ROLLED TOWEL BIOASSAY TO IDENTIFY ROUNDUP READY® TRAIT IN COTTON SEED

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Abstract

Breeding and quality assurance programs need accurate and cost effective methods to test for the presence of target and non-target genes in transgenic cotton varieties. The objective of this paper is to detail the Roundup Ready® (RR) Rolled Towel Bioassay procedure for cotton seed and demonstrate the reliability of this test in identifying the RR trait in cotton seed. Planting media is prepared by adding 2250 ml of a 0.30% Roundup Ultra® solution to 1000 g of germination towels. Fifty seeds are planted in germination towels. Towel rolls are placed in a vented plastic container, and then vertically positioned in a 30°C germinator for seven days. Seedlings are evaluated as either Roundup tolerant, susceptible, dead, or hard seed. Roundup tolerant seedlings produce normal seedlings with all essential structures, although growth is usually inhibited by 25-30% compared to a standard germination test. Susceptible seedlings have a shortened hypocotyl-radicle length with characteristic black lesions on hypocotyls. RR Rolled Towel Bioassay results averaged 99.80% purity compared to 99.84% for RR lateral flow strip tests, across 678 truckload samples. The RR Rolled Towel Bioassay is an accurate and cost effective way to detect the presence of the RR trait in cotton seed. Future work will include a referee project to determine repeatability between laboratories and error rates.

Introduction

Many methods exist to test for the presence of any given transgenic trait. These methods may include immunoassays, such as lateral flow strip tests and ELISA's, DNA extraction/PCR techniques, and bioassays. Bioassays are routinely used to determine purity of herbicide resistant traits, including RR corn and RR soybean (AOSA Cultivar Purity Testing Handbook #33, 1991). Monsanto conducted preliminary work on a bioassay for RR cotton. Stoneville Pedigreed Seed Company has refined the method over the past three years, and has successfully utilized the RR Rolled Towel Bioassay in their quality assurance program. The objectives of this paper are to: 1) illustrate the RR Towel Bioassay procedure for cotton, and 2) provide evidence of reliability of testing for presence of RR gene in cotton seed.

Materials and Methods

Planting media is prepared by adding 2250 ml of a 0.30% Roundup Ultra® (3 ml Roundup Ultra to 1 liter of water) solution to 1000 g of germination towels (Anchor Paper Company, 25 cm x 38 cm, 38#, unbleached) in a 20-liter plastic container. The container is covered and the towels and solution are allowed to equilibrate for at least one hour. Fifty seeds are planted per towel roll sub sample, with two towels below seeds and one towel placed on top of seeds. Towels are rolled and rubber bands are placed around the middle of the roll. The process is repeated until desired number of sub samples is planted.

Towel rolls are placed in a plastic container, such as a No. 7 Rubbermaid 3.78 liter container (34 cm x 34 cm x 25 cm) with two 6-mm air vents in lid. This container can hold up to 12 rolls plus one check sample roll. Container is placed upright in a 30°C germinator for seven days. A known susceptible check, such as ST 474, is always planted to verify that the Roundup Ultra solution was mixed properly and to provide a visual standard for susceptible seedlings. One towel of a check sample is included in each container. Towel rolls may need to be re-hydrated slightly on about the fourth day of the test cycle.

At the end of the seven-day test duration, seedlings are evaluating as either Roundup tolerant, Roundup susceptible, dead, or hard seed. Roundup tolerant seedlings produce normal seedlings with all essential structures, although growth is usually inhibited by 25-30% compared to a standard germination test and minimal secondary root growth occurs. Roundup susceptible seedlings have shortened hypocotyl-radicle lengths with obvious black lesions on hypocotyls (Figure 1). Dead seed fail to produce essential roots and hypocotyls, which are soft, water-soaked and discolored. Hard seed do not imbibe moisture from towels.

The percent RR purity is calculated by dividing the number of Roundup tolerant seedlings by the number of seed sprouted, and then multiplying by 100. Dead or hard seed are not counted in the number of seed sprouted. For example, if a 400 seed sample is planted, and 373 seedlings are RR tolerant out of 376 that sprouted, then 373/376 x 100 equals 99.2% RR purity.

Results

RR Rolled Towel Bioassay results were very similar to lateral flow strip tests, which are designed to detect the CP4 EPSPS protein produced by a gene derived from *Agrobacterium* sp. strain CP4 (Cotton Lab Test Kit CKRCOT, part number 7902000, Strategic Diagnostics Inc., Newark, DE). Six-hundred seventy-eight truckload samples produced the same results with both the RR Rolled Towel method and the lateral flow strip method. RR Towel Test averaged 99.80% purity compared to 99.84% for RR strips, ranging from 97.2 to 100% and 98 to 100%, respectively. The standard deviation was lower in the RR Towel Test (0.369%) compared to RR strips (0.585%).

Discussion

The RR Rolled Towel bioassay is a reliable and repeatable test to determine the presence of the RR trait in cotton seed. It is also very cost effective, which facilitates testing more seeds per sample and a greater confidence level in results, when compared to lateral flow strip tests or PCR methods. A second benefit of the RR Rolled Towel Bioassay is that the test provides an estimate of viability. It is important to note that about 25-30% growth suppression occurs compared to germinations with no Roundup Ultra introduced in the system. Two limitations of the RR Rolled Towel Bioassay are: 1) a seven-day test duration, and 2) non-germinating seed are not assayed. The main advantage of lateral flow strip tests is speed, as RR purity results can be obtained in about 30 minutes.

Summary

The RR Rolled Towel Bioassay is an accurate and cost effective way to detect the presence of the RR trait in cotton seed. Stoneville Pedigreed Seed Company, some other commercial seed companies, and private and public seed-testing laboratories currently use this method in breeding and quality assurance areas with confidence. Future work will include a referee project to determine repeatability between laboratories and error rates. Submission for inclusion in AOSA Cultivar Purity Testing Handbook is in preparation.

References

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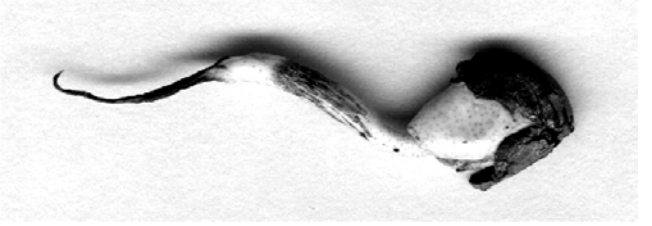

Figure 1. Roundup susceptible cotton seedling with shortened hypocotyl-radicle length and characteristic black lesions on hypocotyl at final evaluation in Roundup Ready Rolled Towel Bioassay.