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Tests of Linkage Between Necrotic Root Locus *Rn1* and Homozygous Chromosome Translocation KS172-11-3

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Introduction

Twenty linkage groups have been identified in soybean that are designated as the classical genetic linkage (CGL) groups. Several linkage groups have only two loci, and two linkage groups have nine loci.

Six translocation (interchange) genetic stocks have been cytologically characterized in soybean (Mahama et al., 1999). These chromosome translocations have been used to place gene order for mutants of CLG 6 and 8. In fact, these translocations indicated that linkage groups 6 and 8 were the same linkage group (same chromosome) (Mahama and Palmer, 2003). Thus linkage group 6 has been merged with linkage group 8, and is no longer considered a separate linkage group.

A small F₂ population (48 plants) suggested linkage of KS172-11-3 (homozygous chromosome translocation) with necrotic root, *Rn1* locus (A. A. Mahama, unpublished results). One of the KS172-11-3 chromosome translocation breakpoints was linked to mutants on CLG 8 (Mahama and Palmer, 2003), which is molecular linkage group (MLG) F. The location of the other breakpoint is not known. Our objective was to test for linkage between the necrotic root locus (*Rn1*) and chromosome translocation KS172-11-3.

Materials and Methods

Plant materials

The KS172-11-3 chromosome translocation line was crossed as female parent with grafted plants of T328 (*rnl, rnl*) (normal rootstock, necrotic root scion) as male parent. The F₁ plants were about 50% pollen and ovule sterile (semisterile) which indicated successful hybridizations. Necrotic root mutant plants are usually lethal when field-grown. Thus about half of the F₂ seed from self-pollination of each F₁ plant was planted in the field (summer, 2006). The homozygous

recessive necrotic root plants died. The surviving F₂ plants were individually identified and pollen fertility/sterility determined by I₂KI staining. If all the pollen grains from a plant were well-stained, the plant was classified fertile. This means that the fertile F₂ plants were either homozygous normal chromosomes or homozygous translocated chromosomes. An F₂ plant with about equal numbers of well-stained pollen and aborted pollen grains was considered heterozygous for the chromosome translocation. All the F₂ plants were threshed individually and 20 F₃ seed were germinated in a growth chamber. After eight days, the seedling roots were examined. The genotype of the F₂ plants was determined to be homozygous dominant for normal root (*Rn1*, *Rn1*) or heterozygous (*Rn1*, *rn1*) based upon the segregation of normal and necrotic root phenotypes.

The remainder of the F₂ seed from self-pollination of each F₁ plant was germinated in a growth chamber. Only the necrotic root seedlings were saved and transplanted to pots in the USDA greenhouse (summer, 2006). These F₂ plants were individually identified and pollen fertility/sterility determined by I₂KI staining, as was done with the non-necrotic root field grown plants. Based upon pollen grain staining phenotypes, the necrotic root plants were classified as either completely fertile or heterozygous for the chromosome translocation; ie, about 50% pollen and ovule sterile.

Results

Linkage test

A total of 221 field-grown non-necrotic F₂ plants were classified for pollen/sterility by I₂KI staining. There were 107 fertile: 114 semisterile plants which was a good fit to the expected 1:1 ratio; $\chi^2 = 0.22$, $P = 0.64$. In the USDA greenhouse, 74 necrotic root plants gave 35 fertile: 39 semisterile plants. This was a good fit to the expected 1:1 ratio, $\chi^2 = 0.22$, $P = 0.64$. The combined field and greenhouse data gave a good fit to the expected 1:2:1:2:1:1 ratio; $\chi^2 = 0.70$, $P = 0.98$ for the combined segregation of normal and necrotic root and for the chromosome translocation.

Unexpectedly, seven greenhouse-grown necrotic root F₂ plants were highly male sterile, as determined by I₂KI staining. Repeated sampling of these seven plants on different days, gave pollen sterility values between 50% to near 100%. A few selfed seed were harvested from each of the seven plants grown in the summer of 2006, in the USDA greenhouse. These seeds were planted in January of 2007, in the USDA greenhouse and the plants flowered in April, 2007. All seven plants had both fertile pollen progeny and semisterile pollen progeny.

Discussion

Linkage test

The *rn1* locus was not linked to either of the breakpoints in chromosome translocation KS172-11-3. The *rn1* locus is on MLG G (R. G. Palmer et al., submitted). The KS172-11-3 showed linkage with mutants on CLG 8 (Mahama and Palmer, 2003), which is MLG F. Additional

linkage studies with KS172-11-3 are necessary to determine the other chromosome involved in the translocation.

The seven necrotic root greenhouse F₂ plants that had varying levels of sterility greater than 50% were all heterozygous chromosome translocation plants; ie, semisterile plants. Also only 7 of the 39 semisterile necrotic root plants expressed this higher level of pollen sterility and only on certain days. The necrotic root plants are weak plants. Thus it was not possible to collect floral buds to check meiosis. The pollen grain morphology of the plants with very high levels of sterility was similar to pollen morphology from homozygous asynaptic and desynaptic soybean mutants. An explanation for this observation awaits more detailed studies.

References

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- Mahama, A. A. and R. G. Palmer. 2003. Translocation breakpoints in soybean classical genetic linkage groups 6 and 8. *Crop Sci.* 43:1602-1609.