Gene Flow from Transgenic Roundup-Ready Soybean to wild Soybean

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Abstract

The study was conducted in 2000 to 2003 in Nanjing, the target of the study was we want to find the possibility of gene flow between roundup ready soybean and their native crops, include wild soybean. After the experiment was finished, we can find that one plant of wild soybean had been got the round-up ready gene which was flow from the original roundup-ready soybean, even the flowing rate was very low. So the scientists and the government should put more eyes on how to protect the original wild soybean germplasm in China in case of the gene flow from the native crops, if we want to plant roundup ready soybean in China.

Key Words

Wild soybean, roundup-ready gene, gene flow

Introduction

Weeds are major agricultural pests that can devastate a crop if not properly controlled or managed. In China, about 1,500,000 t's soybean is lost every year, about 15% of the total yield. In USA, Basil, Agential, farmers use roundup to get rid of the weeds because they plant

roundup-ready soybean, this kind of soybean got roundup-ready gene by transgenic gene method, so farmers can easily to get rid of the weeds compare with that of in China .Every year we import more than 20 million t's soybean product from USA, But we don't plant roundup-ready soybean in China.

Biotechnology may alter our future in many ways. One is by altering crop species to provide new traits that have been impossible. Imparting herbicide resistance to normally herbicide-susceptible crops to produce herbicide-resistant crops has been the most intensively exploited area of plant biotechnology. Glyphosate (another name roundup) is a used in leaves, universality and whole-killed (no selection) herbicide, the mechanism of the roundup is specially control the activity of the EPSPS in the plant or bacteria, one of the enzyme to compose of the fragrnant amino acid, the activity center of the enzyme situate in the chloroplast. If the activity of EPSPS is restrained, the composed of the amino acid is stopped, and the plant would be died. Because all the plants need the EPSPS to catalyse the compose of the amino acid, so if the plant was sprayed with the roundup, all the plants were died. The main method of the roundup-ready soybean genetic engineering is to use the target albumen that decorates the function of the herbicide; made that kind of soybean doesn't sensitive to the herbicide, means made the EPSPS produce more target albumen or take out the coding dot of the albumen. Until the June of 2001, about 70% of the soybean in USA, 95% soybean in Agentia, 30% soybean in Basil is roundup ready soybean.

They are different paths of gene flow in the crops. Jenczewski et al. (1999) conducted an experiment compare the pattern of population differentiation within and among the wild and cultivated gene pool with respect to both allozymes and quantitative traits .They found that populations E064 and E147 could be differentiated from the typical wild populations nor from the cultivated landraces with respect to allozymes. This result strongly suggests that gene flow occurred from crop to wild populations. Andrew et al.[4]investigated the rate and extent of gene flow from cultivated sorghum (Sorghum bicolor L. Moench.) to "johnsongrass" , in a preliminary screen of 11 S. bicolor accessions with 39 RFLPs , he found considerable variation among the races of S. bicolor. Although the specific S. bicolor accession(s) which gave rise to johnsongrass was unknown. A fifty-year- old study done by Mitten[2] indicated

herbicide resistance gene can flow from the herbicide resistance rice to wild red rice even rice-rice out crossing rates were less than 0.5%. Roberto [5] did two different experiments using AFLP markers. The first study was done on 70 genotypes of wild and cultivated common bean from Mexico. The second was on 24 populations of wild and cultivated common bean collected on a single plant basis at different levels of sympatry. The evaluation of gene flow was based on the population structure analysis. The results obtained show that, in general, gene flow between wild and cultivated common bean populations is an event that rarely occurs, as could be expected in the presence of a low out crossing rate and with divergent selection between the wild and domesticated environments.

A lack of out crossing was observed by Russian scientists on the gene flow from soybean to the wild soybean [8].They kept track of the roundup-ready soybean pollinated to wild soybean, and they were suspicious on two seeds. However, the F1 had no round up-ready gene. Papa and Gepts (2003) analyzed the genetic structure of wild and domesticated common bean from Mesoamerica at different geographical levels to test the hypothesis of asymmetric gene flow and to investigate the origin of weedy populations. They showed by admixture population analyses that gene flow was about three- to four-fold higher from domesticated to wild populations than in the reverse direction, yet the rate of gene flow was very low.

Other research work was discussed on the influence of the environment on the gene flow. RRS may become a weed itself and contaminate to the other crops, especially in rotation. This is a particular pertinent question in cropping systems where one crop is resistant to a common used herbicide in the rotation crop. Problems might also develop if all crops in the rotation have the roundup-ready gene and confer resistance to the same herbicide. In such cases, rotational and weed management plans would need to be carefully considered to avoid weed population shifts or evolution of herbicide resistant weed iotypes. An additional potential problem related to the use of RRS is severe crop injury resulting from drift or unintentional application of a nonselective herbicide to a non-RRS [1, 3].

In order to find the gene flow between the roundup ready soybean and the soybean , wild soybean, we do some experiment as below.

Materials and Methods

The experiment was started at the June of the 2000, we selected Nanjing (south of Jiangsu), Dafeng (nearby the sea), Xuzhou (north of Jiangsu) as the three situation, represent the different climate of the province.

The roundup ready soybean line is ARS08 introduce from the Nedia Company, Agential. We use jsy008 (wild soybean), xudou08 (xuzhou), sudou04 (Dafeng and Nanjing), roundup was buy from the department of Monsanto company located in China.

In the middle of the field of each place, we planted the AGS008(white flower) in five circles, with the radius of 0.5m to 2.5m.Then any other legume crops(purple flower) were planted in the eight directions, from the space 5m to 50m,each plot has 25 plants. At the same time, we planted the RRS and the common soybean in the field by three repetitions, each repetition with six lines and 25 plants in each line, use different kind of herbicide to spray in the field, about three weeks later we calculate the weeds' kind and the fresh weight. Another experiment is we spray the roundup-ready directly to the common soybean in different concentration, and observe what is the dead concentrion of the roundup to the soybean.

After finished all the experiment in this year, we sprayed the roundup-ready to all the weeds in the experiment in order to get rid of the gene flow weeds.

The second year, all the soybean and wild soybean were taken from every where to Nanjing and signed the place and the lines we got last year, and planted them in the field. After one month, we sprayed high concentration of the roundup (about 3 times compare with the normal dead concentration) in the plants, and selected the surviving leaves to the lab for DNA extraction. And wrote down the number of the lines and the situation which we got last year in order to find the distance of gene flow.

The third year, we planted the surviving beans' plants in the field and checked the flower color, the fourth year we did the same experiment again.

We used CTAB for the DNA extraction, and AFLP for PCR reaction, a pair of primers were composed by the Boya gene company in Shanghai and the sequence was below:

EPSP125 cctttaggatttagcatcagtg

EPSP1210 ggagttcttccagaccgttcat

All the DNA extraction and PCR reaction was done in the gene lab of the institute of genetic and physiology, Jiangsu Academy of Agricultural Sciences.

Result and conclusion

There are several results as below:

The experiment of the concentration of the roundup

In order to found the dead concentration of the beans', we used 10% of roundup for 5 levels to spray in the plants; 7.5l /ha,10l/ha,12.5l/ha,15l/ha,17.5l/ha.The herbicide was sprayed at the three leaves' stage of the beans'. As a result, all the soybeans die at the level of 10l/ha, all the beans include weeds were die at the level of 12.5l/ha, so we can use 12.5l/ha as a dead concentration for all the beans'.

The experiment of the cross possibility between soybean and wild soybean

An experiment of the cross possibility between the RRS and the common soybean, the RRS and wild soybean were made in the field, and the succeed rate between two soybeans was 23.8%, and the succeed rate between the RRS and wild soybean was 12.3%, even it was lower compared with the former.

The infection of the roundup-ready soybean to the weeds

From the experiment we can know when we planted RRS and sprayed roundup, the weeds' kind would be changed, the multi-years' weeds like bulrush would be increase compare with the field which were planted common soybean sprayed ethylation, and it seemed difficult to get rid of bulrush in the field if they became more and more even through the fresh weight of the weeds had no prominence difference.

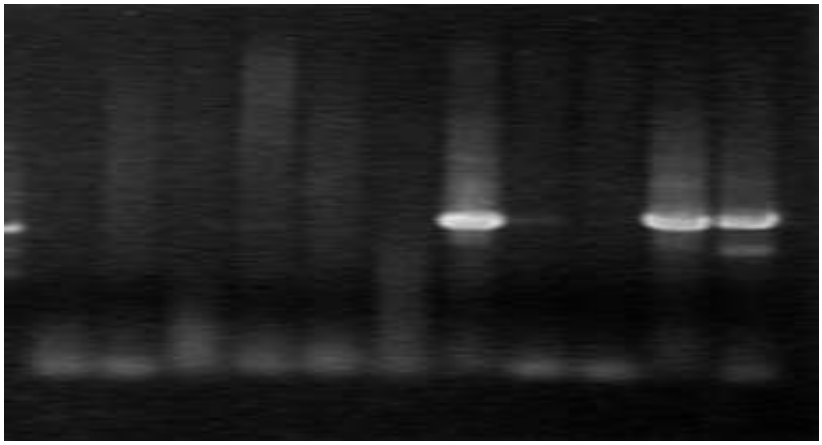
The surviving soybean and wild soybean

We got 12038 soybean seeds and 38690 wild soybean seeds from the three experiment fields, and when we planted them next year and after sprayed the roundup, 220 plants of the soybeans' leaves were yellow (no green leaves surviving), 661 wild soybean plants' leaves

were yellow and another one plant of the leaves were green, this plant we got from the Dafeng, near by the sea side, and more insects were there. The furthest place of the surviving beans we got was 10 m from the RRS; most of them were 5 m far.

PCR reaction of the gene flow from the RRS to the wild soybean

From the map above, we could see that the wild soybean had the same band like the RRS. And we planted the surviving wild soybean the next year and the next two year together with the original wild soybean, the flowers' color of the gene flow wild soybean were purple at the first year and had a rate of 3 purple: 1 white after the second year, compare with all the flowers got same purple color in two years, in this way we can make sure that the gene was flow from the RRS to wild soybean.



map 1: PCR reaction of the gene flow wild soybean (No. 7 is RRS, No. 10 and 11 was the leaves of the gene flow wild soybean ,took from the same plant, any others were the original wild soybean.)

Discussion

Since China is the center origin of the wild soybeans, the alien gene flow to them may create super weeds if roundup-ready soybean was planted every year. The government and the farmers must consider this problem if they want to plant roundup-ready soybean in China. It seemed the gene flow was because of the bees or insects because the cross possibility was also low. The gene flow to the wild soybean and not to the common soybean may be the wild

soybean's plant was higher and had more flowers compare with the common ones, and it seemed more attractive to the bees or insects.

Any other surviving soybean and wild soybean plants may be got some fragments from the RRS. The band was not clear in PCR. For the kinds of the weeds were changed and it seemed more difficultly to get rid of that kind of weeds, so we should find a good way to get rid of them.

Scientists and the goverment should put more eyes on how to protect the original wild soybean germplasm in China in case of the gene flow from the native crops, if we want to plant roundup ready soybean in China.

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