Progress in mapping QTLs responsible for resistance to Sclerotinia head rot and stalk rot in two segregating sunflower populations

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ABSTRACT:
Two segregating F_2 populations were developed by selfing a USDA experimental hybrid (HA 441 x RHA 439) and a proprietary commercial hybrid (Proseed 9405). These two populations were grown in three research plots during the 2005 growing season. The first plot (Mapleton, ND) was inoculated with *Sclerotinia*-infested millet seeds for stalk rot development under natural conditions. Only eight and ten plants out of the 192 plants developed stalk rot in Proseed 9405 and HA 441/RHA 439 populations, respectively. The second plot (Fargo, ND) was inoculated with an ascospore solution (~5000 spores/ml) for studying head rot, which was developed under artificial misting irrigation. For disease reading, we used the zero (resistant) to five (susceptible) rating system. The population could be divided into roughly two groups (0 and 5), and with a few plants scored between 1 and 4. We tested 192 plants for each population. There were 105 and 70 plants with disease scores of zero in the HA 441/RHA 439 and Proseed 9405 F_2 populations, respectively. This could be the result of the fact that the USDA hybrid possesses more resistance genes than Proseed 9405, because the former produced more resistant progeny. Between each pair of parents, about 600 high quality polymorphic TRAP markers have been scored, which will be sufficient to construct linkage maps for QTL mapping. The third plot (Fargo, ND) was for generating F_3 seeds, and selfed seeds were harvested from 300 individuals for the replicated F_2-F_3 row field tests to locate QTLs conferring head rot tolerance in these two populations during the 2006 growing season. It will be possible to pyramid the tolerant QTLs into an elite USDA sunflower line if the two populations have different QTLs responsible for *Sclerotinia* tolerance.

INTRODUCTION

Sclerotinia sclerotiorum causes three, distinctly different diseases on sunflower: a stalk rot, incited by root infection (unique to sunflower), mid-stalk rot and a head rot caused by airborne ascospores. The genetics of resistance to these two diseases are completely different, and thus one cannot select for resistance to stalk rot and achieve head rot resistance concurrently. Since no completely resistant cultivated sunflower line exists, genetic analysis of *Sclerotinia* resistance focused on detecting genetic factors from partially tolerant breeding lines. These detected factors are called QTLs (quantitative trait loci), which can be defined as chromosomal regions that co-segregate with variation of the measurable trait of interest.

Several public researchers have made significant progress in mapping natural resistance to Sclerotinia in the past several years. Mestries et al. (1998) identified four QTLs for leaf resistance and two QTLs for head rot resistance. Gentzbittel et al. (1998) reported that *Sclerotinia* resistance showed multigenic inheritance, with one major QTL explaining about 50% of the genetic variation. The gene was located on linkage group one at the mapping position of a kinase-like locus. Bert et al. (2002, 2004) detected that a total of 15 resistant QTLs across several linkage groups, from 220 F2-F3 families derived from two inbreds, 'XRQ' and 'PSC8'. The range of phenotypic variability explained by each of these QTLs was from 7 to 41%. As expected, the map position of the QTLs for *Sclerotinia* resistance was the same in 2 years of tests. From the 150 F2-F3 families descended from 'FU' and 'PAZ2', they mapped seven QTLs for resistance to *S. sclerotiorum* terminal bud attack, each explaining less than 10% of the phenotypic variance, and four QTLs for head attack were identified, each explaining up to 20% of the variation. In summary, the resistance to this disease was governed by as many as 25 QTLs located on 14 of the 17 linkage groups. We have identified a DNA marker that is associated with *Sclerotinia* head rot susceptibility in a three-way cross F2 population (Chen et al., 2004).

This poster reports the progress of our project of mapping QTLs responsible for resistance to Sclerotinia head rot and stalk rot in two segregating sunflower populations during 2005.

MATERIALS AND METHODS

Two segregating F2 populations were grown in three research plots during the 2005 growing season. The first was developed by selfing a USDA experimental hybrid (HA 441 x RHA 439) and the second was from a proprietary commercial hybrid (Proseed 9405).

About 200 F2 plants were planted in two locations for disease tolerance study. For stalk rot, the population was planted in a commercial field about two miles north of Mapleton, ND. The plots were artificially inoculated with Sclerotinia-infested millet seed (Gulya et el., 2004) and disease developed under natural conditions. Only eight and ten plants out of the 200 plants developed stalk rot in the two populations, Proseed 9405 and HA 441 x RHA 439, respectively. For head rot the population was planted in Fargo, ND, in our mist irrigation nursery. At 25% bloom stage, about 5 ml of ascospores (~5000 spores/ml) were inoculated onto sunflower heads. Disease was developed under artificial misting irrigation commencing immediately for 5 min every half hour, 24 h/day for 3 wk following inoculation. For disease reading, we used the 0-5 rating system (Van Becelaere and Miller, 2001).

For screening the polymorphism levels among the parents, DNA samples were extracted from seedlings grown in the greenhouse with the Qiagen DNeasy Plant Mini. For marker detection, we will follow the established TRAP protocol (Hu and Vick, 2003) in our laboratory.

RESULTS AND DISCUSSION

Response to Sclerotinia head rot of the segregating population in the field during 2005

The distribution of the disease scores of the two populations is shown on Figure 1. The distribution does not fit the expected pattern of a quantitative trait. The population could be divided into roughly two groups, susceptible (0) and resistant (5), with a few plants rated between 1 to 4. The resistant group could contain two types of plants, one with resistant genes and the other without resistant genes. The decrease of the middle class could be due to excessive rain in the 2005 growing season. In the Fargo area, the normal precipitation is 19 inches. In 2005, there was 30 inches of rain, 33% more than a normal year. The resulting higher humidity favored head rot development and produced more susceptible plants. It seems that the USDA hybrid (HA 441/RHA 439) possesses more resistance genes than Proseed 9405 because the former produced more resistant progeny in the segregating F_2 population.

Polymorphism among the two pairs of parents at the DNA level

We have tested the polymorphism levels among the parents of the two populations. The number of polymorphic markers amplified by the TRAP marker technique ranged from zero to five per primer combination. This low level of polymorphism is expected since the hybrids are intervarietal crossed. Figure 2 shows a portion of a gel image displaying DNA fragments amplified by eight primer combinations from the four parental lines and the two F_1 hybrids. About 600 high quality polymorphic markers have been scored between each pair of parents, which will be sufficient to construct linkage maps.

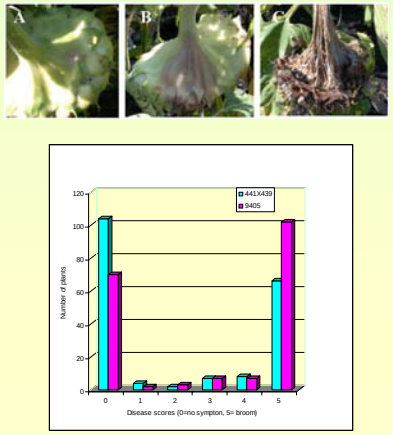

Figure 1. Upper panel: Three sunflower heads with different head rot disease scores (A: no symptom, score=0; B: 50% destroyed, score=3 and C: 100% destroyed, score=5);
Lower panel: Distribution of *Sclerotinia* head rot scores (0 to 5) of plants in the two F_2 populations in the 2005 growing season in Fargo under misting conditions after artificial inoculation.

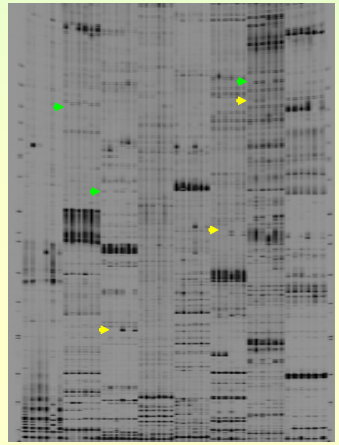

Figure 2. TRAP profile of eight primer combinations of a population of the four parental lines and two hybrids (HA 441, RHA 439, Hybrid HA 441 x RHA 439, A line, R line and Hybrid Proseed 9405, Lanes 1 to 6 in each panel from the left). The labeled primer is TRAP03-700 (sequence: 5'-CGTAGCGCGTCAATTATG-3') and the fixed primers are A10B18a A10B18b, A11D14a, A11D14b, A11D14F1, A11D14F2, A11I24a and A11I24b, for panels one to eight from the left, respectively. Size standards on each side are from 50 to 700 bases. Green arrows indicate the same polymorphic markers in the two populations and yellow arrows indicate the polymorphic markers in one of the two populations.

Plans for 2006

In 2006, we will continue the genetic analysis of Sclerotinia resistant QTLs in the two populations by using the F_2:F_3-row populations, which have proven very efficient for estimating additive and dominant gene action for QTL analysis. We will conduct replicated field trials for Sclerotinia head rot resistance at different locations. We will inoculate the F_3 heads twice within a week to minimize any escape events and change the scoring method by measuring the size of the rot area of each head. It will be possible to pyramid the tolerant QTLs into an elite USDA sunflower line if the two populations have different QTLs responsible for *Sclerotinia* tolerance.

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