Smooth cordgrass synthetic seeds:

Production, storage and potential use for coastal erosion controls

By Herry S. Utomo, Ida Wenefrida, and Timothy P. Croughan
Louisiana State University

An acre of coastal wetlands in Louisiana is lost to erosion every 20 minutes. Synthetic seeds of smooth cordgrass (*Spartina alterniflora Loisel (Poaceae)*) would be a useful adjunct approach to the hand-transplanting employed in coastal erosion control.

The synthetic seed production process can be automated, therefore synthetic seeds can expand the vegetative propagation of smooth cordgrass: an acre of land can be planted by airplane in eight seconds, compared to a minimum of 24 man-hours to plant the same area by hand transplanting.

Smooth cordgrass is a perennial warm-season grass found along shorelines. It grows vigorously at seawater salinity and has a extensive root system, but it can grow in both fresh and saltwater environments. It tolerates fluctuating water levels and is adapted to many soil types. Smooth cordgrass can provide an effective buffer that disperses wave energy, reduces shoreline scouring and entraps floating sediments and other solids.

Smooth cordgrass is sterile with little or no viable seed production. As a result, erosion control materials are produced through vegetative propagation. Divided plants can be placed in one-gallon containers to produce new stems before transplanting. Vegetative propagation and hand-transplanting is labor intensive and expensive.

Cordgrass synthetic seeds

Development and potential use of synthetic seeds, or artificial seeds, has been a hot topic since Murashige introduced the concept in 1977. The definition of synthetic seed refers to the encapsulation of somatic embryos that functionally mimic the behaviors of true seeds and sprout into seedlings under suitable conditions. Under a broader definition, synthetic seeds include encapsulated buds, bulbs or any form of meristem that can develop into a plant (Li, 1993).

Hydrogelling agents used for encapsulation include sodium pectate, Gel-rite, agar, guar gum, carrageenan, and sodium alginate.

Currently, plant regeneration through somatic embryogenesis has been achieved in more than 200 species and therefore the production of synthetic seeds from these species is theoretically possible. Efforts to produce synthetic seeds have involved a range of species, including papaya (Castillo et al. 1998), pistachio (Onay et al. 1996), white spruce (Fowke et al. 1994), celery and lettuce (Sanada et al. 1993), and alfalfa (Redenbaugh 1993, Fuji et al. 1992). Despite progress in this research area, synthetic seed germination rates remain a major problem.

Research in smooth cordgrass synthetic seeds has been directed towards improving seed viability. Synthetic seed technology developed for smooth cordgrass may serve also as a model for other grass species.

Tissue culture

Somatic embryogenesis can be a rapid propagation tool, since a large quantity of embryogenic callus capable of producing plants can be obtained quickly in either a semi-solid or liquid medium. The amount of callus tissue cultured in liquid medium triples within a one-week period of subculture. A tissue culture protocol has been developed for clonal propagation of smooth cordgrass in which a half gram of smooth cordgrass callus tissue can be induced to

regenerate into 25 or more plants.

The medium for producing somatic embryos and micro-plantlets contains R4 salts (Chaleff and Stolarz, 1981) supplemented with 0.5 g l-1 casein acid hydrolysate, 100 mg l-1 inositol, 1 mg l-1 biotin, 0.4 mg l-1 thiamine.HCl, 64 g l-1 maltose, and 6 g l-1 agarose, with the pH adjusted to 5.8 before autoclaving. Cultures are maintained at 25oC in a light regime of 16:8 light/dark with a light intensity of 15 mE.m-2.s-1. Resulting somatic embryos and micro-plantlets are then encapsulated to produce synthetic seeds.

Embryogenesis

Embryogenic tissues of smooth cordgrass are formed during callus initiation and proliferate to produce more embryogenic tissues. A part of the tissues develops into somatic embryos which further undergo a maturation process.

A maturation phase is the most critical period in which fully developed, germinable somatic embryos are formed. The mature embryos enter a phase that involves elongation of the hypocotyl-root axis and emergence of the radicle, resulting in a seedling-like somatic plantlet. The somatic plantlet (or "micro-plantlet") becomes the plant material of choice for developing high germinating synthetic seeds. Micro-plantlets are small in size, facilitating easy encapsulation, and they are already growing.

Encapsulation

Encapsulation protects both embryos and micro-plantlets. It provides favorable conditions for handling, storage and mechanical seeding. It also provides a suitable environment to support acclimatization of autotrophic micro-plantlets to field conditions.

Alginate is an excellent encapsulating agent for smooth cordgrass synthetic seeds. Calcium alginate gel beads are produced by dropping alginate solution into calcium chloride solution. Sodium alginate will complex when mixed with calcium chloride to form calcium alginate. Ionic bonds will be formed between carboxylic acid groups on the glucoronic acid molecules of alginate. Solidified alginate will form after 20 minutes in 50 mM calcium chloride solution. Gel beads with an average diameter of 6 mm are produced.

An alginate concentration of 2 percent is adequate for smooth cordgrass encapsulation. The growth of non-encapsulated and encapsulated micro-plantlets under the same environment were similar, indicating that encapsulating material hardness did not affect the micro-plantlet's growth. The use of hollow encapsulations to encourage normal growth as suggested by Patel et al. (2000) may not be necessary.

Sugar and other constituents such as vitamins, myo-inositol and biotin can be incorporated into the capsule. Without such constituents, somatic embryos of smooth cordgrass will not always germinate. Incorporation of these chemicals into the capsule, however, provides favorable conditions for the growth of microorganisms.

The beads become rapidly contaminated with microbes when they are transferred into non-sterile environments. Preventive measures using a combination of biocides, bacteriocides and fungicides can provide complete control of microbial growth.

The use of a hydrated alginate gel for the encapsulation of natural seeds has also been reported to improve germination and survival in the soil. Encapsulating materials can carry a variety of pesticides, fungicides and fertilizers. Encapsulation also provides moisture and can be used to add weight to natural seeds that are relatively light.

Germination of synthetic seeds

Using micro-plantlets for producing synthetic seeds is more desirable than using somatic embryos for the following reasons.

First, synthetic seeds derived from micro-plantlets have about a two-week maturity advantage over embryo-derived synthetic seeds. Second, somatic embryos are more sensitive to growth environments and nutritional composition changes than micro-plantlets. Therefore, microplantlet-derived synthetic seeds perform better under the conditions of field planting.

Data from germination studies show that higher rates of viability were obtained when synthetic seeds were produced from micro-

Germination studies indicate that higher rates of viability were obtained when synthetic seeds were produced from micro-plantlets rather than somatic embryos.

plantlets rather than somatic embryos. A germination rate of 86 percent was produced with micro-plantlets, while only 6 percent of encapsulated somatic embryos germinated (Fig. 1). Seedling establishment was more rapid with micro-plantlet-derived synthetic seeds. Under controlled environments, encapsulated micro-plantlets grew to 6-cm shoot lengths with root lengths of 4 cm in ten days. This seedling establishment would be ideal for revegetating intertidal coastal marshes.

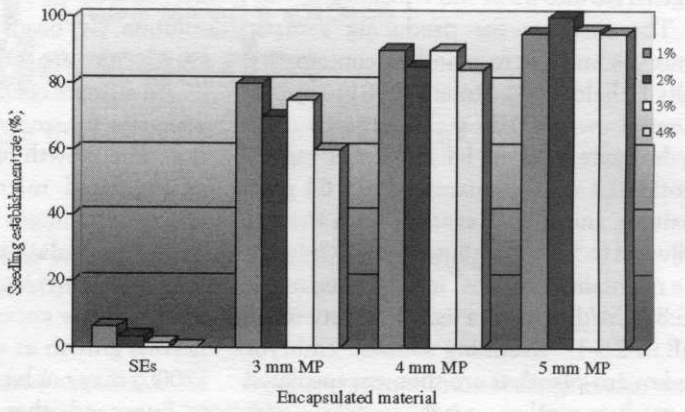

Figure 1. Seedling establishment rate for somatic embryos (SEs) and 3, 4, and 5 mm micro-plantlets (MPs) encapsulated with 1, 2, 3, and 4percent (w/v) alginate.

Storage

A storage method providing enough time for the accumulation of sufficient synthetic seeds for planting a large area is another important aspect in synthetic seed production. Preservation and storage methods include induction of slow growth using plant growth regulators (Ammirato, 1986), low temperatures (Molle et al., 1990; and Nadel et al., 1990) and hypoxia (Engelmann, 1990). Long-term preservation through freezing and desiccation that provides reversible arrest of embryo development to mimic true seed behavior has also been studied (Senaratna et al., 1989).

Despite significant progress in this research area, germination rates of synthetic seeds remain a major problem.

Encapsulated micro-plantlets can be stored at 1 to 5o C inside closed containers under a low light intensity of 5 mE.m-2.s-1 for 6 weeks without loss of viability (Fig. 2). With the potential for automating the production process of smooth cordgrass synthetic seeds, a six-week period should provide enough time for the accumulation of sufficient synthetic seeds to plant a large area.

Potential use for erosion control

Research progress involving in vitro clonal propagation of smooth cordgrass shows that this technique can mass-produce vegetative clones in a short period. Synthetic seeds can capture the benefits of this rapid plant multiplication. Individual plants possessing desirable characters can produce synthetic seeds.

Genetic diversity has been an issue related to the use of plants for coastal stabilization and wetlands reclamation. Populations with low genetic diversity may have greater vulnerability to sudden changes in macro/micro climate caused by disease, insects, drought, high salinity and fire. Resistance to these stresses favor long-term survival and need to be preserved. Genetic diversity among subpopulations of smooth cordgrass can be assessed using DNA fingerprinting techniques.

Besides possessing desirable characters, parental lines can be selected to represent the genetic diversity of the original populations in the target areas. The number of parental lines will not be a limiting factor in the synthetic seed production and as many as required can be included to keep the genetic diversity in the target regions. Parental line composition and the proportion of synthetic seeds derived from each parental line can be adjusted so that resulting synthetic seeds are tailored to provide the right degree of genetic diversity.

Herry Utomo is a postdoctoral researcher at the Biotech Lab at the Rice Research Station of Louisiana State University Agricultural Center, Crowley, LA. He has a Ph.D. in Agronomy from LSU and has been conducting research in "Biotechnology applications to biological control of wetlands erosion" in the last five years. Ida Wenefrida also is a postdoctoral researcher at the Center. Her Ph.D. degree is in Plant Pathology and Crop Physiology. Dr. T. P. Croughan is an Endowed Professor of Excellence in Plant Biotechnology at LSU. He received his Bachelor's in biology from Reed College, then attended graduate school at the University of California at Davis, where he received a M.S. in agronomy and a Ph.D. in plant physiology. They can be reached by fax at 337/788-7553.

REFERENCES

Ammirato, P.V. 1986. Control and expression of morphogenesis in culture. In: Plant Tissue Culture and Its Agricultural Applications, Withers, L.A. and Alderson, P.G. (Eds.). Butterworths. London. Pp. 23.

Castillo, B., Smith, M.A., Yadava, U.L. 1998. Plant regeneration from encapsulated somatic embryos of Carica papaya L. Plant Cell Rep. 17:172-176.

Engelmann, F. 1990. Use of atmospheres with low oxygen contents for the storage of oil palm (Elaeis guineensis Jacq.) somatic embryo cultures. C.R. Acad. Sci. Paris, Ser. III, 310:679.

Fowke, L.C. Attree, S.M., Pomeroy, M.K. 1994. Production of vigorous desiccation-tolerant white spruce (Picea glauca [Moench] Voss.) synthetic seeds in a bioreactor. Plant Cell Rep. 13:601-606.

Fuji. L., Slade D., Aguirre-Rascon, J. 1992. Field planting of alfalfa artificial seeds. In Vitro Cell Devel Biol Plant 28:73-80.

Li, X.Q. 1993. Somatic embryogenesis and synthetic seed technology using carrot as a model system. In: Redenbaugh K (Ed.) Synseeds: Applications of synthetic seeds to Crop Improvement. CRC. Press. Boca Raton, Fla. Pp.289-304.

Molle, F., Freyssinet, G., Giroud-Abel, B. 1990. In: Seed: Genesis of Natural and and Artificial Forms, Le biopole vegetal, Ed., Le Conseil Regional de Pirandie, Amiens, France. Pp.172.

Murashige, T. 1977. Plant cell cultures as horticultural practices. Acta Hort. 78:17-21

Nadel B.L., Altman, A., Ziv, M. 1990. Cold storage and efficient conversion of somatic celery embryos into transplantable plants. Sci. Hort. 4:9-14

Onay, A., Jeffree C.E., Yeoman, M.M. 1996. Plant regeneration from encapsulated embryoids and an embryogenic mass of pistachio, Pistacia vera L. Plant Cell Rep. 15:723-726.

Patel, A.V. Pusch, I., Mix-Wagner, G. and Vorlop, K.D. 2000. A novel encapsulation technique for the production of artificial seeds. Plant Cell Rep. 19:868-874.

Redenbaugh, K. 1993. Introduction. In: Redenbaugh K (Ed.) Synseed: Application of synthetic seeds to crop improvement. CRC Press, Boca Raton, Fla., pp 3-10.

Sanada, M., Sakamoto, Y., Mashiko, T., Okamato, A., Ohnishi, N. 1993. Celery and lettuce. In: Redenbaugh K (Ed.) Synseeds: Application of synthetic seeds to crop improvement. CRC Press, Boca Raton, Fla. Pp 305-327.

Senaratna, T., Bryan, D., McKensie, B.D., Bowley, S.R. 1989. Desiccation tolerance of alfalfa (Medicago sativa L.) somatic embryos. Influence of abscisic acid, stress pretreatment and drying rates. Plant Sci. 65: 235.

TURFGRASS TRENDS

Name:

Title:

Business:

Address:

City: State: Zip:

Phone: ( )

Fax: ( )

TURFGRASS TRENDS • 131 WEST FIRST STREET • DULUTH, MN 55802-2065 01/01

ORDER

▶ YES, Send the **TURFGRASS** TRENDS subscription that I have marked.

(12 issues per year)

❑ **6 months @ $105.00**

❑ **1 year @ $199.00**

❑ **1 year overseas @ $230**

❑ **Payment Enclosed**

❑ **Please Bill Me**

▶ For faster service please call: 1-888-527-7008 or FAX your completed form to: 1-218-723-9417 or 9437