Recovery of *Rhizoctonia solani* Resistant Creeping Bentgrass Using the Host-Pathogen Interaction System

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Goals:

- *Recover Rhizoctonia solani selected variants of creeping bentgrass using the Host-Pathogen Interaction System (HPIS).*
- *Screen and grade recovered R. solani selected creeping bentgrass variants using an in vitro whole plant disease screening system.*
- *Establish a clonal repository of in vitro screened R. solani resistant creeping bentgrass variants.*
- *Verify whole plant resistance of in vitro screened variants using greenhouse studies for determining R. solani resistance.*
- *Select parents that exhibit resistance to R. solani in conjunction with other desirable turf characteristics.*
- *Evaluate progeny for resistance to R. solani.*

The Host-Pathogen Interaction System (HPIS) is an *in vitro* cell selection system developed in conjunction with efforts to obtain creeping bentgrass with resistance to *Rhizoctonia solani.*

The primary objective throughout our USGA research projects has been to verify HPIS as a valid *in vitro* cell selection system. With this objective achieved, we can recover bentgrass germplasm from HPIS selections and evaluate those genotypes in the field, with confidence that some will segregate from the population and exhibit enhanced disease resistance.

The first step in achieving our primary objective was to obtain disease resistant bentgrass callus via HPIS selection. HPIS refinement studies associated with our initial USGA research project confirmed selection of resistant callus. Callus mortality increased significantly as PENNCROSS calli were co-cultured in HPIS with a virulent isolate of *R. solani.* Small numbers of plantlets were regenerated from resistant callus compared to high numbers of plantlets recovered from control populations.

With bentgrass germplasm successfully regenerated from resistant calli, our USGA research project progressed from there to determine whether enhanced resistance could be exhibited at plantlet and whole plant levels. Plantlets were evaluated for tolerance to *R. solani* using two *in vitro* screening techniques:

1) **HPIS Chamber** - Plantlets were placed in an HPIS Chamber, exposing them to *R. solani* for two weeks. More than 33% of the plantlets did not survive.

2) **Leaf Bioassy** - Leaves of plantlets were exposed to exudate produced by *R. solani.* Plantlets recovered from resistant callus displayed significantly less leaf necrosis as compared to control plantlets. Plantlets surviving both screening techniques were subsequently transferred to soil (whole plant) and maintained in a greenhouse.

Selected bentgrass plants [variant (s)] were inoculated with *R. solani* using growth chamber techniques and evaluated for disease response. Preliminary results indicated that some variants expressed enhanced resistance to *R. solani.* Based on these findings, variants were established in the field under putting green conditions.

Establishment and turf quality data indicate the majority of variants are similar to or better than PENNCROSS. Concurrent with establishment, variants were rated for brown patch resistance based on natural infection. Preliminary observations indicate enhanced resistance may exist among some variants. Several plots displayed brown patch symptoms while adjacent plots had no symptoms.

Bentgrass variants will be evaluated under various stress and environmental conditions through two successive brown patch seasons (2 years). Natural infection and field inoculations will occur under natural putting green conditions. Results from these evaluations will provide us the opportunity to confidently identify variants exhibiting enhanced resistance to brown patch. Selected variants will be used to improve existing creeping bentgrass gene pools by incorporating genes with enhanced *R. solani* resistance. A clonal repository of HPIS germplasm is maintained in an environmentally controlled greenhouse at Mississippi State University. To date, almost 200 genotypes are established under putting green conditions at locations in Mississippi and North Carolina.

Callus is cultured on regeneration medium under continuous low light to induce plantlet regeneration. Bentgrass germplasm is recovered from callus that survived co-culture with *R. solani* in HPIS (left plate). By comparison, more that 60 plantlets regenerated from control callus (right plate) that was not co-cultured with *R. solani.*

HPIS refinement studies have confirmed HPIS as a valid *in vitro* cell selection tool, giving us confidence that some bentgrass variants will be identified as having enhanced resistance brown patch.