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2008 FINAL REPORT

TITLE: Predicting success of genome placement of a herbicide resistance gene to minimize movement of the gene from wheat to jointed goatgrass

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TIMELINE: July, 2005 to August, 2009

HYPOTHESIS: The placement of a glyphosate gene on either an A or B genome chromosome of wheat should lead to the eventual loss of the gene in a jointed goatgrass backcross due to chromosome elimination once self-fertility is restored.

SUMMARY OF PROGRESS: Evaluation of BC₂S₁ generation of plants derived from backcrossing jointed goatgrass to wheat carrying the gene for glyphosate resistance on either the A or B genome has shown that the gene is transmitted through pollen and that homozygosity for the resistance gene can be restored in the BC₂S₂ generation regardless of genome location of the resistance gene. A new GISH protocol has been identified that should allow completion of this study to determine the level of introgression of A and B genome chromatin that has occurred.

OBJECTIVES:

1. Determine if the glyphosate resistance gene located on either the A or B genome is transmitted through pollen.
2. Determine the frequency of retention of the glyphosate resistance gene in the BC₂S₁ and BC₂S₂ generations of lines that carry the glyphosate resistance gene on either the A or B genome.
3. Do cytological analysis of any line that appears homozygous for the glyphosate gene to determine if the stable resistance is due to a disomic alien chromosome addition or a translocation.

EXPERIMENTAL DESIGN:

Objective 1: Determine if the glyphosate resistance gene located on either the A or B genome is transmitted through pollen.

Plants from the BC₂S₁ and BC₂S₂ of the A genome and B genome populations that are known to carry the glyphosate resistance gene will be used as the male parent in crosses to susceptible jointed goatgrass. Reciprocal crosses using susceptible jointed goatgrass as the pollen parent and the resistant backcross line as the female parent will also be made to get the corresponding female transmission rate. Seedlings produced by these crosses sprayed at the four leaf stage with 24 oz./acre of Round-up Ultra. Resistance/susceptibility will be scored once the susceptible jointed goatgrass parent planted as a control shows symptoms.

Objective 2: Determine the frequency of retention of the glyphosate resistance gene in the BC₂S₁ and BC₂S₂ generations of lines that carry the glyphosate resistance gene on either the A or B genome.

Up to 30 BC₂ lines from each genome population will be screened for retention of the glyphosate resistance gene. Ten BC₂S₁ seedlings per replication and 4 replications per line will be sprayed at the 4 leaf stage with 24 oz./acre of Round-up Ultra. Plants will be scored for resistance based on survival. Plants that survive will be vernalized at 4 C for six weeks and then placed back in the greenhouse and allowed to mature and set seed to produce the BC₂S₂ generation. Forty BC₂S₁ lines will then be selected from the survivors for the next generation of evaluation. Evaluation of the BC₂S₂ generation will follow the same protocol that was used for the BC₂S₁ generation. Rate of retention of the A or B genome chromosome will be based on whether the frequency of retention of the glyphosate resistance gene decreases with each generation of selfing as predicted by Wang (2000).

Objective 3: Conduct cytological analysis of any line that appears homozygous for the glyphosate gene to determine if the stable resistance is due to a disomic alien chromosome addition or a translocation.

A subset of lines in the BC₂S₁ and BC₂S₂ generations found to be homozygous for the resistance gene will be analyzed cytologically to determine the source of the homozygosity. Mitotic analysis will be used to determine if the stable retention of the glyphosate resistance gene is due to a translocation (based on the homozygous line having 28 chromosomes) or a disomic chromosome addition (based on the homozygous line having 30 or more chromosomes). The presence of the A or B genome chromatin carrying the glyphosate resistance gene will be confirmed using genomic *in situ* hybridization (GISH) as described by Wang et al. (2000) and molecular marker analysis for molecular markers specific for the chromosomes (6A and 4B) that carry the glyphosate resistance gene.

PROGRESS TO DATE

Objective 1: Determine if the glyphosate resistance gene located on either the A or B genome is transmitted through pollen.

B genome: BC₂S₁ populations carrying the gene on the 4B chromosome have been tested for pollen transmission of the glyphosate gene. Pollen transmission was found to occur with all BC₂S₁ populations with the transmission ranging 0 to 100% within a population. Comparing results from reciprocal crosses indicated that there were differences in the transmission rate between male and female gametes but within each population at least one line showed homozygous transmission through both reciprocal crosses indicating stable introgression of the resistance gene.

A genome: BC₂S₁ populations carrying the gene on the 6A chromosome have been tested and while transmission has occurred through the male gamete it was at a lower percentage than that observed through the female gamete or compared to transmission when the gene was on a B genome chromosome. No homozygous transmission was observed when the

resistant jointed goatgrass was used as the male parent and only two of the populations had lines that showed homozygous transmission through the female gamete. The percent seed set and the percent viable seed also was lower than that observed for the B genome lines indicating that aneuploidy for the A and/or B genome chromosomes still existed in these lines and that the resistance gene may not have been introgressed into a C or D genome chromosome.

Objective 2: Determine the frequency of retention of the glyphosate resistance gene in the BC₂S₁ and BC₂S₂ generations of lines that carry the glyphosate resistance gene on either the A or B genome.

Homozygosity for the herbicide resistance gene was found in B genome BC₂S₂ plants at a higher frequency than in the A genome BC₂S₂ plants.. Cytogenetic analysis needs to be completed on these lines to confirm that the resistance is due to introgression and not maintenance of aneuploidy.

Objective 3: Conduct cytological analysis of any line that appears homozygous for the glyphosate gene to determine if the stable resistance is due to a disomic alien chromosome addition or a translocation.

Chromosome counts were initiated on the A and B genome lines to determine if the resistance observed is due to translocation or chromosome addition. Redirection of the principal investigator's time continues to delayed completion of this objective. A new shorter GISH protocol developed at the University of California Riverside should make it possible to complete this objective by mid-summer, 2009.

SUMMARY/CONCLUSIONS

Based on results to date it does not appear that genome placement of a transgene will prevent movement of the gene from wheat into jointed goatgrass. Results from this study show that in the second self-generation of both the A and B genome lines that transmission of the transgene could occur though differences existed on frequency of transmission depending on genome location. Cytological analysis of a portion of these lines needs to be done to confirm segregation data that there has been introgression of the gene in the majority of the B genome lines and whether chromosome addition or chromosome introgression is responsible for the resistance observed in in the A genome lines.

PUBLICATIONS in 2008: None

TECHNOLOGY TRANSFER ACTIVITIES:

Results from this study will be published in refereed journals and presented at regional and national meetings when completed. The results will also be incorporated where appropriate into extension management guides associated with the National Jointed Goatgrass Initiative.

BUDGET: No additional funds needed to complete the research.