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Taifun Soy Info

Specialist information for soy producers and processors

Taifun Zentrum für Sojaanbau

Testing soybeans for genetic engineering with rapid strip tests?

Introduction

Since the first transgenic crops were approved in 1996, genetically modified organisms (GMOs) have become increasingly important. Genetically modified soy was first cultivated in 1997 and now accounts for 80% of total soy cultivation in the USA. In Argentina and Uruguay, it even accounted for almost 100% of the cultivated soy in 2013.

Also for local growers it is nowadays important to be able to test the crop for possible contaminations as quickly and cheaply as possible. In the case of conventional crops, the legal tolerance limit for contamination by components from approved GMOs is 0.9% (only accidental and technically unavoidable components). This tolerance limit also applies to organic products. Yet, during the organic monitoring program in Baden-Wuerttemberg, GMO contents exceeding 0.1% were never found in organic maize or soy products. It is therefore assumed that higher values can be regarded as "technically avoidable" (CVUA Freiburg, 2013).

In addition to the PCR method, which must be performed in the laboratory by specialised personnel, there are also rapid strip tests that can be performed directly in the field or during soy processing. These tests detect the proteins formed by the modified genetic material and optically indicate a positive test result.

There are now five different, approved changes ("events") in the genetic makeup of soybeans that cause resistance to certain herbicides or insects:

- LibertyLink®: soybeans with resistance to Bayer CropScience's herbicide Liberty® (A5547-127 (LL55))

- Roundup Ready® soybeans with resistance to Monsanto's herbicide Roundup® (GTS 40-3-2 (RR1)) - the world's most widespread herbicide

- Roundup Ready® soybeans with resistance to Monsanto's herbicide Roundup® (MON89788 (RR2))

- DP356043: Soybeans with Roundup® resistance from Pioneer

- MON87701: Soybeans with resistance to various lepidopteran pests (moths) from Monsanto

For the first three events there are common strip tests. The other two are currently only detected by PCR analysis.

Costs

The strip tests are cheaper and faster than the usual PCR tests. For example, 100 test strips for LibertyLink® soybeans from Romer Labs® cost 499 €, strips for Roundup Ready® soybeans cost 399 €. Strip tests for combined testing for RR1, RR2 and LL55 cost about 140°€ for 20 strips. However, as different detection limits apply for the individual events, such combinations must be viewed very critically. A PCR analysis for the events LL55, RR1 and RR2 costs about 170 €.

Accuracy

If you want to be 99% sure that the tested lot does not contain more than 0.1% Roundup Ready soybeans, five subsamples of 1000 soybeans must be tested (see table 1). In this case 5 x 7.00 € = 35.00 € have to be calculated for the test strips. On top of this, the cost of grinding 5 x 200 g soybeans must be added. For a 95% certainty, three subsamples must be examined. Furthermore, the cauliflower mosaic virus (CaMV) cannot lead to false positive results in protein tests. In PCR screenings, the 35S promoter that originates from the CaMV is tested for, but this sequence only exists at DNA level, so protein tests are not affected.

In a very detailed study, strip tests were tested under field conditions. During the study, soybean samples containing varying amounts of genetically modified grains have been blindly tested at 20 different grain processing companies, which also routinely carry out strip tests. In order to save time and costs, only one test was carried out per sample. The results showed that under such realistic conditions, GMO contamination could only be detected effectively and reliably (without false results in all companies) from 10 % upwards using strip tests. The lowest amount of false results (6.7 %) for impurities below 10 % were found in the samples containing 0 % impurities. Apart from the insufficient sample size and repetition, the human factor in sample processing was highlighted as the biggest error in this study (Fagan et al. 2001).

Coarse contamination, such as that caused by a complete truck with contaminated goods, can be avoided by means of a quick strip test in harvest stress. However, the accuracy that can be achieved with a quick test must be considered here. Also breeders who want to exclude gross inputs of GMO contaminations can test their harvested material quickly and cost-effectively using strip tests.

Advantages and disadvantages

The advantages of the strip tests are obvious: They can be carried out without a laboratory and therefore by anyone - at the elevator or directly in the field. The results are quickly available. In addition, strip tests are cheaper than PCR tests, provided that traces of GMOs in the range < 0.1% are tolerated

A major disadvantage of strip tests are the detection limits: if the accuracy and sensitivity should match a PCR analysis, a large number of tests and a large amount of sample material are required, driving up costs. In Germany, according to the Official Collection of Test Methods pursuant to § 28b Gentechnikgesetz (Genetic Engineering Act), only PCR methods are approved for the verification of the GMO-free status of seeds.

It should further be noted that strip tests sometimes only have a shelf life of four to six months. For occasional tests, the purchase of packages containing at least 20 strips can be uneconomical for this reason alone.

Further information

Implementation:

The sample is finely ground (e.g. with an electric coffee grinder) so that no grains are left, and water is added. The mixture of soy-flour and water is shaken and left to stabilize. After a short time, a liquid supernatant is formed at the top. Approximately 0.5 ml of the supernatant is now removed with the pipette contained in the kit and added to the tube (also contained in the kit). The test strip is then held in this tube for approx. 5 minutes. Now the result can be read from the strip. If one line (= control line that the test itself has worked) shows up, the result is negative, if two lines show up the test result is positive (see fig. 1). The waiting time of 5 minutes should not be exceeded as the results are no longer meaningful after 60 minutes. In any case, the tests should only be carried out by trained personnel in order not to obtain false results.

Functioning of a PCR and the detection method used in strip tests:

In a PCR, primers, a heat-resistant polymerase, deoxyribonucleoside triphosphates as building blocks for the new DNA and a buffer are added to the sample material containing the DNA to be amplified. The mixture is first heated to 94-96 °C to break hydrogen bonds and separate the DNA double strands. The sample is then cooled down again and kept at a primer-specific temperature. The primers determine the starting point for the new synthesis of DNA and are adapted to the sequences where they are supposed to "attack". The polymerase is then applied and connects the opened sequences with the corresponding nucleotides. This procedure is repeated 20-50 times to produce the appropriate amount of the specific DNA section. The DNA sections produced in this way are then identified based on their size (number of base pairs) by means of gel electrophoresis. The DNA is placed in an agarose gel to which a voltage is applied. The DNA strands now migrate through the gel, with shorter fragments being faster than longer ones. For comparison, DNA pieces of known size are applied ("DNA ladder"), which run along with the samples. If the piece you are looking for is present, a band will appear in the gel at the corresponding position

The immunobiological method used in the strip tests works with antibodies. The sample is mixed with an appropriate buffer and then moves along the strip to a layer which is coated with antibodies. If the GMO protein is present in the sample, it binds specifically to an antibody. This complex then moves further until it binds to a second antibody and stops. This causes a line to appear indicating the presence of genetically modified material.

The advantages of PCR, besides the high sensitivity, is also the small sample size required (due to the sensitivity). Furthermore, DNA molecules are stable and, in contrast to proteins, cannot be denatured as easily, which is particularly important for detection in processed products. The disadvantages of the PCR are the costs, the time until results are available (approx. 1 day) and the fact that it cannot be performed in the field or at the elevator. Furthermore, the high sensitivity can lead to false positive results (Adugna and Mesfin, 2008).

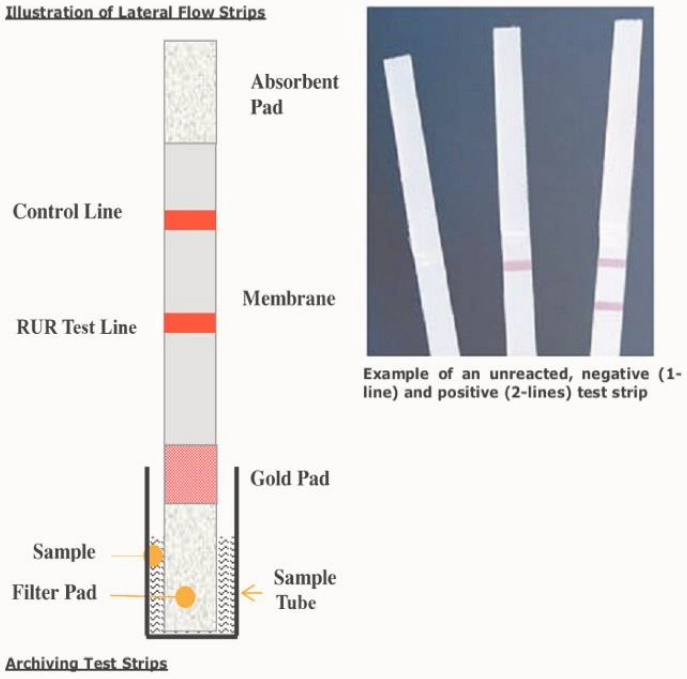

Figure 1: Schematic diagram of a RuR test strip (left) and examples of an unreacted, negative (one line) and positive (two lines) test strip (source: Romer Labs®, Inc., 2010).

Quantity subsamples of 1000 grains each	Maximum percentage of RR-soybean, with a sub-sample size of 1000 grains at five different confidence intervals (%)				
	50	75	90	95	99
1	0.070	0.139	0.231	0.300	0.461
2	0.035	0.070	0.116	0.150	0.231
3	0.024	0.047	0.077	0.100	0.155
4	0.018	0.035	0.058	0.075	0.116
5	0.014	0.028	0.047	0.060	0.093
6	0.012	0.024	0.039	0.050	0.077
7	0.010	0.020	0.033	0.043	0.066
8	0.009	0.018	0.029	0.038	0.058

Table 1: Maximum percentage of RR soybeans contained in 1000 grain subsamples at different confidence intervals (Source: Romer Labs®, Inc., 2010)

Sources

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For comprehensive information on all aspects of soy cultivation visit:

www.sojafoerderring.de

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