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Effects of Chemical Growth Retardants on Growth and Development of Sweetpotato (*Ipomoea batatas* (L.) Lam.) in Vitro

R. L. Jarret*

Research Horticulturist, USDA/ARS, Griffin, GA 30223 USA

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Abstract. Plant growth retardants were evaluated for their ability to reduce the growth rate of sweetpotato (*Ipomoea batatas* (L.) Lam.) in vitro. Nodal sections of cv. Jewel were cultured for 30 days on medium containing NDA, ancymidol, phosfon, TIBA, difenzoquat, chlormequat, ACC, mepiquat chloride, or daminozide at 0, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, or 10^{-8} M. Difenzoquat, NDA, phosfon, and TIBA, at 10^{-4} M, were lethal to axillary bud explants. A low concentration (10^{-8} M) of chlorflurenol or NDA stimulated shoot elongation. The effective concentration range for most growth retardants was 10^{-5} to 10^{-6} M. Small (2- to 4-mm diameter) storage root-like swellings were observed on roots in cultures containing TIBA or ancymidol. The growth-inhibiting effects of ancymidol and NDA were transitory and did not persist through a 180-day culture period. Shoots cultured on medium containing 10^{-5} M phosfon, TIBA, or difenzoquat were significantly shorter than control plants after a 180-day culture period. Culture on medium containing TIBA, NDA, ancymidol, or ACC resulted in abnormal leaf and stem development. Plants derived from nodal explants cultured on medium containing either phosfon or chlormequat were near normal in appearance but with some plants exhibiting interveinal chlorosis and reduced root system development.

Key Words. Sweetpotato—Germplasm maintenance—Growth inhibition—Growth retardants—Tissue culture

In vitro storage of tropical root crop germplasm including potato (*Solanum tuberosum*), sweetpotato (*Ipomoea batatas*), yam (*Dioscorea* spp.), and others (Hanson 1988) provides an alternative means for maintenance and distribution of virus-free propagules of these plant materials (Towill 1988, Withers and Williams 1986). Protocols reducing in vitro growth rates, in support of in vitro plant germplasm maintenance activities, generally use sugar alcohols such as mannitol or sorbitol (Westcott et al. 1977) or abscisic acid (Jarret and Gawel 1991a), and these compounds continue to be used extensively to reduce the frequency of the cyclic reculture of individual accessions.

A wide range of synthetic growth retardants inhibit shoot elongation without causing plant malformation or damage (Cathey 1964). Many growth retardants exert their influence by inhibiting cell division in the subapical zones of the shoot apex and subsequent cell enlargement, resulting in reduced stem elongation. Certain classes of growth retardants such as the triazoles, pyrimidines, quaternary ammonium compounds, and norbornenodiazetine derivatives interfere with the biosynthesis of sterols and gibberellins, thus affecting stem elongation (Sauerbrey et al. 1987). ACC, whose effects are correlated with the release of ethylene, reduces stem and internodal elongation predominantly via an inhibition of cell elongation (Sauerbrey et al. 1987). Growth retardants have numerous agricultural applications (Hicklenton 1990, Nickell 1982, Starman 1990, Zhang et al. 1990).

The objectives of this study were (1) to evaluate the growth-inhibiting effects of various growth retardants on stem elongation and plantlet growth characteristics of sweetpotato in vitro, and (2) to evaluate the efficacy of

Abbreviations: NDA (tetcyclacis), 5-(4-chlorophenyl)-3,4,5,9,10-pentaaza-tetracyclo-5,4,1,0,0-dodeca-3,9-diene; ACC, 1-aminocyclopropane-1-carboxylic acid; DPC (mepiquat chloride), 1,1-dimethyl-piperidiniumchloride; daminozide, butanedioic acid mono(2,2-dimethylhydrazide); ancymidol, α-cyclopropyl-α-(4-methoxyphenyl)-5-pyrimidinemethanol; phosfon, tributyl-2,4-dichlorobenzylphosphonium chloride; TIBA, 2,3,5-triiodobenzoic acid; chlormequat chloride, 2-chloroethyltrimethyl ammonium chloride; difenzoquat, 1,2-dimethyl-3,5-diphenyl-1-pyrazolium methyl sulfate; chlorflurenol, methyl 2-chloro-9-hydroxyfluorene-9-carboxylate.

*Address for correspondence: USDA/ARS, Plant Genetic Resources Unit, 1109 Experiment St., Griffin, GA 30223 USA.

Table 1. Effect of growth retardants at various concentrations on plant height, fresh weight, and number of nodes of cv. Jewel grown in vitro for 30 days ($n = 30$).

Growth retardant (M)	Height (cm)	Shoot fresh weight (g)	No. of nodes
Chlorflurenol			
0	3.74a	0.47a	8.6a
10^{-8}	5.23b	0.59a	9.2a
10^{-7}	1.72c	0.11b	6.4a
10^{-6}	0.00d	0.00c	0.0b
10^{-5}	0.00d	0.00c	0.0b
10^{-4}	0.00d	0.00c	0.0b
Chlormequat chloride			
0	4.60a	0.39a	10.8a
10^{-8}	5.10a	0.45a	11.1a
10^{-7}	4.79a	0.45a	10.5a
10^{-6}	4.59a	0.41a	11.8a
10^{-5}	3.95a	0.41a	11.1a
10^{-4}	3.44a	0.40a	11.1a
Daminozide			
0	5.86a	0.45a	5.86a
10^{-8}	4.95a	0.44a	4.95a
10^{-7}	5.35a	0.47a	5.35a
10^{-6}	5.33a	0.47a	5.33a
10^{-5}	5.11a	0.44a	5.11a
10^{-4}	3.46b	0.52a	3.46b
Difenzoquat			
0	3.29a	0.32a	7.8a
10^{-8}	3.24a	0.35a	8.6a
10^{-7}	2.90a	0.33a	7.9a
10^{-6}	2.00b	0.20b	6.2b
10^{-5}	0.52c	0.04c	4.7c
10^{-4}	0.00c	0.00c	0.0d
Mepiquat chloride			
0	3.13a	0.40a	7.8a
10^{-8}	3.52a	0.38a	9.2a
10^{-7}	2.82a	0.30a	7.4a
10^{-6}	2.43a	0.24a	7.4a
10^{-5}	1.96a	0.27a	6.5a
10^{-4}	1.16b	0.21a	6.2a
NDA			
0	2.26a	0.31a	6.7a
10^{-8}	4.31a	0.43a	8.6a
10^{-7}	3.19a	0.36a	9.8a
10^{-6}	3.45a	0.35a	7.6a
10^{-5}	0.14c	0.18b	1.0b
10^{-4}	0.00c	0.00b	0.0b
Phosfon			
0	4.2a	0.38a	9.6a
10^{-8}	4.6a	0.42a	10.3a
10^{-7}	4.2a	0.39a	9.6a
10^{-6}	3.7a	0.35a	9.3a
10^{-5}	0.4b	0.05b	3.5b
10^{-4}	0.0b	0.00b	0.0b
TIBA			
0	3.8a	0.35a	9.6a
10^{-8}	4.6a	0.41a	9.9a
10^{-7}	3.7a	0.36a	9.9a
10^{-6}	2.2a	0.18b	5.9b
10^{-5}	0.2b	0.02c	1.0c
10^{-4}	0.0b	0.00c	0.0c
ACC			
0	2.9a	0.27a	7.8a
10^{-8}	3.1a	0.34a	7.9a
10^{-7}	2.9a	0.31a	7.7a
10^{-6}	2.7a	0.28a	7.0a
10^{-5}	0.8b	0.05b	5.7ab
10^{-4}	0.7b	0.02b	4.8bc
Ancymidol			
0	3.2a	0.32a	8.0a
10^{-8}	2.9a	0.27a	8.4a
10^{-7}	2.6a	0.32a	8.9a
10^{-6}	2.4a	0.23a	7.0a
10^{-5}	1.1b	0.32a	8.0a
10^{-4}	0.0c	0.00b	0.0b

Note. Numbers in columns followed by the same letter are not significantly different ($p = 0.05$).

chemical growth retardants in extending the culture period of sweetpotato germplasm maintained in vitro.

Materials and Methods

Nodal explants of cv. Jewel were utilized in all experiments. Individual nodes approximately 0.5–1.0 cm in length, containing a single axillary bud, were excised from plantlets selected at random from an in vitro population maintained as described by Jarret and Gawel (1991a).

The basal medium in all experiments consisted of Murashige and Skoog (1962) inorganic salts, vitamins (Gamborg et al. 1968), 100 mg/liter *i*-inositol, 30 g/liter sucrose, adjusted to pH 5.8 and solidified with 7 g/liter Difco Bacto agar. Individual explants (30/treatment) were cultured on 20 mL of medium in 25- × 150-mm culture tubes, capped with kaputs and incubated at 28°C on a 16-h photoperiod under cool-white fluorescent lights (50 μ Em^{-1} s^{-2}). At specified intervals, plantlets were removed from culture, blotted with paper towels, weighed, and measured. Data were analyzed (ANOVA) using Minitab.

Growth Retardants

Chemical growth retardants evaluated included NDA (tetcyclacis), chlormequat, mepiquat chloride (DPC), ACC, daminozide, ancymidol, phosfon, TIBA, difenzoquat, and chlorflurenol. Growth retardants were dissolved in ddH_2O, 50% methanol, or 50% acetone, as appropriate, filter sterilized, and added to the previously sterilized culture medium.

Effective Concentrations

Growth retardants were evaluated for their effects on plantlet morphology and their ability to inhibit growth (shoot height, shoot weight, number of nodes/shoot, and plantlet weight). Growth retardants were tested at 0.0, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, and 10^{-4} M, and data were collected 30 days after culture initiation. Retardants suppressing growth at one or more of the previously tested concentrations were evaluated further

over a narrower range of concentrations (0.0, 10^{-6}, 3×10^{-6}, 6×10^{-6}, and 10^{-5} M) after a 180-day culture period.

Results and Discussion

Most of the compounds evaluated reduced shoot elongation over a 30-day culture period, at one or more of the tested concentrations (Table 1). At higher concentrations (10^{-4} or 10^{-5} M) individual growth retardants varied in their toxicity. For example, a concentration of 10^{-4} M difenzoquat, NDA, phosfon, or TIBA was lethal to axillary buds of sweetpotato. This same concentration (10^{-4} M) of ancymidol and chlorflurenol inhibited further axillary bud development but was not lethal. Chlormequat chloride at 10^{-4} M had no significant effect on axillary bud viability or shoot growth. Daminozide at 10^{-4} M reduced shoot growth and internodal elongation minimally. In contrast, chlorflurenol, mepiquat chloride, NDA, phosfon, TIBA, and ACC stimulated stem elongation at a concentration of 10^{-8} M, although in all instances this stimulatory effect was not statistically significant. The effective concentration range for most growth retardants was 10^{-5} to 10^{-6} M.

Growth retardants differentially affected the growth parameters that were evaluated. For example at 10^{-5} M, TIBA and ancymidol significantly reduced shoot growth primarily as a result of reduced internodal elongation. At this concentration plantlets were reduced in stature, and stems were thicker, giving the plant a rosette-like appearance. Whole plant fresh weight values were not significantly different from control plants (Table 1). In contrast, phosfon and NDA appeared to exert their growth-inhibitory effects principally through an inhibition of cell division. NDA inhibited both shoot growth and ethylene formation in sunflower plants cultured in vitro (Sauerbrey et al. 1987). NDA-induced height reduction of sunflower plants correlated positively with a reduction in the length of the first internode, and stunting was primarily due to an inhibition of cell elongation at low concentrations (Grossman et al. 1982). NDA inhibited cell division at high concentrations (>10^{-6} M) (Grossman et al. 1983).

Plantlets developing from axillary buds of sweetpotato cv. Jewel cultured on media containing 10^{-8} or 10^{-7} M TIBA had fewer but thicker roots than control plantlets. Numerous bead-like microstorage roots (2–4 mm in diameter) developed along the length of these roots during the 30-day culture period. Several microstorage roots gave rise to shoots while immersed in the culture medium. The accumulation of nonstructural carbohydrate in the root systems as a result of changes in carbohydrate partitioning after treatment with growth retardant was noted by Hicklenton (1990). However, although the stimulatory effects of various growth retardants on tuberization in potato (*S. tuberosum* L.) are well documented (Harvey et al. 1991, Levy et al. 1993), sweetpotato storage root formation in vitro has not been reported previously. The formation of microstorage roots also occurred at a lower frequency when shoots were cultured on medium containing low concentrations of ancymidol or NDA.

Several growth retardants including daminozide, chlormequat chloride, and mepiquat chloride were ineffective in reducing the growth rate of sweetpotato in vitro. Daminozide is generally regarded as an effective growth retardant that inhibits internode expansion at concentrations that are often considerably higher than those of other growth-retarding compounds (Hicklenton 1990, Starman 1990). Therefore, it seems likely that the highest concentration of daminozide (10^{-4} M) utilized in this study was insufficient to induce significant growth retardation. The apparent ineffectiveness of chlormequat chloride in reducing stem elongation in sweetpotato may be associated with the fact that this compound is metabolized readily in plants; its application resulted in only a transient inhibition of in vitro stem elongation in potato (Harvey et al. 1991).

The growth-inhibiting effects of several growth retardants were less evident after a 180-day culture period (Table 2). Of the six compounds tested over this time period at the concentrations indicated, only shoots cultured on medium containing phosfon, TIBA, or difenzoquat chloride at 10^{-5} M were shorter than control plants. Shoots cultured on medium containing phosfon were near normal in appearance, although some plants exhibited interveinal chlorosis and reduced root system development (Fig. 1).

Several growth retardants resulted in the development of morphologically aberrant shoots with poorly developed or no petioles or leaf blades. These effects were most pronounced on plants cultured on medium containing TIBA, but they were also evident on shoots cultured on medium containing NDA, ancymidol, and ACC. Increasing concentrations of these compounds increased the leaf length/width ratio, resulting in arrow-shaped leaves with little or no leaf blade development. Douglas and Paleg (1974) noted that repeated doses of phosfon affected leaf width/length ratios in tobacco. In this study, phosfon had no apparent effect on leaf size or shape.

Increased chlorophyll synthesis was observed in stem and leaf tissue of plantlets cultured on medium containing ACC, ancymidol or TIBA. The stimulatory effects of growth retardants on chlorophyll biosynthesis have been reported previously (Cathey 1964). In contrast, interveinal chlorosis was a common feature of leaves on shoots cultured on media containing either phosfon or difenzoquat. NDA and ancymidol promoted the development of an extensive fibrous root system in contrast to phosfon and TIBA, which stimulated the development of thickened cord-like roots on most shoots.

The growth-retarding effects of most compounds evaluated were transitory. Ancymidol reduced internodal

Table 2. Effect of growth retardants on shoot fresh weight (SFW) (g) and height (cm) of sweetpotato (cv. Jewel) plantlets after 180 days of in vitro culture.

Growth retardant	Concentration (M)									
	0.0		10^{-6}		3×10^{-6}		6×10^{-6}		10^{-5}	
ACC										
SFW	2.06	(0.5)	1.58	(0.47)	1.42	(0.67)	1.25	(0.35)	1.16	(0.81)
Height	12.3	(2.4)	12.7	(1.9)	10.8	(2.5)	11.5	(1.9)	11.3	(3.5)
Ancymidol										
SFW	2.01	(0.5)	1.95	(0.32)	2.35	(0.79)	2.54	(0.22)	2.20	(0.5)
Height	12.3	(2.3)	13.9	(1.5)	13.7	(2.6)	14.1	(1.7)	13.4	(2.6)
Difenzoquat										
SFW	1.94	(0.5)	1.42	(0.27)	1.21	(0.21)	1.17	(0.4)	0.79	(0.27)
Height	12.3	(2.4)	12.3	(1.9)	13.1	(1.5)	11.8	(1.3)	9.3	(3.5)
NDA										
SFW	1.98	(0.43)	1.84	(0.27)	1.61	(0.39)	1.48	(0.59)	1.49	(0.37)
Height	12.7	(2.1)	13.0	(1.4)	12.7	(2.1)	10.9	(3.1)	13.5	(1.5)
Phosfon										
SFW	1.74	(0.84)	1.63	(0.26)	0.64	(0.27)	0.48	(0.21)	0.25	(0.12)
Height	12.3	(2.3)	13.1	(1.9)	9.24	(2.7)	6.15	(2.2)	3.48	(1.1)
TIBA										
SFW	2.00	(0.5)	0.57	(0.76)	0.88	(0.42)	0.48	(0.55)	0.24	(0.21)
Height	12.3	(2.3)	9.95	(5.2)	8.18	(4.0)	6.48	(4.5)	4.26	(2.5)

Note. Results are the mean of 20 cultures ± S.E. (in parentheses).

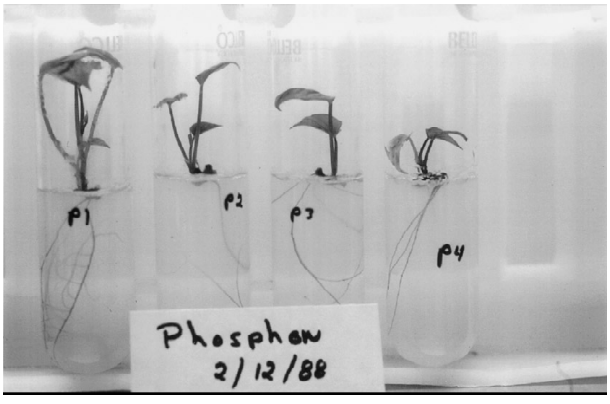

Fig. 1. Effect of phosfon on growth of sweetpotato cv. Jewel cultured in vitro for 30 days. Phosfon concentrations are, from *left* to *right*, 0.0, 10^{-6}, 3×10^{-6} and 6×10^{-6} M.

elongation noticeably during the initial 30-day culture period resulting in rosette-like plants. The effects of ancymidol on plant morphology mimicked those of mannitol, a sugar-alcohol routinely used to reduce the growth of potato and cassava in vitro, and some shoots appeared hyperhydrated. However, by the end of the 180-day culture period, the initial growth-inhibitory effects of ancymidol were not evident, and shoots had elongated to a length equal to those of the control cultures. The growth-inhibiting effects of phosfon, TIBA, and to a lesser extent difenzoquat, were stable over a 180-day culture period.

For purposes of plant germplasm maintenance in vitro, a superior treatment is one that is stable over time, results in uniform growth reduction and with minimal distortion of plant morphology. Based on these criteria, only phosfon and difenzoquat chloride, among those evaluated in this study, appear to be suitable for further evaluation as growth retardants for sweetpotato germplasm maintenance in vitro.

The efficacy of individual growth retardants is likely to vary with genotype (Hicklenton 1990) and confound efforts to define a single protocol/growth retardant concentration that is applicable across the wide array of genotypes in a germplasm collection. It remains to be determined whether more effective growth reduction can be accomplished in vitro by the use of growth-inhibitory chemicals alone, by manipulation of the culture incubation environment and form and concentration of normal medium constituents (Jarret and Gawel 1991b), or a combination of these systems which capitalizes on synergistic effects. The growth rate of sweetpotato cultured in vitro is correlated negatively with the culture incubation temperature (Jarret and Gawel 1991b). Although sweetpotato is relatively cold sensitive, growth retardant-induced cold tolerance (Huang et al. 1981) might permit further reductions in incubation temperature without a loss of culture viability while prolonging the culture period.

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