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An overview of progress and accomplishments in 1988 for the project **"A Realistic Whole Plant Microculture Selection System for Turfgrasses".**

Overall Project Goals and Objectives:

Phase I
Development of uniform, working whole plant microculture systems for key turfgrass species

Phase II
Design and implementation of controlled screening tests for stress tolerance on the whole plant microculture level

Phase III
Exploitation of the whole plant microculture system as donor tissue for biotechnological research (somaclonal variation and cell-level selection)

Summary of Progress to Date:

Our progress within the past year has resulted in the completion of all the objectives in Phase I of the experimental plan, and strong headway towards the goals outlined for Phase II. A broad range of grasses with variations in stress resistance traits were obtained from fellow researchers in Florida and Texas, familiar with the field responses of the lines. Paired sets of salt and or drought tolerant genotypes of 4 turfgrass species were prepared as vegetative plugs (Bermudagrass, St. Augustinegrass, Zoysia), grown as greenhouse specimens, and explanted via tillers or nodal segments into whole plant microculture, or germinated as seed (Bentgrass) directly into the microculture environment. Each were adapted into continuous whole plant microculture maintenance and propagation systems, based on uniform subculture of vegetative nodal segments or tillers. The introduction of these grasses into whole plant microculture systems is a unique accomplishment, as some of these grasses have never been explored at all in vitro, even at the callus level. To pave the way for future work in callus regeneration/somaclonal variation with these genotypes, we have been exploring environmental stimuli leading to the initiation of inflorescences both in the greenhouse and for in vitro whole plants. Immature inflorescences provide ideal, highly regenerative callus for biotechnological research.

Optimization of complete whole plant microculture systems for the new turfgrass selections has required in depth analysis of microenvironmental conditions. To simulate natural physical environmental conditions, we have used connected Magenta GA7 vessels with 4 sterile couplers to create unique microchambers approximately 3″ x 3″ x 8″ for maintenance of whole plant turfgrass cultures in a realistic setting. Unlike conventional test tubes, this adapted microchamber is ideally suited to the grasses, providing excellent root

system area and adequate aerial atmosphere so that the plants can respond to imposed stress with natural mechanisms of defense.

The stress resistance screening outlined in Phase II of this research program requires that whole plant microcultures be adapted for quantitative analysis of stress resistance and susceptibility traits. We have therefore worked to develop novel methods for whole plant microculture screening, analysis, and non-destructive quantification using an adaptation of microcomputerized video image analysis (2 manuscripts in preparation; oral paper presented at the recent American Society for Horticultural Science annual meeting in East Lansing, MI). Using this technology, individual turfgrass microculture replicates are imaged within a matter of seconds, and data is collected based on the visual density and morphometric size and shape of the video and digitized images. We have demonstrated that this unique technique permits accurate non-intrusive measurement of turfgrasses in vitro that correlates very well with conventional measurements of tiller length and fresh weight, foliage color, and texture. Image data from control and salt stressed turfgrass treatments has yielded clear and accurate growth curves illustrating the quantitative impact of stress on growth in terms of overall length (analysis of stunting response) and overall plant fresh weight. It therefore allows time-course evaluation of turfgrass stress screening experiments without destroying experimental replicates at each observation.

In addition, our analysis of chemical microenvironmental parameters led to the substitution of Gelrite gellum gum as the solidification agent for the whole plant microcultures. Unlike agar, Gelrite creates a very clear substrate around the root zone of individual turfgrass plants, and has aided our visual and image analysis time course observations of root system development and reaction to stress (e.g. root response to increased salt concentration in the root zone-stunting or discoloration responses can be clearly visualized and quantified).

In order to validate the whole plant microculture screen as a system for identification and characterization of tolerant turfgrass lines, it is necessary to directly compare the system to other evaluation techniques. The responses of different genotypes within each species have been pinpointed by our colleagues in field plot research, however the inherent environmental inconsistencies and complex interactions in field evaluations precludes in depth analysis or characterization of these lines. We have also designed and implemented a more rigorously controlled and uniform new solution batch culture system to concurrently evaluate experiments in both solution and whole plant microculture regimes. In current research, we have been working to comparatively evaluate solution culture and microculture methods to identify salt tolerant grass selections (manuscript in preparation). In this line of experimentation, rooted in vitro plantlets are transferred to solution culture, and evaluated in side by side test treatments in microculture for reaction to increasing levels of salt stress over time.

Current Research Initiatives:

1) In parallel tests, evaluate the response of field-proven salt tolerant and susceptible lines to artificially induced salt stress in solution and whole plant microculture screens. Evaluate salt stress influence on osmotic adjustment, relative growth rate (RGR), and effect on appearance - each criteria difficult to

determine in field tests of the same nature.

2) Determine the ability of different salt stress agents (NaCl, Na_2SO_4, or artificial sea salt) to elicit realistic, field correlated salt responses in the grasses in both microculture and solution culture.

We recently built an elegantly simple batch solution culture system within a new Conviron environmental growth chamber for simultaneous evaluation of paired sets of grasses. The setup includes evaluation of 4 distinct salt stress levels (10 bottles [replicates] and 5 plants [subreplicates] per genotype/salt level per experiment). In each aerated solution culture bottle, salt levels are gradually (over a 4 week period) increased to the maximum level of the stress treatment, with readings on grass response taken at intervals throughout the process. Salt concentrations encompass the range from 2.4 to 32.4 Ds m^{-1}. In our current research, we have concentrated on NaCl as the stress agent, although evaluation of alternative salts are planned. St. Augustinegrass vegetative plugs and bentgrass seedlings have been the initial material processed through the screen. The data collected includes initial readings of plant cell osmolarity, relative growth rate, and visual parameters (image analysis), with repeated readings at 4 and 8 weeks per experiment. Parallel growth and stress conditions are repeated in vitro, with gradual increments in salt concentration provided through periodic subculture to supplemented medium. This line of research contributes valuable new information to the turfgrass industry. Some advantages and new opportunities afforded by this system and previously unavailable to turfgrass researchers include:

1. an evaluation of RGR response of turfgrasses (lnDW regressed over time). The RGR parameter yields revealing information about turfgrass resistance characteristics, not provided through the more typical FW or DW data information.

2. Aeration is more uniform than previously reported solution culture research, and root responses are directly evident since the roots are not embedded in an artificial substrate.

3. Concrete information about the osmotic adjustment response is provided (previously in turfgrass research, an osmotic mechanism has merely been alluded to in research reports).

4. Image analysis used in the solution culture and microculture phases of the research provides quantitative data to support observations on growth rate and color responses.

Projected Research for the Coming Year:

We intend to thoroughly screen the broad range turfgrass lines in both solution culture and microculture systems to document their demonstrated ability to identify and characterize salt tolerance. In addition, we will determine the contributions of osmotic, root morphological, and other mechanisms to salt tolerance in the grasses via analysis of osmolarity (cell sap extraction), RGR, and visual parameters in experimental plants.

Although NaCl has been successfully imposed as the salt stress agent in tests to date, other researchers have utilized Na_2SO_4 or artificial sea salt to simulate natural stress. We will determine turfgrass responses to each unique agent, and determine the most suitable salt to supplement solution culture and microculture screens for the turfgrass research.

Germplasm will be next evaluated in the form of seedlings, young vegetative plugs (solution culture), microcultured whole plants, or callus in parallel salt screening tests to demonstrate comparative reliability of each screen for identifying field tolerance characteristics.

Somaclonal methods of regeneration of turfgrasses for callus will be investigated using established methods, and somaclonal variant populations screened for somaclonal variants salt stress using the previously established whole plant microculture technique. The germplasm in the uniform, presterile and highly juvenile status similarly provides excellent donor material for protoplast and genetic engineering high technology research on turfgrass germplasm - a donor tissue previously unavailable in turfgrass.

Continued Funding Requirements:

Annual stipend for one dedicated graduate research assistant (Ph.D. candidate), and related supplies = $9,000.

Continued support will permit completion of the research program of the current Master of Science candidate contributing to this project, and will allow uninterrupted assignment of a new Ph.D. candidate in the fall when the current student has graduated. A Ph.D. candidate is the preferred recruit for continuation of the research initiative, since the research will require in depth analysis of key cellular and whole plant responses to stress, as well as implementation of some biotechnological routes of investigation.