Turfgrass Improvement Through Cell Culture

by DR. JEFFREY V. KRANS
Assistant Professor, Mississippi State University

CULTURE OF a turfgrass species and/or cultivar is tied directly to genetic makeup of that species/cultivar which, in turn, dictates its performance in the environment. Development and introduction of new and improved turfgrass varieties is the ultimate goal of the plant breeder and others who strive to produce improved turfgrasses. The availability of improved turfgrass varieties is growing and has resulted in grasses which show improved resistance to pest and environmental stresses as well as more desirable turf-type features (prostrate growth, fine texture, etc.). The majority of the new and improved turfgrass varieties that are available now originated from field selections of naturally occurring variants. These variants were recognized in their natural habitats by exhibiting outstanding characteristics noticeable to the observer.

Future progress in improving the desirability of turfgrass species will undoubtedly involve other techniques in the discovery and screening of improved turfgrasses. Plant breeding techniques including planned cross- and/or self-fertilization schemes, induced mutations and plant cell and tissue culture will all play a role in the continued improvement of turfgrasses.

Plant cell and tissue culture techniques are a recently employed tool to improve the genetic desirability of a plant species. Although cell and tissue culture technology has been used in various plant species, turfgrass species have received only limited attention. The Carolinas Golf Association, through the USGA Green Section Research and Education Fund, is supporting research at Mississippi State University to investigate the use of plant cell and tissue culture in creeping bentgrass (*Agrostis palustris* Huds.). This research is designed to improve the genetic desirability of creeping bentgrass for all but especially southern golf greens by generating and screening improved varieties.

CELL AND TISSUE CULTURE TECHNOLOGY

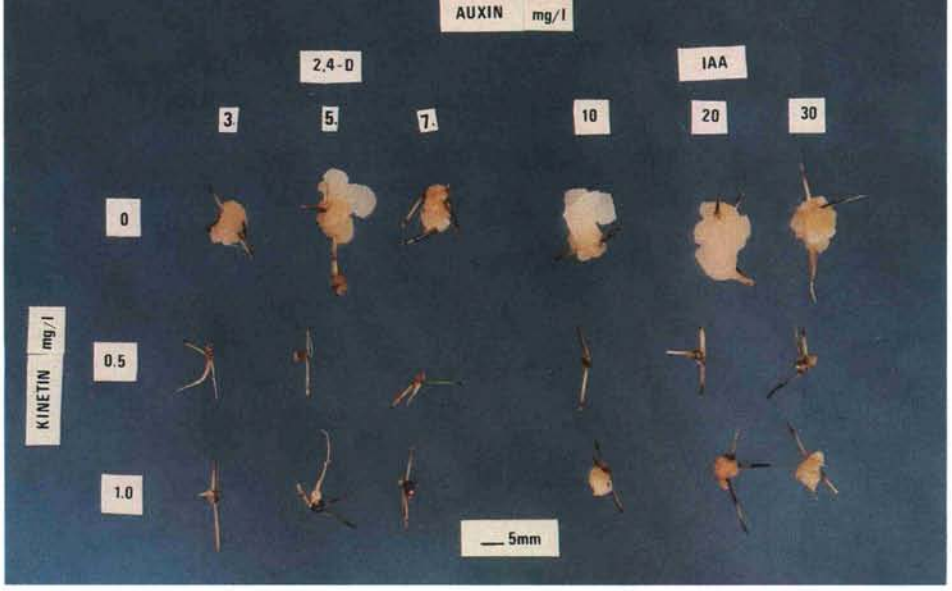
Plant cell and tissue culture deals with the culture of aggregates or individual plant cells in a vessel containing a substrate (media) which supplies nutritional elements and support for cell or tissue growth, replication and/or differentiation (Figure 1). The composition of the media used to culture plant cells consists of a variety of components, including inorganic salts, sucrose, vitamins and plant hormones (Table 1). The change in component or concentration of a constituent in the media dictates the growth and/or differentiation of the given tissue or cell culture. Throughout

Figure 1: Callus (unorganized aggregate of cells) of Kentucky bluegrass growing in a sterile vessel on defined media of inorganic salts, sucrose, vitamins and plant hormones.

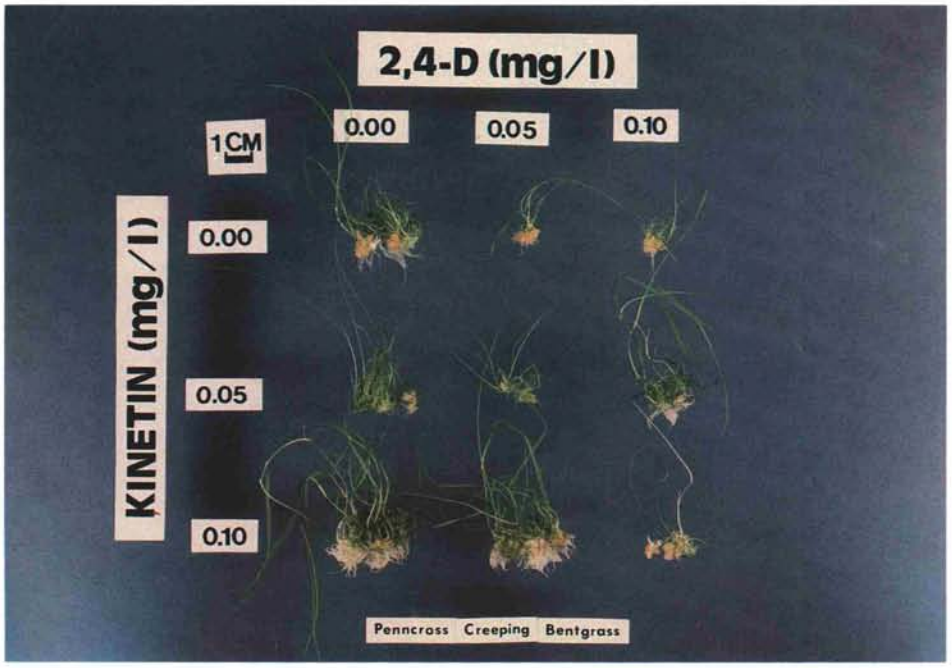
TABLE 1
Components of Media including Inorganic Salts, Sucrose, Vitamins and Hormones used for Callus Induction and Maintenance and Plantlet Regeneration of Creeping Bentgrass[1]

Media Constituents and Concentrations							
Inorganic Salts		**Vitamins**		**Hormones**		**Others**	
	—mg/l—		—mg/l—		—mg/l—		—g/l—
NH_4NO_3	1650	Thiamine•HCl	0.1	2,4-D	0-10	Sucrose	10-30
KNO_3	1900	Myo-inositol	100	Kinetin	0-1	Agar	6-10
$CaCl_2•H_2O$	440	Nicotinic acid	0.5				
$MgSO_4•7H_2O$	370	Pyrodoxine•HCl	0.5				
KH_2PO_4	170						
Na_2EDTA	37.3						
$FeSO_4•7H_2O$	27.8						
H_3BO_3	6.2						
$MnSO_4•4H_2O$	22.3						
$ZnSO_4•7H_2O$	8.6						
KI	0.83						
$Na_2MoO_4•2H_2O$	0.25						
$CuSO_4•5H_2O$	0.025						
$CoCl_2•6H_2O$	0.025						

[1]Murashige, T., and F. Skoog. 1962. A revised medium for rapid growth and bio-assay with tobacco tissue cultures. Physiologia Plantarum 15:473-497.



(Top, left) Figure 2: Callus which formed from the nodes of excised stolon segments of "Tifgreen" bermudagrass. Degree of callus formation is dictated by the addition of the plant hormones (2,4-D, IAA, and kinetin) in combination with defined media composed of inorganic salts, sucrose, and vitamins.



(Below) Figure 3: Callus which has formed from the embryo region of the seed and along the coleoptile (shoot) of a 4-week-old creeping bentgrass seedling. Callus was induced to form by the addition of 5 milligrams of 2,4-D in combination with defined media composed of inorganic salts, sucrose, and vitamins.

(Center, left) Figure 4: Plantlets regenerated from callus of creeping bentgrass. Degree of shoot and root formation is determined by the relative composition of plant hormones (2,4-D and kinetin) in combination with defined media composed of inorganic salts, sucrose, and vitamins.

(Below, left) Figure 5: Twelve-week-old creeping bentgrass plants which originated from callus cultures previously incubated and induced to form root and shoot structures on defined media composed of inorganic salts, sucrose, vitamins and plant hormones.

all phases of this research, techniques to destroy (sterilize) and exclude (asepsis) contaminating microorganisms are critical. Although elaborate facilities are not necessary to conduct cell and tissue culture research, controlled techniques and specialized equipment are required.

In order for tissue culture to be used in a given plant species, that species must be capable of manipulation such that callus (unorganized aggregate of cells) can be induced from an excised part of that plant (explant), callus growth after the explant has been excised can be continued and regeneration of whole plants (plantlets) from the callus is readily obtainable. Plant parts commonly used for callus induction include shoot or root tips from seedlings or mature plants, mature or immature embryos, leaf sections, etc. (Figure 2).

In creeping bentgrass, callus has been initiated from mature embryos (whole seeds). Callus forms from the embryo region and along the coleoptile up to the first node (Figure 3). Callus growth in creeping bentgrass following separation from the explant can increase in size six to 10 times over a six-week period. These callus cultures are subcultured or split into smaller pieces at four- to six-week intervals and placed on fresh media. Subculturing is required to maintain vigorous and healthy callus as well as a means to propagate additional callus. Plantlet regeneration in creeping bentgrass is accomplished by transferring callus to a media containing a specified hormone balance [auxin (2,4-D) to cytokinin (kinetin) ratio] (Figure 4). The resulting plantlets generally show 95 percent normally developed root and shoot structures. These plantlets can readily be transferred to soil and grown to full maturity (Figure 5).

Once the media requirements for callus induction, maintenance and plantlet formation are known for a given plant species, additional cell culture technology can be applied to improve the desirability of that species. However, it should be noted that not all plant species/cultivars are adapted to

manipulation in cell or tissue culture. Table 2 lists those turfgrass species which have been shown to produce callus and/or plantlets.

UTILIZATION OF CELL AND TISSUE CULTURE TECHNOLOGY

Avenues of use of cell or tissue culture vary depending on the objectives desired. Tissue culture is currently most widely used to propagate plant varieties rapidly. Although not used in turfgrass species, numerous horticultural crops depend on tissue culture as the principal means of propagation.

Currently, a major application of cell and tissue culture technology in addition to rapid clonal propagation involves the improvement of the genetic desirability of plant species. This can be achieved in several ways. The most widely used method involves induction and screening of mutants at the cellular level. This system involves the exposure of aggregates or single cells to chemical [i.e., ethyl metlanesulfonate (EMS)] or radioactive (i.e., cobalt 60) mutagens or maintenance of cultures over a period of time which inherently results in random mutations. This use of chemical or radioactive mutagenic agents to generate mutations in plants is not unique to tissue culture. The generation of mutations using these external mutagens has been used in various crop species, including turfgrasses. However, mutant induction in tissue culture provides the opportunity to mutate large numbers of cells as well as screen for desirable mutants rapidly. This screening process involves the application of a selection pressure (chemical or environmental condition) on the mutant cells in order to separate non-mutants or undesirable mutants from desirable mutants. The actual method of this selection process generally involves the incorporation of a substance into the media or location of the holding vessels in a specific environment not usually conducive to normal (non-mutant) cell growth. Those cells showing resistance grow in the presence of the selection pressure and are later isolated. These isolates are induced to form plantlets which are usually representative of the resistant cells and retain the desirable characteristic originally selected for at the cellular level. This system of generating and screening for resistance in cell culture has been demonstrated in various crop species and is currently under way in creeping bentgrass. Items used in this screening process which have been utilized for developing resistance in other plant species include fungal diseases (most fungi are associated with a chemical toxin which is used to select for resistance), salt concentrations, antibodies, chemical toxins, drugs, and environmental stresses. Although this screening process may appear straightforward, various complications arise which limit widespread application to all plant species.

In addition, cell and tissue culture can be applied in other ways to improve the desirability of plant species. Perhaps the most popular and attractive of these tissue culture techniques is somatic (vegetative or non-germ origin) hybridizations. Somatic hybridization has generated widespread popular attention to tissue culture. This technology involves the fusion of plant cells in culture and bypassing normal cross-fertilization as the means of producing a hybrid. Although somatic hybrids of several plant species have been reported in tissue culture, this technology will undoubtedly require additional investigation to play a significant role in crop improvement. Other uses of plant cell and tissue culture include induction of haploid (one-half the normal chromosome number) plants and freeze-preservation of plant cells (cryogenic storage) at the temperature of liquid nitrogen (-196C°).

Plant cell and tissue culture technology is not a substitute for other techniques used to propagate or improve the desirability of a plant species. It will, however, be a part of the future development and discovery of more desirable plant species, including turfgrasses. Current research efforts in creeping bentgrass have established the necessary prerequisites, which enables the use of tissue culture techniques for improving its genetic desirability. Continued research efforts will determine the extent to which plant cell and tissue culture technology will play in the improvement of various economically important plant species.

TABLE 2
Turfgrass Species which have been shown to produce Callus and/or Plantlets using Plant Cell and Tissue Culture Techniques

Turfgrass Species	Callus Induction	Plantlet Formation	Reference
Annual Ryegrass	yes	yes	1,3
Tall Fescue	yes	yes	4
Creeping Bentgrass	yes	yes	3
Kentucky Bluegrass	yes	no	3
Common Bermudagrass	yes	no	3
St. Augustinegrass	yes	no	5
Rough Bluegrass	yes	no	2
Red Fescue	yes	no	2
Chewings Fescue	yes	no	3
Meadow Fescue	yes	no	2
Perennial Ryegrass	yes	no	2,3
Colonial Bentgrass	yes	no	2

ACKNOWLEDGMENT

The experimental investigation conducted within the Mississippi Agricultural and Forestry Experiment Station is funded in part by the Carolinas Golf Association through the USGA Green Section Research and Education Fund.

REFERENCES

1. Ahloowalia, B. S. 1975. Regeneration of ryegrass plants in tissue culture. Crop Sci. 15:449-452.
2. Atkins, R. K., and G. E. Barton. 1973. The establishment of tissue culture of temperate grasses. J. Esp. Bot. 24:689-699.
3. Krans, J. V., K. C. Torres and V. T. Henning. 1978. The effects of nutritive and environmental parameters on callus initiation and growth of selected turfgrasses. Agron. Abst. 70:118.
4. Lowe, K. W., and B. V. Conger. 1979. Root and shoot formation from callus cultures of tall fescue. Crop Sci. 19:397-400.
5. Torres, K. C., and J. V. Krans. 1979. The effects of carbohydrate sources and concentration on callus initiation and growth of St. Augustinegrass. Agron. Abst. 71:124.