Mapping of Quantitative Trait Loci (QTL) for Winter Hardiness in Perennial Ryegrass

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Introduction

Perennial ryegrass (*Lolium perenne* L) is an important turf species. It has a fast establishment rate, strong seedling vigor, good tolerance to both traffic and low mowing which makes it a good choice for use on golf course fairways and athletic fields. However, perennial ryegrass has a poor ability to survive in the severe winter, which limits its use in the far northern areas of United States, including Iowa. One important breeding objective for perennial ryegrass is to improve its winter hardiness.

Winter hardiness is a complex quantitative trait that is controlled by multiple genes with each having minor genetic effect. In addition, the expression of such genes is often affected by the environment, which makes it difficult to identify such genes. With the development of DNA marker techniques, it is now possible to locate these genes (quantitative trait loci, QTLs) that are associated with winter hardiness. There are abundant DNA marker variations present in natural population; some of these markers are in the same chromosome as the genes responsible for winter hardiness. These markers often transmit together with the winter hardiness genes into their progenies. The DNA markers are stable and relatively easy to identify compared to winter hardiness genes that are influenced by the environment and difficult to identify with classic genetics. The long-term goal of this project is to facilitate germplasm improvement of perennial ryegrass with enhanced winter hardiness through marker-assisted selection (MAS). The specific objectives of this research are to identify the QTLs that are associated with winter hardiness in perennial ryegrass.

Materials and methods

*Plant materials:*A segregating population of 174 genotypes was created by crossing a perennial ryegrass cultivar 'Manhattan' with an annual ryegrass cultivar 'Floregon.' While Manhattan has good winter hardiness, 'Floregon' is very sensitive to winter killing. This population was maintained in our research greenhouse at 20-21°C and was fertilized with Miracle Gro to prevent nutrient deficiency. Irrigation was provided as needed. In May 2003, four clones of each genotype were planted in the field within each replication in an α lattice design with three replications. The distance between individual clones of a genotype is 30 cm, and the distance between each genotype is 60 cm. The distance between rows is 90 cm.

Data collection

Fall regrowth: Plants were mowed on July 17, August 14, and September 22, respectively. Fall regrowth was measured as the vertical height of regrowth in centimeters on November 14. Fall regrowth may be correlated with winter hardiness.

Freezing tolerance: Freezing tolerance was assessed by measuring ion leakage. One clone of each genotype was removed from the field on November 30, 2003, and was placed into a sealed plastic bag with a wet paper towel in a corner and then stored at a 4 °C in a walk-in cold room. Individual tillers of similar size were separated from sampled plants, and were then washed quickly in deionized distilled water to remove the soil and blotted dry in a paper towel. The aerial parts and roots were trimmed to 2 cm and 0.5 cm respectively. For each genotype, 18 individual tillers were prepared and then placed into glass tubes (16 x 125mm) with 2 tillers in each tube. Eight different temperature treatments (-6°C, -10°C, -14°C, -18°C, -20°C, -24°C, -28°C, -32°C) were applied to every two trimmed tillers using a ScienTemp programmable freezer (Model:8.5-3.1), and the 4°C treatment was used as a control. The freezer was first equilibrated at -2°C, -3 °C and -4 °C for 30 minutes, respectively. The duration at each test temperature is 15 minutes. The temperature was cooled at a rate of 2°C per hour until -10°C when the temperature started to lower at a rate of 4°C per hour until it reached -32°C. Samples were taken out at each test temperature and thawed on ice overnight. The conductivity of these samples was measured to calculate ion leakage. The freeze-treated samples were infiltrated twice for 4 min each after adding 7ml ddH_2O and shaken horizontally for 1 hour at 250 rpm. The conductivity was then measured with an YSI conductance meter (model 3403). Total conductivity for each sample was determined by measuring the autoclaved samples. Percentage of ion leakage was plotted as a function of the freezing temperatures. LT_{50} value was determined from the midpoint between the maximum and minimum (control) ion leakage obtained for each genotype.

Winter survival: Winter survival was evaluated at the end of April using a scale of l - 5 with 1 being completely dead and 5 being no injury.

QTLs analysis: Data for the same characters will be measured again in 2004. Once the second-year data becomes available, QTL analysis will be conducted. First, a single-factor Analysis of Variance (ANOVA) analysis will be computed for each pair wise combination of quantitative traits and maker loci. The trait values of all individuals having a marker will be compared with those without this marker by using an F test. Then the interval mapping method will be used to find the more robust position of QTL's.