EXECUTIVE SUMMARY TO THE USGA - NOVEMBER, 1991

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New methods for assessing the responses of grass lines to salinity in the root zone are required to help identify selections with potential for use on marginal sites. The response of a turfgrass cultivar to salt stress is an important evaluation criterion, because the demand for new selections that will tolerate saline conditions is escalating. A variety of systems have been developed to rate the response of grasses to salts in the root zone, yet the plant growth stage and the selection criteria have varied between tests. Inconsistencies in environmental test conditions also tend to bias the ratings and preclude direct comparisons. Due to the experimental complexity involved, few screens have attempted to gauge comprehensive salt stress reactions of entire grass plants over time. Some plant adaptations, notably those in the root zone, are particularly difficult to observe or quantify.

Solution batch culture and whole plant microculture techniques were comparatively evaluated for paired cultivars from 3 turfgrass species (bermudagrass, creeping bentgrass and St. Augustinegrass) to evaluate shoot and root zone (using machine vision), and osmotic adjustment responses within the test environments. While all of the turfgrass cultivars exhibited growth reductions under conditions of elevated salinity stress, the degree of response was more dramatic for cultivars of bermudagrass and St. Augustinegrass which had previously been rated as salt-sensitive in field evaluations. Morphometric shoot growth evaluations between solution culture and whole plant microculture tests exhibited similar trends, while root responses were more variable in microculture. Osmotic adaptation responses were highly correlated between solution culture and whole plant microculture. In general, the whole plant microculture system provided a simpler, smaller scale test environment which allowed non-intrusive evaluation of salt stress adaptation over the course of the screening test.

Now that we have validated the system using lines with established salt tolerance [ST] or salt susceptible characteristics (as identified by breeders in extensive field tests), we see the project extending in two important ways.

First, the WPMC system can be used to test the ST of unique germplasm developed using the tools of biotechnology. In vitro methods for manipulating turfgrass lines have very recently been worked out for many important species. Cell level screening for ST (with callus cells growing on high salt media) can be an effective way to rapidly isolate unique lines with cell level tolerance to salt. It has traditionally been difficult to make the transition to a field population for whole plant level testing of the new grass line, to verify that it has good field tolerance for salt. The WPMC system can provide an excellent vehicle to facilitate that intermediate testing stage; to quickly and efficiently identify and isolate cell lines selected through biotechnology, and demonstrate practical benefits of the grass selection. Towards this objective, I currently have a student working with callus generation/regeneration of turfgrass callus on high salt media, and testing these new regenerated lines using the WPMC system. As you observed in my laboratory, image analysis is used both at the callus stage and the whole plant microculture stage to rigorously evaluate experimental performance.

Secondly, the WPMC system we have developed can be an effective tool to test turfgrasses for other stresses (heat, drought tolerance, etc.). We can establish an effective link with traditional turfgrass breeders at this point for pretesting new lines they have developed, prior to scaling up for full field testing of the turf. This would be testing of new selections (potential commercial lines) produced by conventional breeding techniques, rather than by any in vitro biotechnology. The same very practical, controlled test approach can be utilized.

ANNUAL REPORT TO THE USGA - NOVEMBER, 1991

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Current project status:

WPMC

Final evaluations of data derived from a practical, alternative whole plant microculture [WPMC] screening system tested in direct parallel with solution batch culture tests for cultivars of creeping bentgrass (Agrostis palustris Huds), bermudagrass (Cynodon dactylon (L.) Pers.), and St. Augustinegrass (Stenotaphrum secundatum (Walt) Kuntze) that were ranked as relatively salt resistant or susceptible in field observations were completed. Detailed assessments of the growth (morphometric and dry weight adjustment) and osmotic adjustment responses of each grass line to NaCl stress were provided, comparisons between plant response in vitro and in solution culture were made, and the additional advantages and limitations of using a WPMC system for screening novel germplasm were considered. The growth and developmental responses and adaptations under gradually increasing salinity stress were monitored repeatedly over time-course evaluations during the salt screening tests. Machine vision was adapted to facilitate the evaluations and permit non-destructive interim evaluations.

The batch nutrient culture system (based on the design of Hershey and Merritt, 1986, was constructed in a Conviron (model PGW36) walk-in growth chamber (3.3 m^2). Individual plants were placed in pretreated white foam plugs and five plants were inserted into fitted holes in the plastic lid of one liter vessels (Meyer et al., 1989). Uniform aeration between vessels was supplied through bubblestones by an oil-less diaphragm pump and regulated with a pressure gauge. Initially, the photosynthetic photon flux (PPF) reaching the transplants (provided by 50% high-pressure sodium lamps [Sylvania Lumalux 1500W] and 50% metal halide lamps [Sylvania Leviton 1500W]) was reduced to 269 $\pm$ 25 μmol m^{-2} s^{-1} to facilitate transplant acclimation to the new environment. After the first day, PPF was raised to 576 $\pm$ 50 μmol m^{-2} s^{-1} measured 48 inches below the lamp bank, with a 15 hour photoperiod, 28 $\pm$ 2°C light/dark temperature, and 55 $\pm$ 5% relative humidity for the duration of the experiments. Solution conductivity was gradually adjusted within treatments by decanting and replacing solutions at weekly intervals until all four treatment conductivity levels (2.4, 12.4, 22.4, and 32.4 dS m^{-1}) were established.

For WPMC, nodal stolon segments (2.5-3.5 cm) of bermudagrass and St. Augustinegrass cultivars were harvested from greenhouse plants and stirred in a mild detergent solution for 20 min. Explants were rinsed for 10 min under running tap water, immersed in a solution of 1.3% sodium hypochlorite and 0.1% Tween 20 (polyoxyethylene sorbitan monolaurate), and stirred for 10 min. Bentgrass caryopses were surface disinfested by a similar procedure, but the sodium hypochlorite concentration was reduced to 0.8%. Plants were allowed to proliferate in vitro at 25 $\pm$ 2 °C under cool white fluorescent lamps providing 40-50 μM m^{-2} s^{-1}, and nodal segments were subcultured to fresh medium three times at 1-2 week intervals prior to use in salt stress experiments. The culture medium for subculture and propagation of bermudagrass explants was supplemented with 0.5 mg l^{-1} IBA to facilitate establishment of these cultivars in culture.

To facilitate gradual acclimation to increasing salinity in vitro (and avoid salt "shock" responses, which would be less useful in evaluation), the plantlets were trimmed at subculture every 2 weeks and introduced to fresh media with higher conductivity until final treatment levels were reached.

Evaluations were facilitated using machine vision techniques previously described for the USGA. From the digitized image, precise measurements of shoot and root system length and area were obtained. Shoot weighted density was also recorded for WPMC samples. In order to capitalize on the opportunity for non-intrusive data collection without disturbing the experimental sequence, intact specimens in test tubes were image analyzed just prior to each subculture to higher salt concentration. This weekly evaluation was analyzed to determine how quickly salt stress responses became evident in each cultivar. Image analysis was finally conducted on WPMC experiments after one week of growth at final treatment conductivity levels, and again after subculture and an additional week in culture. Dry weight (DW) analysis was subsequently conducted on experimental plants from solution culture by drying samples for two days at 75 °C in a forced air oven.

Reductions in growth were evident in both solution and WPMC with elevated salinity for all of the test grass cultivars. Typically, the field resistant cultivar in the paired set from each species exhibited less dramatic reduction in shoot size/length than the more susceptible cultivar, although this was not the case for bentgrass. Correlations between trends in solution culture and WPMC were excellent. For roots, the field-evaluated resistant cultivars of St. Augustinegrass and of bermudagrass ('Seaside' and 'Tifway', resp.) were capable of producing longer roots in response to the salinity stress in solution culture, whereas the salt susceptible cultivars were not. This observation was <u>not</u> paralleled in the WPMC screenings, most likely due to interference from the gel matrix.

In general, the trends in shoot growth were very similar between WPMC and solution culture, whereas the root trends were not the same. If plants are supported in soil or aggregate culture during salt stress screenings, the distribution of salts in the rhizosphere can be difficult to control due to irregular accumulation on the substrate. Even careful excavation of root systems for evaluation will intrude on the growing environment and disturb subsequent plant development. In both the solution batch culture and WPMC screening systems, distribution of salts around the roots was extremely uniform. In solution culture, roots were continually bathed in the saline medium, whereas in WPMC, salts contacted the roots by diffusion through the medium or root growth to new supplies of medium (similar to the situation in soil culture). Easier access to the root systems (without interference from medium gelling agent) makes a solution culture system more practical for analysis of dry weight. Although root data was more variable in WPMC, many of the same trends existed for roots of all three grass selections in both WPMC and solution culture. Root length increases as a consequence of salinity level, as one mechanism of salinity tolerance, were more obvious in solution culture. This is to be expected, since root growth was unrestricted in solution, whereas the gel matrix impeded root length in WPMC. Variability in root growth throughout the WPMC screening tests is likely due to the necessity for trimming roots prior to subculture, and a temporary "transplant shock" that precluded immediate root response to increasing salt levels.

Earlier weekly image analysis observations taken for the WPMC tests during the gradual subculture of treatments up to final salinity treatment levels, in general, indicated enhanced growth for bermudagrass and St. Augustinegrass cultivars at the 12.4 dS m^{-1} treatment level, although at the time of the final evaluation, these differences were no longer evident. In general, shoot growth decreases coincident with salt treatment level were more clearly defined at the time of the final observation at the end of the screening test, rather than at any earlier observation period.

Cell-level selection tests

WPMC offers an additional advantage for realistic evaluation of novel turfgrass germplasm from biotechnological research during early stages of development (while still in vitro). Cell culture techniques are currently being explored on many fronts to improve turfgrass quality and performance. The WPMC evaluation system described here may permit promising new selections from in vitro research to be identified, screened, and rated prior to plant acclimatization and scale-up for field trials.

At the present time, the M.Sci. student dedicated to this project (Kuo) has generated callus from immature inflorescences of several turfgrass cultivars within zoysia, bermudagrass, and St. Augustinegrass. He has been evaluating the regenerative potential of callus derived from immature inflorescences vs. mature caryopses and internode segments. He has succeeded in development of an efficient protocol for callus induction in the dark with standard temperature and physical microenvironment.

As part of this work, a 2-phase screening of putative ST lines selected in cell culture will be implemented. Cell level selection trials will be followed immediately by WPMC tests on regenerated, putative ST selections. This intermediate step will provide a valuable prescreen prior to time-consuming and expensive field trials, and will effectively "weed-out" selections from culture that are not realistically superior at the whole plant level.

Need for continued support:

My requirements to maintain the program as planned are simple and as before: I have need of continued funding [$9,000/year] for graduate student stipend. I request a one year extension of my current project to support a completed thesis for the student now on board through at least fall, 1992. I have been able always in the past to augment my turfgrass research with Hatch funds for supplies, and have been awarded JBT scholar research award support for some other aspects of the project. Although we utilize some sophisticated instruments in the development of the turfgrass ST testing systems (including vapor pressure osmometry and image analysis), I have been able to purchase major equipment from other funds. I hope that overall that the Research Committee will consider this project to be an efficient use of resources, and will elect to continue support in the future so that we can continue towards the goals we've outlined.

Future plans beyond the scope of the present project:

Following the cell level regeneration tests, cell-level salt screening/regeneration, and channeling of regenerates from cell culture into a WPMC screening system, we will be prepared to begin a new phase of our research, and develop a unique project for consideration by the USGA. In this initiative, we intend to extend the WPMC system to interaction with other USGA sponsored projects, as discussed with Dr. Mike Kenna during a recent site visit. In particular, we hope to interact with salt-tolerance turfgrass breeding projects, to be able to evaluate new selections from these projects using our systems and to correlate the evaluations to obtain new, valuable insight on the characteristics of tolerant grasses. In addition, we envision adaptation of the system to evaluation of other turfgrass stress disorders (including, for example, heat tolerance or drought), and to similarly work with the key breeders in these arenas.