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Optimizing sweet potato [*Ipomoea batatas* (L.) Lam.] root and plantlet formation by selection of proper embryo developmental stage and size, and gel type for fluidized sowing

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ABSTRACT

Potassium starch polyacrylamide, potassium acrylate, a copolymer of potassium acrylate and acrylamide, and hydroxyethylcellulose carrier gels were tested to find a fluid drilling material suited for synthetic seeding of sweet potato (*Ipomoea batatas* (L.) Lam.) somatic embryos. Somatic embryo developmental stage and size, and maturation (incubation) time were also evaluated to improve plantlet formation. All embryos suspended in the fluidized hydroxyethylcellulose gel were viable after six days and 7% developed into plantlets after two weeks. Up to 97% of the somatic embryos suspended in acrylate and/or acrylamide gels died within six days. Root development was at least 10% and plantlet development at least 30% greater when embryos were subcultured on basal medium for 16 instead of 25 days prior to placement and suspension in hydroxyethylcellulose gel. Up to 25% more plantlets were obtained from embryos at the elongated torpedo stage than those at the cotyledonary or torpedo stages of development. When suspended in hydroxyethylcellulose gel embryo length had no effect on the percentage of plantlets obtained.

ABBREVIATIONS

2,4-D = 2,4-dichlorophenoxyacetic acid; MS = Murashige and Skoog medium (1962); PC = copolymer of potassium acrylate and acrylamide; PSA = potassium starch polyacrylamide; PA = potassium acrylate; HEC = hydroxyethylcellulose; ODR = oxygen diffusion rate

INTRODUCTION

Patterns of somatic embryony in sweet potato were recently determined (Chée and Cantliffe, 1988a). The authors identified three embryo developmental stages which had the potential to form plants 1) torpedo, 2) cotyledonary, and 3) elongated torpedo. Embryos capable of developing into a plantlet usually ranged from 1 to 4 mm in length.

Selection of the proper developmental stage and size of somatic embryo can improve germination and subsequent plantlet formation. Gray et al. (1987) determined that orchardgrass *(Dactylis glomerata)* somatic embryos must have well-developed scutellar and coleoptilar regions for germination to occur. Morphological features of the embryos were suggested by Stuart et al. (1985) to be an identifiable marker in determining which embryos would more readily convert to plantlets. Embryo length has been positively correlated with germination and/or plant formation in zygotic and somatic embryos (Gray et al., 1987; Austin et al., 1969; Soffer and Smith, 1974; Stuart et al., 1985).

Several delivery methods have been proposed for direct-field sowing of somatic embryos: 1) encapsulation of single embryos in an alginate gel capsule (Redenbaugh et al., 1984, 1986); 2) embryo desiccation of orchardgrass and grape (Gray et al., 1987; Gray, 1987); 3) embryo encapsulation in a soft gel capsule and their subsequent desiccation (Jeon et al., 1986); 4) simultaneous desiccation of embryos in a water soluble resin (Kitto and Janick, 1985); 5) automated direct transfer of embryos to the greenhouse (Levin et al., 1988) and 6) fluid drilling (Drew, 1979; Baker, 1985; Schultheis and Cantliffe, 1988).

Desiccation and encapsulation has reduced the percentage of embryos which formed plants, especially after prolonged storage periods (Gray et al., 1987; Kitto and Janick, 1985; Redenbaugh et al., 1987). Fluid drilling is a planting method involving the sowing of pregerminated or dry seeds suspended in a fluidized gel (Bryan et al., 1978; Gray, 1981). The potential advantages of fluid drilling over conventional drilling which uses dry seeds are; 1) the seeds are germinated under optimum conditions prior to sowing which reduces the variable effects of a seedbed environment at planting, and 2) the gel which suspends the seeds can be amended with pesticides, nutrients, and hormones to promote earlier, more uniform seedling emergence. By hastening emergence, fluid drilling minimizes the risk of prolonged exposure to attack by pathogens or unfavorable weather conditions. Improved crop establishment has resulted in earlier and improved yields in several seeded vegetable corps (Gray, 1981). Specifically, fluid drilling of somatic embryos would allow embryos to be planted in either an actively growing or desiccated state (Cantliffe et al., 1987). Drew (1979) was one of the first to suggest fluid drilling as a delivery method for somatic embryos but few researchers have investigated its usefulness for synthetic seeding (Baker, 1985; Schultheis and Cantliffe, 1988).

The goals of this research were to find a carrier gel for somatic embryos of sweet potato which would allow normal plant development and to determine the effect of embryo development and size on root and plantlet development when suspended in a fluidized gel carrier.

MATERIALS AND METHODS

Embryo Production

Embryogenic callus was initiated from sweet potato, cv. White Star, shoot apices and proliferated as described previously (Chée and Cantliffe, 1988b) on 20 ml agar-solidified basal medium containing 10 μM 2,4-D. The basal medium contained the inorganic salts of Murashige and Skoog (MS) (1962), 500 μM myo-inositol, 5 μM thiamine•HCl, 10 μM nicotinic acid, 5 μM pyridoxine•HCl, 3% sucrose, and 0.7% 'Phytagar' (GIBCO Lab., Grand Island, NY). The pH was adjusted to 5.8 using 1.0 N NaOH solution, then autoclaved for 20 min at 121°C and 1.1 kg cm^{-2}. Incubation of cultures was in the dark at 27°C with unmonitored light interruptions during daily observation.

After eight weeks, embryogenic callus was transferred to Petri plates containing basal medium plus 10 μM 2,4-D and 1 μM 6-benzylaminopurine. Six to eight weeks later, embryogenic callus was placed into liquid basal medium and gently separated into its component cell aggregates with slight pressure from a glass slide. The resulting suspension was fractionated using phosphate-bronze sieves with square mesh openings of 710 and 355 μm. The 355 to 710 μm fraction was used to produce embryos on modified solidified MS basal medium containing 10 mM ammonium nitrate, 0.8% Phytagar, and without growth regulators. Incubation was one week in the dark with unmonitored light interruptions, then in light in a 10/14 h light/dark cycle at 27°C until embryos were selected. Cell aggregates larger than 710 μm were recycled for embryogenic callus production.

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Growth Response to Carrier Gel

The gels were prepared by mixing a solution containing MS inorganic salts, 500 μM myo-inositol, 5 μM thiamine•HCl, 3% sucrose, and adjusted to pH 6.5, with a gel material added at 2.0%. The carrier gels compared were: 1) copolymer of potassium acrylate and acrylamide (PC) (Planta-gel, Nepera, Inc. Harriman, N.Y.); 2) potassium starch polyacrylamide (PSA) (Liqua-gel, Miller Chemical Co., Hanover, Pa.); 3) potassium acrylate (PA) (Hydrozorb 30, American Colloid, Arlington Heights, Ill.); and 4) hydroxyethylcellulose (HEC) (N-gel H, Hercules, Wilmington, Del.). The gels were autoclaved for 20 min. Eight ml of each gel were dispensed into each separate quadrant of 100 x 15 mm x-plate plastic Petri dishes (Falcon, Fisher Scientific, Orlando, Fla.). The pH was adjusted to 6.5 for all gels prior to autoclaving. After autoclaving the pH was 0.5 to 1.0 unit lower for the HEC and PA gels, the PSA gel remained at 6.5, and the PC gel increased to 7.0. One embryo was suspended in fluidized gel within each quadrant. Ten plates were used in each of three replications. After three and six days of culture, embryos were rated as healthy when green, unhealthy when some browning was visible, or dead when completely brown or black. After 14 days embryos were observed for root or plantlet development. Hydroxyethylcellulose gel was tested at 1, 2, 2.5, 3, 4, and 5% and observed for root and plantlet development after 14 days. Data were analyzed using analysis of variance and means separated with Duncan's multiple range test.

Influence of Stage of Embryo Development on Growth, Embryo Size, and Embryo Age

Embryos at the torpedo, cotyledonary, and elongated hypocotyl torpedo stage of development were selected (Fig. 1). One of each embryo type was suspended in hydroxethylcellulose gel in a 25 x 100 mm Petri plate (Lab-Tek, Nunc, Inc., Naperville, Ill.) containing 20 ml of carrier gel. The gel was amended with the inorganic nutrients of MS, and the same organic constituents used for callus production.

Fig. 1. Torpedo embryo stage of sweet potato with rudimentary cotyledons (left), cotyledonary embryo stage with small lobed cotyledonary-like appendages (middle), and elongated torpedo stage with elongated hypocotyl (right). Size bar = 1 mm.

The length and diameter of the embryos were measured using a stereomicroscope with a calibrated ocular lens (Table I). Embryos were observed for root and plantlet development at 6, 12, 21, and 31 days after suspension in the gel. Roots had to be at least 2 mm long to be recorded. Embryos with a well-developed shoot apex (apical bud and at least one separated leaf primordia) and a root were counted as plantlets. This consideration was based on the fact that plantlets grew into plants in greenhouse or field conditions. This is the definition common to what a viable germinating seed is, as considered by the Association of Official Seed Analysts (Yaklich, 1984). Two separate experiments were used to evaluate embryos of different ages. In experiment 1 the embryos were 25 days old and in experiment 2 they were 16 days old. A total of 90 embryos were evaluated in each experiment -- 30 torpedo, 30 cotyledonary, and 30 elongated torpedo embryos. There were three replicates and each embryo developmental stage contained 10 embryos. Significance between means were determined using standard error analysis.

Table I. Relationship of embryo developmental stage to embryo size.

Embryo developmental stage	No. obs.	Embryo size (mm) Length	Width
		- - Experiment 1 - -	
Torpedo	29	2.10 ± 0.07[a]	1.03 ± 0.03
Cotyledonary	29	2.57 ± 0.15	1.12 ± 0.05
Expanded torpedo	29	3.21 ± 0.09	1.00 ± 0.04
		- - Experiment 2 - -	
Torpedo	29	1.87 ± 0.06	0.92 ± 0.04
Cotyledonary	29	2.47 ± 0.11	1.11 ± 0.04
Expanded torpedo	25	3.55 ± 0.15	0.93 ± 0.04

[a]Average mean ± standard error.

In a third experiment, somatic embryos at the three stages of development were randomly separated into three categories by length, 1.0 to 1.95, 2.0 to 2.95, and 3.0 to 3.95 mm and cultured in the carrier gel as described above. Root and plantlet formation were determined at 31 days and the data were compared using least significant differences statistical analyses.

RESULTS

Growth Response to Carrier Gel

All embryos placed in the HEC gel were healthy (green) after six days (Table II). However, at least 23% of the embryos grown in the acrylamide or acrylate gels were partially necrotic after three days. Nearly all embryos were dead after six days in PC and PA gels while the PSA gel still had 40% healthy embryos.

Table II. Embryo survival three and six days after placement in carrier gel.

	% response					
	3 days			6 days		
Gel[a]	green	necrotic	dead	green	necrotic	dead
Hydroxyethyl cellulose	100a[b]	0	0	100a	0b	0c
Potassium Copolymer	16c	57a	27b	0c	13b	87a
Potassium starch polyacrylamide	70b	23ab	7b	40b	37a	23b
Potassium acrylate	3c	40a	57a	0c	3b	97a

[a]Murashige and Skoog inorganic salts, 500 μM myo-inositol, 5 μM thiamine•HCl, and 3% wt/vol sucrose were incorporated in gels.

[b]Means separated within columns using Duncan's multiple range test, p = 5%.

Seven percent of the embryos grew into plantlets after being cultured 14 days in HEC gel (Fig. 2). No root, shoot, or plantlet formation was observed from embryos placed in the other gels. Thus, in subsequent experiments, the HEC gel was used. A concentration of 2.5% was used instead of 2.0% because the thicker consistency (2.5%) allowed for the addition of more nutrients and resulted in similar root and plantlet development (data not given).

Influence of Stage of Embryo Development on Growth, Embryo Size and Embryo Age

Elongated torpedo embryos gave rise to the most rapid root development compared with the cotyledonary and torpedo embryo stages (Fig. 3A). After six days, 37% of the elongated embryos had roots compared with 7% for the torpedo and 7% for the cotyledonary embryos. After 31 days 88% of the elongated torpedo stage embryos had roots compared to 58% for the torpedo and 52% for the cotyledonary embryos.

Plantlet formation was similar at 6, 12 and 21 days regardless of embryo developmental stage used (Fig. 3B). However, the percentage of embryos which converted to plantlets at 31 days was 38% for elongated torpedo embryos and only 21% and 17%, respectively, for torpedo and cotyledonary embryos.

The effect of development stage on root and plantlet formation was less pronounced in experiment 2 (Fig. 4) than in experiment 1 (Fig. 3). However, root development again occurred earlier in the elongated torpedo than either the cotyledonary or torpedo embryos

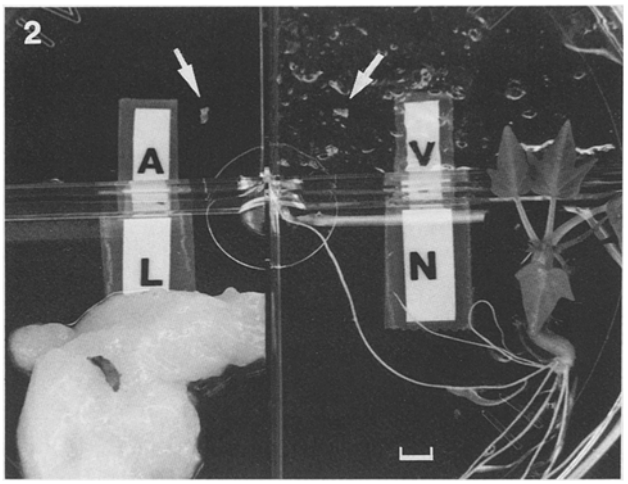

Fig. 2. Lack of embryo growth in the acrylamide and/or acrylate gels (upper left and right quadrants, and lower left quadrant) and plantlet formation from somatic embryos grown in hydroxyethylcellulose gel (lower right quadrant). A = American Colloid Hydrozorb 30 gel, L = Liqua-gel by Miller Chemical Co., V = Viterra (Planta-gel) by Nepera Inc., N = N-gel by Hercules Inc. Size bar = 0.6 mm.

after 12 days (Fig. 4A). The final percentage root formation was greater from embryos at the elongated torpedo development stage than those at the cotyledonary development stage. As in experiment 1, the percentage of embryos which grew into plantlets through day 21 were similar regardless of embryo development stage (Fig. 4B). However, in this experiment more embryos at the elongated or torpedo stage grew into plantlets compared with those in the cotyledonary stage of development (63 and 59 vs. 41%, respectively).

Embryos at the elongated torpedo stage (Fig. 1) consistently improved root and plantlet development. To test the relationship of development to size, embryos at all three developmental stages (1) torpedo, 2) cotyledonary, and 3) elongated torpedo were combined then separated according to length (Table III). Developmental stage did not always correlate with embryo length. The percentage of embryos which formed roots and plantlets were similar regardless of embryo size. Thus, embryo to plantlet formation was more closely associated with developmental stage than to embryo length or size.

Table III. Effect of embryo length on percentage root and plantlet formation after 31 days in culture. Embryos were sorted by size regardless of developmental state.[a]

Embryo length (mm)	No. Embryo	Embryo size (mm) Length	Width	% Response Root	Plantlet
1.00-1.95	63	1.71 ± 0.02[b]	0.92 ± 0.02	67[c]	39[d]
2.00-2.95	76	2.38 ± 0.03	1.04 ± 0.02	74	38
3.00-3.95	41	3.39 ± 0.04	1.00 ± 0.03	79	43

[a]Data pooled from 2 experiments.
[b]Average mean ± standard error.
[c]LSD (P=0.05) for comparing root formation means = 16%.
[d]LSD (P=0.05) for comparing plant formation means = 15%.

DISCUSSION

The superior embryo growth and plant formation obtained in the HEC gel could be attributed to a number of factors. Frazier et al. (1982) determined that seed viability across gel types was related to the oxygen diffusion rate (ODR). They, along with Kubik (1986) reported the HEC gel had a better ODR than Laponite, (magnesium silicate clay) Planta-gel or Permasorb gels. In addition to the high ODR, the HEC gel maintained its viscosity when amended with salts. Ghate (1982) reported that low levels of fertilizer salts reduced the

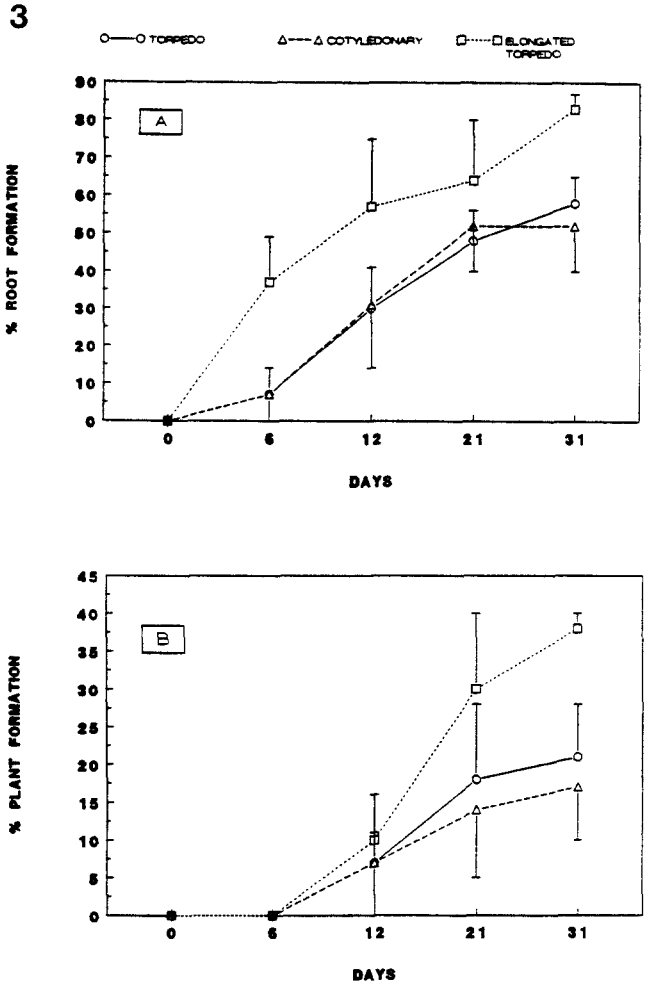

Fig. 3. Growth of sweet potato somatic embryos from three developmental stages after 25 days maturation and placement in hydroxyethylcellulose gel with the inorganic salts of Murashige and Skoog medium, 500 μM myo-inositol, 5 μM thiamine•HCl, and 3% wt/vol sucrose, experiment 1.
A. Percentage root development of embryos at the torpedo, cotyledonary, and elongated torpedo developmental stage at 0, 6, 12, 21, and 31 days.
B. Percentage plantlet development of embryos at the torpedo, cotyledonary, elongated torpedo developmental stage at 0, 6, 12, 21, and 31 days.

viscosity of gels tested in his experiments and thus, their usefulness as a gel carrier was reduced. The HEC gel however, was not included in his study. It has been reported that HEC gels maintain their viscosity better than polyacrylamide gels (Ward, 1980). The maintenance of gel viscosity is critical for synthetic seeds since additives must be incorporated into the gel to replace the missing "growth factors" needed by the naked somatic embryo.

The occurrence of faster root formation from elongated embryos was probably due to their more advanced developmental stage (maturity) or the induction of precocious germination as evidenced by elongated hypocotyls. For all developmental stages, the percentage of embryos that formed plantlets was much less than the percentage that formed roots. This indicated that the shoot meristem was not as well organized or developed as the root meristem. Chée et al. (1990) reported the lack of shoot meristem often coincided with root formation in sweet potato somatic embryo to plantlet development studies. Precocious germination has been reported to commonly occur in somatic embryos resulting in stunted and abnormal embryos in species such as carrot and caraway (Ammirato, 1985). Further development of the apical meristem is necessary during embryo maturation for improved and more consistent plantlet formation since roots were produced more readily than shoots.

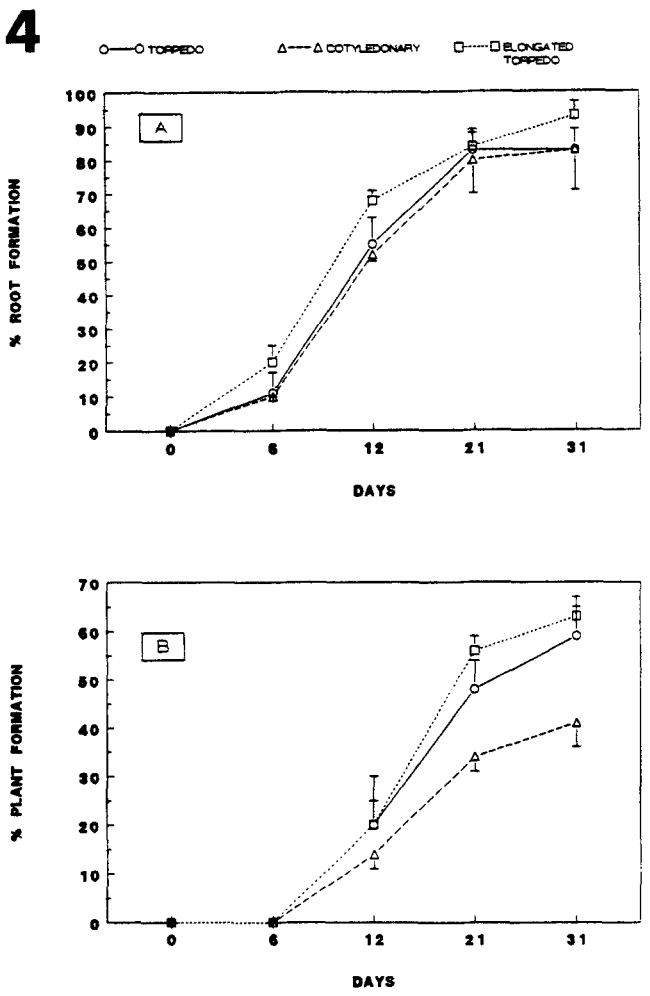

Fig. 4.Growth of sweet potato somatic embryos from three developmental stages after 16 days maturation and placement in hydroxyethylcellulose gel with the inorganic salts of Murashige and Skoog medium, 500 μM myo-inositol, 5 μM thiamine•HCl, and 3% sucrose, experiment 2.

A. Percentage root development of embryos at the torpedo, cotyledonary, and elongated torpedo developmental stage at 0, 6, 12, 21, and 31 days.

B. Percentage plantlet development of embryos at the torpedo, cotyledonary, elongated torpedo developmental stage at 0, 6, 12, 21 and 31 days.

Embryo size has been correlated to improved seed vigor in carrot (Austin et al., 1969) and lettuce (Soffer and Smith, 1974) or somatic embryo quality (ability to form plantlets) in alfalfa (Stuart et al., 1985), but this was not found to be the case in the present study. The ability of sweet potato somatic embryos to form plantlets was apparently more influenced by stage of maturity (embryo age) than embryo length. This is an important consideration for proper selection of somatic embryos for studies related to container or field sowing. Redenbaugh et al. (1987) reported embryo quality (ability of the embryo to become a plant) as the most important factor for somatic embryo to plant conversion.

The use of shorter maturation time periods during embryo development, the selection of embryos at the proper stage of development, and the determination that HEC gel is compatible with somatic embryos for fluid drilling are important steps towards the development of synthetic seeding technology. Seed or embryo quality is the key to the successful establishment of any crop, whether by conventional zygotic or synthetic (somatic) seed sowing. In the future, studies will need to be focused on methods to promote synchronous somatic embryo maturation in order to increase the success rate of embryo to plantlet conversion and the production of more vigorous plants.

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