a given selection quickly. Shoot cultures, at times, developed roots which were not specifically attached to shoots and they were not successfully transferred to soil. Specific rooting was initiated by excising single shoots from multiplication cultures and transferring them to a 1/2 strength rooting medium, BDS-G-Rm (table 2). After two weeks, roots began to develop at the base of the shoots. Rooted shoots were transplanted into peat pellets and covered with a beaker where they grew and were subsequently transplanted into soil where they were grown to maturity.

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