



(86) Date de dépôt PCT/PCT Filing Date: 1994/08/05
(87) Date publication PCT/PCT Publication Date: 1995/02/23
(45) Date de délivrance/Issue Date: 2005/03/29
(85) Entrée phase nationale/National Entry: 1996/02/09
(86) N° demande PCT/PCT Application No.: EP 1994/002598
(87) N° publication PCT/PCT Publication No.: 1995/005082
(30) Priorité/Priority: 1993/08/12 (P 43 27 056.5) DE

(51) Cl.Int.⁶/Int.Cl.⁶ A01N 57/20
(72) Inventeur/Inventor:
DONN, GUNTER, DE
(73) Propriétaire/Owner:
HOECHST SCHERING AGREVO GMBH, DE
(74) Agent: SMART & BIGGAR

(54) Titre : METHODE POUR AMELIORER LE RENDEMENT DE PLANTES CULTIVEES RESISTANT AUX HERBICIDES
(54) Title: METHOD OF IMPROVING THE YIELD OF HERBICIDE-RESISTANT CROP PLANTS

(57) **Abrégé/Abstract:**

Method of improving the yield of crop plants which are resistant to glutamine synthetase inhibitors, in which plants are treated with glutamine synthetase inhibitors at low application rates, and to the use of glutamine synthetase inhibitors for improving the yield of transgenic crop plants.

Abstract of the Disclosure

Method of improving the yield of herbicide-resistant crop plants

Method of improving the yield of crop plants which are resistant to glutamine synthetase inhibitors, in which plants are treated with glutamine synthetase inhibitors at low application rates, and to the use of glutamine synthetase inhibitors for improving the yield of transgenic crop plants.

75749-16

- 1 -

Description**Method of improving the yield of herbicide-resistant crop plants**

The compound glufosinate (glufosinate-ammonium, ammonium DL-homoalanin-4-yl(methyl)phosphinate, Schwerdtle et al., Z. Pflanzenkr. Pflanzenschutz., 1981, Special Edition IX, page 431) acts as a glutamine synthetase (GS) inhibitor since it is a structural analog of glutamic acid. GS plays a central role in the metabolism of all plants. It is responsible for the detoxification of NH_3 , and, as a consequence, all terrestrial plants are damaged severely or destroyed after application of glufosinate since the assimilation of ammonia is inhibited.

Plants which are resistant to the herbicidal activity of GS inhibitors were successfully produced by transferring and expressing a glufosinate acetyltransferase gene isolated from from strains of Streptomyces which produce bialaphos (phosphinothricin-alanyl-alanine) (EP-B1-0 242 236 and EP-B1-0 257 542). Stands of such transgenic, herbicide-tolerant crop plants can be kept weed-free in an efficient manner by post-emergence treatment with glufosinate.

Unexpectedly, field trials with such transgenic plants showed that the glufosinate-treated plants give a measurably higher yield than untreated plant stands. This higher yield is not a result of the excellent weed control by glufosinate and its complete compatibility with the stands of transgenic crop plants, but a positive effect of the herbicide treatment on growth and yield.

The invention therefore relates to a method of improving yield of crop plants which are resistant to glutamine synthetase inhibitors, which comprises treating the crop plants with at least one glutamine synthetase inhibitor at an application rate which is not harmful to the plants.

In particular, the invention relates to a method in which glutamine synthetase inhibitors are employed for a yield-improving treatment of plants which are protected against the herbicidal activity of the glutamine synthetase inhibitor by expression of an N-acetyltransferase gene.

The invention furthermore relates to the use of a glutamine synthetase inhibitor for improving the yield of crop plants which are resistant to this inhibitor. In particular, it relates to the use of glutamine synthetase inhibitors for improving the yield of transgenic crop plants.

The glutamine synthetase inhibitor used is preferably the compound glufosinate or bialaphos (Tachibana et al., Abstr. 5th Int. Congr. Pestic. Chem., IVa, Abstract 19; Mase, Jpn. Pestic. Inf., 1984, No. 45, p. 27). In this context, the term glufosinate embraces the racemate (DL-homocalanin-4-yl(methyl)phosphinic acid as well as the biologically active L isomer and the corresponding salts. The herbicide can be employed in the commercially available formulations. A further example of a GS inhibitor is the compound phosalacin (Omura et al., J. of Antibiotics, Vol. 37, 8, pages 939-940, 1984).

The yield-improving effect of the treatment with glufosinate is particularly pronounced when the herbicidal treatment is carried out in the 2 to 8-, preferably the 3 to 6-leaf stage of the crop plants before flowering or, in the case of perennial plants, at any desired point in time.

In the method according to the invention, the plants are treated at least once with the herbicide at application rates as they are also employed for weed control, for example 150 g - 1000 g of glufosinate/ha.

However, the application rate required may vary as a function of the plants, their height and the climatic

conditions.

It is particularly advantageous to carry out the process using application rates of 350 - 700 g of glufosinate/ha. Within this range of application rates, the effect
5 achieved is proportional to the application rate of glufosinate, but not based on differences in the level of weed control. It is possible to achieve a weed control effect which is similar to the effect which can be achieved at higher application rates even when the
10 application rate of PTC is low.

It is particularly advantageous to treat the plants repeatedly with low dosages in the lower range of the application concentrations, the treatment interval being a few days, i.e. between 2 and 30 days, preferably
15 between 5 and 20, particularly preferably between 8 and 15 days. It is particularly advantageous to treat the plants with low dosages, the treatment interval being from 9 to 11 days.

The method according to the invention can generally be
20 used for the treatment of plants which are resistant to GS inhibitors. Resistant plants can also be obtained by conventional breeding methods. If the resistance level of plant obtained by conventional selection is similar to that of the transgenic plants, the plants obtained by
25 conventional selection can also be treated by the method according to the invention. However, the method is particularly suitable for the treatment of glufosinate-resistant plants which have been obtained by transferring a gene for resistance to the herbicide. EP-B1-0 242 236
30 and EP-B1-0 257 542 describe methods for producing such plants.

In this context, the term plants embraces crop plants from the group of the angiosperms and the gymnosperms. The method according to the invention allows individual
35 plants, but also crops of plants, to be treated.

Particularly interesting among the gymnosperms is the class of the conifers.

Particularly interesting among the angiosperms are the plants from the families of the Solanaceae, Cruciferae, 5 Compositae, Liliaceae, Vitaceae, Chenopodiaceae, Rutaceae, Bromeliaceae, Rubiaceae, Theaceae, Musaceae or Gramineae and the order of the Leguminosae. Representatives of the families Solanaceae, Cruciferae and Gramineae are preferably treated.

10 The method is of particular interest for the treatment of crop plants in which high yields are important, such as, for example, maize, soybeans, spring and winter oil seed rape, sugar beet, lucerne, sunflower, cotton, potatoes, wheat, barley and rice. However, it can also be used 15 advantageously in tomatoes and other vegetables, such as cucumber, and fruits, such as melon, strawberries, raspberries, and kiwi fruit.

The use of the method in herbicide-resistant woody species is also particularly important, for example in 20 plantations and nurseries.

Application of GS inhibitors, such as, for example, PTC and its analogs and derivatives, to young specimens of woody species can accelerate the juvenile development. In this context, mention must be made, in particular, of 25 walnut trees, oil palms, fruit trees, poplars and other cultivated plants which are woody species.

The method according to the invention is therefore important both in agriculture and horticulture since application of the herbicidal glutamine synthetase 30 inhibitor allows a clearly measurable increase in yield to be achieved without an additional application of fertilizer and plant growth regulators. The term increase in yield means in this context that the plant yield up to 50% more. Herbicides having different mechanisms of

action either do not show such an effect or, frequently, have an adverse effect on yield.

The growth-enhancing activity of the glufosinate treatment can be measured in field trials and pot trials, by comparing yields of stands of plants which are treated with conventional herbicides or which were kept free from weeds by non-chemical methods.

The examples which follow are intended to illustrate the invention without thereby imposing any restriction.

10 Example 1

Transgenic glufosinate-tolerant maize or soybean plants were planted in plots (10 m²) and, in the 3 - 5 leaf stage, treated with various amounts of glufosinate. The weed control level was scored 42 days after the application. When the crops were ripe, the plots were harvested, and the seed yield was determined by weighing the kernels obtained.

When the maize plants were examined, Laddock[®] (a mixture of atrazine and bentazone) was employed as comparison product. Two products were employed for the treatment of the soya bean plants. Comparison product 1 contained a mixture of 134 g of fenoxaprop-P-ethyl/ha and 425 g of fomesafen/ha; comparison product 2 contained 2240 g of metolachlor/ha and 840 g of Storm[®] (a mixture of bentazone and acifluorfen)/ha. The comparison products are known from "The Pesticide Manual", 9th Edition, Brit. Crop Prot. Council, 1991.

The treatment described in Table 2, in which two low glufosinate dosage rates were used, was carried out at a 10 day interval.

Table 1

| | Application rates of Comparison glufosinate (g of active substance/ha) | | | Comparison product | |
|----|--|-----|-----|-----------------------|-----|
| 5 | 150 | 450 | 650 | | |
| | Weed control level in % | 92 | 97 | 98 | 78 |
| 10 | Yield in % of the plot with the comparison product | 118 | 121 | 125 | 100 |

Table 2

Grain yield of glufosinate-tolerant soybean plants after application of glufosinate

| | Application rates of glufosinate: g of active substance/ha | | | | | | | | | | Comparison product | |
|---|---|-----|-----|-----|-------------|-------------|-------------|-------------|-----|----|--------------------|--|
| | 350 | 450 | 550 | 650 | 150 +150 | 250 +250 | 350 +350 | 450 +450 | 1 | 2 | | |
| Weed control level in % | 85 | 93 | 95 | 94 | 96 | 96 | 98 | 99 | 98 | 90 | | |
| Yield in % (based on plot with comparison product 1) | 88 | 108 | 120 | 132 | 104 | 132 | 148 | 152 | 100 | 92 | | |

75749-16

- 8 -

CLAIMS:

1. A method of improving yield of crop plants which are resistant to glutamine synthetase inhibitors, which comprises treating the crop plants with at least one
5 glutamine synthetase inhibitor at an application rate which is not harmful to the plants.
2. The method as claimed in claim 1, wherein glufosinate and salts thereof are employed for the yield-improving treatment of crop plants which are protected
10 against the herbicidal property of the glutamine synthetase inhibitor by expression of an n-acetyl-transferase gene.
3. The method as claimed in claim 1 or claim 2, wherein the crop plants are treated at least once with the active substance at an application rate as also employed for
15 weed control.
4. The method as claimed in any one of claims 1 to 3, wherein the crop plants are treated at least once using 150-1000 g of glufosinate/ha.
5. The method as claimed in any one of claims 1 to 3,
20 wherein the crop plants are treated at least once using 350-700 g of glufosinate/ha.
6. Use of a glutamine synthetase inhibitor for improving yield of crop plants which are resistant to this inhibitor.

SMART & BIGGAR
OTTAWA, CANADA

PATENT AGENTS