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The Syngenta logo features the word "syngenta" in a lowercase, sans-serif font. A small green leaf icon is positioned above the letter 'g'.

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**Opposition to European Patent No. EP-1 069 819-B1 (Appl. No. 99915886.8)**

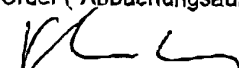
<u>Publication Number:</u>	EP - 1 069 819 - B1
<u>Proprietor:</u>	PLANT BIOSCIENCE LTD Norwich, Norfolk NR4 7UH (GB)
<u>Title:</u>	<b>METHOD FOR SELECTIVE INCREASE OF THE ANTICARCINOGENIC GLUCOSINOLATES IN BRASSICA SPECIES</b>
<u>Application Number:</u>	99915886.8
<u>Date of Filing:</u>	April 8, 1999
<u>Priorities:</u>	April 9, 1998 (US 81169 P)
<u>Publication of the Grant of the Patent:</u>	July 24, 2002; Bulletin 2002/30

#### **NOTICE OF OPPOSITION**

In the name of **Syngenta Participations AG, Schwarzwaldallee 215, 4058 Basle, Switzerland**, the above-identified European Patent is opposed pursuant to Art 99 EPC. The following is requested:

- (1) **Revocation** of the patent **in its entirety** (all 18 claims granted); and
- (2) Oral proceedings if the Opposition Division intends to reject the opposition or to maintain the patent in amended form.

The opposition is based on Art 100, particularly **Art 100 a), b) and c) EPC**. It is requested that the **opposition fee** of EUR 610,- is debited from our Deposit Account No. 28110232 with the EPO in Munich (see enclosed Debit Order ("Abbuchungsauftrag") ).

  
Representative: **Smolders, Walter, et al; GA No. 42789.**  
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## GROUNDS OF OPPOSITION

### **1. Priority**

#### **1.1. Claims 17 and 18 are not entitled to the priority date of the opposed patent.**

Claims 17-18 are drawn to "A *Brassica* plant cell". These claims were not present in the priority document but were added to the international application WO 99/52345, which formed the basis for the specification of the opposed patent.

There is no support in the priority application US 81169 P for "A plant cell" as claimed in claims 17-18. US 81169 P merely refers to "plant cell extracts" at the first paragraph of the Summary of the Invention (page 8), but does not specifically refer to plant cells. Nothing in US 81169 P otherwise points towards plant cells.

Consequently, "A *Brassica* plant cell" was not directly and unambiguously disclosed in the priority document US 81169 P as required by G1/98, and is not entitled to the priority date.

The State of the Art for claim 17-18 therefore comprises everything made available to the public before the filing date of the opposed patent, i.e. April 8, 1999.

### **2. Article 83 EPC (Insufficiency of the disclosure)**

#### **2.1. The subject-matter of claims 1-18 is not sufficiently disclosed**

Claims 1-18 recite the feature "elevated" levels of glucosinolates. However, the opposed patent does not define what "elevated" levels mean and only discloses measurements of glucosinolate contents resulting from three crosses (Table 1 of the opposed patent).

The Case Law of the Board of Appeal clearly states that the disclosure of a European patent must allow to perform the subject-matter of a claim in its entire scope (see e.g. T 409/91 and T 435/91).

In the opposed patent "elevated" is an open-ended feature without boundaries. The disclosure of the opposed patent does not sufficiently disclose or enable the entire open-ended range of "elevated" levels of glucosinolates. The person skilled in the art would therefore not be able to practice all embodiments of the claims and the claims are not enabled over their entire scope.

Consequently, claims 1-18 are in contradiction with Art. 83 EPC.

### **3. The Prior Art**

The arguments presented refer to the following documents of evidence. Two hard-copies thereof are forwarded by mail simultaneously (Rule 59 EPC).

As discussed above, claims 17-18 of the opposed patent are not entitled to the priority date of April 9, 1998. Consequently, the state of the art for these claims comprises everything made available to the public before April 8, 1999, date of filing of the European patent application.

- D 1: Juvik (1997) HortScience 32(3), Abstract 733
- D 2: Mithen et al. (1987) Phytochemistry 26:1969-1973
- D 3: Giamoustaris and Mithen (1996) Theor Appl Genet 93: 1006-1010
- D 4: Fahey et al. (1997) Proc. Natl. Acad. Sci. USA 94: 10367-10372
- D 5: Heaney et al. (1983) Z. Pflanzenzüchtg. 91: 219-226
- D 6: Carlson et al. (1987) J. Amer. Soc. Hort. Sci. 112: 173-178
- D 7: Backcross method, Chapter 28 in Principles of Cultivar Development (1987) vol 1: 360-376
- D 8: Cheung et al. (1997) Theor. Appl. Genet. 95:73-82.
- D 9: Camargo et al. (1997) The Journal of Heredity, 88: 57-59.
- D 10: Toroser et al. (1995) Theor. Appl. Genet. 91: 802-808.
- D 11: Ferreira et al. (1994) Theor. Appl. Genet. 89: 615-621.
- D 12: Faulkner et al. (1998) Carcinogenesis 19: 605-609.

### **4. The claims**

The opposed patent contains 18 claims.

Claims 1-8, and 12 are drawn to "A method for the production of *Brassica Oleracea*". Claims 2-8 and 12 all depend on claim 1 directly or indirectly.

Claim 9 is drawn to "An edible *Brassica* plant", claim 10 to "An edible portion of a broccoli plant", claims 11 to "Seed of a broccoli plant". Claims 9-11 all refer to material "produced according to the method of any one of claims 1 to 6".

Claims 13-14 are drawn to "A broccoli plant". Claim 13 is independent, claim 14 depends on claim 13.

Claims 15-16 are drawn to "A broccoli inflorescence". Claim 15 is independent, claim 16 depends on claim 15.

Claims 17-18 are drawn to "A *Brassica* plant cell". Claim 17 is independent, claim 18 depends on claim 17.

## **5. Article 53(b) EPC (Essentially Biological Process)**

### **5.1. Claims 1-3 are in contradiction with Art 53(b) EPC**

Claim 1 refers to a process claim comprising two steps:

Step (a) referring to "crossing wild *Brassica oleracea* species with *Brassica oleracea* breeding lines", and

Step (b) referring to "selecting hybrids with levels of 4-methylsulfinylbutyl glucosinolates, or 3-methylsulfinylpropyl glucosinolates, or both, elevated above that initially found in *Brassica oleracea* breeding lines".

Claims 2 and 3 further incorporate additional steps of "back-crossing" and "selecting".

Thus, claims 1-3 entirely consist of crossing and selecting *Brassica oleracea* plants. According to R.23b(5) EPC, such process of crossing and selecting is an "essential biological process for the production of plants" and therefore the subject-matter of claims 1-3 falls under the exception to patentability set forth in Art 53(b) EPC.

Accordingly, claims 1-3 are not in compliance with Art.53(b) EPC.

## **6. Article 54 EPC (Lack of Novelty)**

### **6.1. Claims 9-11 lack novelty over D2**

Claims 9-11 are set forth in a "product-by-process" format. According to the Guidelines for Examination of the EPO, a claim defining a product in terms of a process is to be construed as a claim to the product as such, and should be interpreted as a product "obtainable" by the said process (GL, C-III, 4.7b).

Claims 9-11 are thus to be interpreted as referring to *B.oleracea* with elevated levels of 4-methylsulfinylbutyl glucosinolates or 3-methylsulfinylpropyl glucosinolates, or both.

D2 describes *B.oleracea* whose levels of 4-methylsulfinylbutyl glucosinolates or 3-methylsulfinylpropyl glucosinolates, or both, are elevated with respect to other *B.oleracea* (see Tables 1 and 2.).

Some of the *B.oleracea* described in D2 are edible (e.g. Cv. Green Duke, a broccoli cultivar, as known from D6, page 176, Table 3), and as such comprise an "edible portion" and produce "seeds".

Consequently, claims 9-11 lack novelty over D2.

## **7. Article 56 EPC (Lack of Inventive Step)**

The Opponent holds the view that the subject matter of the claims lack inventive step.

To assess inventive step, the "problem/solution approach" is normally to be applied in the proceedings before the EPO. The problem/solution approach is set out in the Guidelines for Examination in the European Patent Office ("the Guidelines") at C:IV 9.5 and requires the following three sequential steps:

- determine the closest prior art;
- establish the technical problem to be solved; and
- consider whether or not the claimed invention, starting from the closest prior art and the technical problem, would have been obvious to the skilled person.

#### 7.1. Claim 1 lacks inventive step

##### 7.1.1. Claim 1 lacks inventive step over D3 in combination with D2

D3 can be considered as the closest prior art as it relates to glucosinolates in *B. oleracea* and their anticancer activities.

D3 discloses a cross between *B. drepanensis* and a hybrid of *B. drepanensis* and *B. atlantica* (paragraph bridging pages 1007 and 1008). A selected hybrid resulting from this cross is shown to have levels of 3-methylsulfinylpropyl elevated above that of the hybrid of *B. drepanensis* and *B. atlantica* (page 1008, 2<sup>nd</sup> column, lines 12-13, and Figure 4 A and C).

Therefore, D3 discloses a method according to claim 1 without the provision of *B. oleracea* breeding lines in the crossing step (a).

Starting from D3, the objective problem can therefore be formulated as providing alternative starting materials in a method for the production of *B. oleracea* with elevated levels of glucosinolates.

D3 also discloses *B. oleracea* breeding lines and their use in the crosses reported in D3 (cultivated forms of *B. oleracea*, page 1010, 1<sup>st</sup> column, lines 27-30). The skilled person would therefore not need any inventive activity to substitute the hybrid of *B. drepanensis* and *B. atlantica* in the cross exemplified in D3 with *B. oleracea* breeding lines. Moreover, based on the teachings of D2, the skilled person would know that hybrids between wild and cultivated forms of *B. oleracea* are at least partially fertile (D2 page 1969, 1<sup>st</sup> column, lines 6-8). This would further invite the person skilled in the art to carry out such crosses to arrive at the method of claim 1 without any inventive step.

Consequently, claim 1 lacks inventive step over D3 in combination with D2 under Art.56 EPC.

##### 7.1.2. Claim 1 lacks inventive step over D5 in combination with D3

D5 can be considered as the closest prior art as it relates to levels of glucosinolates in *Brassicacae*.

D5 discloses crosses between *B.oleracea* breeding lines (Brussel sprouts parent lines, known as *B.oleracea* L. *gemmifera*, from D6, page 173, second line of abstract) to produce hybrids progeny possessing a more desirable glucosinolate combination (D5, page 223, lines 12-14). Elevated levels of glucosinolates in the hybrid progeny compared to the parent lines are disclosed (maintaining (or even increasing) the amount of those glucosinolates, D5, page 223, lines 19-20).

D5 suggests that the selection of suitable parent lines is important to produce a desirable pattern of glucosinolate in hybrids (page 223, abstract, lines 7-8) and states that the relative amounts or patterns of glucosinolates in the F1 hybrids generally match the mean value of both parents (page 223, abstract, lines 5-7).

D5 also mentions the anticarcinogenic properties of glucosinolates (D5, bridging paragraph between pages 219 and 220).

D5 is silent about the use of wild Brassica species and does not specifically mention 3-methylsulfinylpropyl or 4-methylsulfinylbutyl.

Starting from D5, the objective problem to be solved may be formulated as improving the anticarcinogenic properties of Brassicas. This problem is addressed in the opposed patent at page 4, section [0018], lines 51-53.

D3 also addresses the problem of improved nutritional properties of glucosinolates in Brassicas, for example anticancer activity (D3, page 1006, abstract, lines 12-15, and 2<sup>nd</sup> column, lines 21-24).

D3 discloses wild *Brassica* species *B.drepanensis* having high levels of 3-methylsulfinylpropyl (D3 page 1008, 1<sup>st</sup> column, first sentence of the Results and discussion section). D3 shows that elevated levels of 3-methylsulfinylpropyl can be transferred to *B.drepanensis* progeny (Figure 4 A and C).

Consequently, the skilled person would apply the teachings of D3 to D5 and substitute one of the parent lines of D5 for *B.drepanensis* in the crosses to produce hybrids with elevated levels of 3-methylsulfinylpropyl.

Based on the teachings of D2, the skilled person would know that hybrids between wild and cultivated forms of *B.oleracea* are at least partially fertile (D2 page 1969, 1<sup>st</sup> column, lines 6-8). The skilled person would also know from D5 that the relative amounts or patterns of glucosinolates in the F1 hybrids generally match the mean value of both parents (page 223, abstract, lines 5-7).

Therefore, by combining the teachings of D5 and D3, the skilled person would arrive at the method of claim 1 without any inventive step.

Consequently, claim 1 lacks inventive step in view of D5 combined with D3 under Art. 56 EPC.

### 7.1.3. Claim 1 lacks inventive step over D2 in combination with D3 or D4

D2 can be considered as the closest prior art as it relates to glucosinolate levels in wild and cultivated forms of *B. oleracea* (D2, page 1969, column 1, lines 6-8).

D2 discloses *B. drepanensis* having high levels of 3-methylsulfinylpropyl and *B. incana* having high levels of 4-methylsulfinylbutyl (D2, Tables 1 and 2). D2 further discloses *Brassica oleracea* breeding lines (cultivated forms of *Brassica oleracea*, see D2, page 1969, column 1, lines 6-8), for example Green Duke. The contents in glucosinolates of these lines is also disclosed in D2 (Tables 1 and 2). D2 also further discloses that crosses between wild and cultivated forms of *B. oleracea* are at least partially fertile (D2, page 1969, 1<sup>st</sup> column, lines 6-8).

D2 is silent about crossing *Brassica* wild species and breeding lines and selecting hybrids with elevated levels of glucosinolates.

Starting from D2, the objective problem to be solved can be formulated as increasing levels of anticarcinogenic glucosinolate derivatives in *Brassica oleracea*. This problem is addressed in opposed patent at page 1, section [0001], lines 1-2.

Although D2 mentions that high levels of glucosinolates within wild species may deter plant breeders from using them, D2 also recognizes that "it may be possible to reduce undesirable glucosinolates" (page 1969, 2<sup>nd</sup> column, lines 31-34). D2 also recognizes that wild *Brassica* species may be sources of genes to use in breeding programs (D2, page 1969, column 2, lines 17-18). Therefore, D2 does not teach away from transferring other genes from wild species to cultivated forms of *B. oleracea*.

The skilled person is well aware of the beneficial effects, in particular anticarcinogenic effects, of certain glucosinolates present in *Brassica oleracea* species. In particular, the skilled person knows from D3 about the anticarcinogenic effects of 3-methylsulfinylpropyl (D3, page 1006, 2<sup>nd</sup> column, lines 24-26) and from D4 about the anticarcinogenic effects of 4-methylsulfinylbutyl (D4, abstract, page 10367, lines 25-29).

The skilled person would recognize that the beneficial glucosinolates described in D3 and D4 correspond to those mentioned in some of the wild *Brassica* species of D2 (Table 2). The skilled person would also know from D2 that crosses between wild and cultivated forms of *B. oleracea* are at least partially fertile (page 1969, 1<sup>st</sup> column, lines 6-8). Therefore, in front of the combined teachings of D2 and D3 or D4, the skilled person would be led to cross wild *Brassica* species and cultivated forms of *B. oleracea*, for example *B. drepanensis* and Green Duke, and arrive at the method of claim 1 without any inventive step.

Therefore, claim 1 lacks inventive step over D2 in combination with D3 or D4.

#### 7.1.4. Claim 1 lacks inventive step over D1 in combination with D3

D1 can be considered as the closest prior art as it relates to variations of glucosinolate contents in *Brassica oleracea* (D1, lines 13-15) and to the development of *Brassica* leading to enhanced cancer chemoprevention (D1, lines 20-22).

D1 discloses a breeding program in brassicas, in particular in *B. oleracea*, aimed at developing germplasm with enhanced cancer chemoprotection (last sentence of D1).

D1 refers for example to sulforaphane, known as the chemoprotective metabolite of glucoraphanin (see D4, page 10367, column 2, 3<sup>rd</sup> paragraph). Glucoraphanin is the common chemical name for the glucosinolate 4-methylsulfinylbutyl (see D2, page 1970, Table 1). The inter-relation between sulforaphane and 4-methylsulfinylbutyl regarding cancer chemoprotective or anticarcinogenic activity is clearly acknowledged in the opposed patent at page 4, section [0016], lines 22-25.

The person skilled in the art knows that a breeding program involves crossing and selecting plants (see for example D7). D1 also refers to crosses (line 17) and to assays for chemical content (line 18). It is also well-known in the art that breeding lines are used in a breeding program and that a cross between two plants results in a hybrid.

Therefore, D1 discloses all the features of claim 1 with the exception of the use of wild *Brassica oleracea* species in the breeding program.

Starting from D1, the objective problem can thus be formulated as providing novel compositions of matter to increase levels of glucosinolates in a *Brassica* breeding program to provide enhanced cancer chemoprotection. This problem is addressed in the opposed patent at page 3, section [0012], lines 49-51.

D3 also relates to the manipulation of *Brassica oleracea* (see title and abstract, lines 12-15) and to the anticancer activity of glucosinolates (D3, page 1006, 2<sup>nd</sup> column, lines 24-26). D3 discloses wild *Brassica* species, such as *B. drepanensis* and *B. atlantica*, and plants having *B. drepanensis* as a parent with elevated level of 3-methylsulfinylpropyl compared to the other parent (see for example Figure 4 A and C, peak 1).

Therefore, the skilled person faced with the problem at stake would use a wild *Brassica* such as *B. drepanensis* disclosed in D3 in a breeding program as set out in D1, and arrive at the method of claim 1 without any inventive step.

Consequently, claim 1 lacks inventive step over D1 in combination with D3 under Art. 56 EPC.



## 7.2. Claim 2 lacks inventive step

Claim 2, which depends on claim 1, further recites a step of backcrossing and a step of selecting, and specifically refers to broccoli breeding lines.

### 7.2.1. Claim 2 lacks inventive step over D3 in combination with D2

D3 also mentions backcrosses (page 1008, 1<sup>st</sup> column, 1<sup>st</sup> sentence). Therefore, the additional steps of claim 2 cannot be considered as imparting any inventive step to the claim. D3 refers to cultivated forms of *B.oleracea* and to cruciferous vegetables (page 1010, 1<sup>st</sup> column, last paragraph). Broccoli is a well-known vegetable of the *B.oleracea* species. Therefore, the recitation of "broccoli" in claim 2 also cannot be considered as imparting any inventive step to the claim.

Consequently, claim 2 lacks inventive step over D3.

### 7.2.2. Claim 2 lacks inventive step over D5 in combination with D3

Claim 2 also lacks inventive step over D5 in combination with D3, as the additional features of claim 2 are disclosed or suggested in D3 (see section 7.2.1. above).

### 7.2.3. Claim 2 lacks inventive step over D2 in combination with D3 or D4 and in view of D7

D7 clearly shows that backcross and selection are commonly used in breeding programs and therefore known to the skilled person. Moreover, D2 discloses Green Duke (Table 2), which is known from D6 as a broccoli cultivar (D6, page 176, Table 3).

Consequently, claim 2 lacks inventive step over D2 in combination with D3 or D4 and in view of D7.

### 7.2.4. Claim 2 lacks inventive step over D1 in combination with D3

Claim 2 lacks inventive step over D1 in combination with D3, as the additional features of claim 2 are already disclosed or suggested in D3 (see section 7.2.1. above).

## 7.3. Claim 3 lacks inventive step

Claim 3, which depends on claim 2, further recites a step of selecting a broccoli line capable of causing a strong induction of phase II enzymes.

The fact that 3-methylsulfinylpropyl or 4-methylsulfinylbutyl, or both, cause a strong induction of phase II enzymes is known from D3 (methylsulphinylalkyl isothiocyanates induce phase-2 detoxification enzymes, D3, page 1006, 2<sup>nd</sup> column, lines 15-17) and D4 (page 10367, Abstract, lines 11-17).

Therefore the induction of phase II enzymes is a consequences of elevated levels of 3-methylsulfinylpropyl or 4-methylsulfinylbutyl, or both, and the additional feature of claim 3 cannot confer an inventive step to the claim.

Claim 3 therefore lacks inventive step over the combinations of references used against the inventive step of claim 2, further combined with D3 or D4.

#### 7.4. Claim 4 lacks inventive step

Claim 4, dependent on claim 1, further recites a step of screening for the specific SI alleles with RFLP markers. Claim 4 also recites that the *Brassica oleracea* breeding lines are broccoli double haploid breeding lines containing specific SI alleles.

According to the opposed patent, combining specific alleles for self-incompatibility is merely useful for seed production strategies (opposed patent, page 6, section [0040], lines 43-44). Thus, this additional feature of claim 4 does not confer any additional technical feature to the claim and cannot contribute to any inventive step of the claim.

Double haploids in broccoli were known in the art as acknowledged in the opposed patent at page 8, Example 1, lines 15-16. Moreover, the screening for SI alleles with RFLP markers was also known in the art as acknowledged in the opposed patent at page 6, section [0040], lines 49-52.

Consequently, the additional features of claim 4 cannot confer an inventive step to the claim. Claim 4 therefore lacks inventive step over the combinations of references used against the inventive step of claim 1.

#### 7.5. Claim 5 lacks inventive step

Claim 5, dependent on claim 1, further recites features already discussed in respect of claims 2, 3 and 4. As mentioned above, these features were known from the prior art and do not contribute to any additional technical effect. Therefore, they cannot impart any inventive step to claim 5.

Consequently, claim 5 lacks inventive step in view of the references cited against claim 1 and further combined with D3 or D4.

#### 7.6. Claim 6 lacks inventive step

Claim 6, dependent on claim 1, further recites additional steps of backcrossing and selecting, broccoli double haploids, and the strong induction of phase II enzymes. These features have already been discussed in respect of claims 2, 3 and 4. As mentioned above, these features do not contribute to any additional technical effect, they were known from the prior art, and thus cannot contribute to any inventive step with respect to claim 6.

Claim 6 also further recites the selection of plants with "the genetic combination encoding the expression of elevated levels of 4-methylsulfinylbutyl glucosinolates, 3-methylsulfinylpropyl glucosinolates, or both". Claim 6 also refers to DNA probes used to select hybrids with such genetic combination.

Starting from the combination of references used to attack the inventive step of claim 1, the objective problem can be formulated as facilitating the selection of *Brassica* lines having appropriate levels of glucosinolates. This problem is addressed in the opposed patent at page 7, section [0044], lines 28-30.

D10 addresses the same problem (D1, page 803, 1<sup>st</sup> column, lines 9-13). D10 discloses a locus on LG20 that contributes to significant amounts of 4-methylsulfinylbutyl (page 805, 2<sup>nd</sup> column, last paragraph). Fig 2a of D10 discloses a number QTLs on LG20. It is noted that the opposed patent acknowledges that the DNA probes or markers used in the opposed patent are derived from D10 or D11 (page 7, section [0043]).

Faced with the problem at stake, the skilled person would know about the QTLs and DNA probes of D10, and that they are linked to glucosinolate levels. The skilled person would use them in a method of producing *B. oleracea*, and in combination with the references used in the attacks against claim 1, would arrive at the subject-matter of claim 1 without any inventive activity.

Consequently, claim 6 lacks inventive step in view of the references cited against claim 1 and further combined with D10.

#### 7.7. Claims 7 and 8 lack inventive step

Claims 7 and 8, which depend on claim 1 to 6, further recite that only 4-methylsulfinylbutyl glucosinolates or 3-methylsulfinylpropyl glucosinolates, respectively is elevated.

It is apparent from the references used in the attacks against claims 1 to 6 above, in particular from D3 and D4, that both compounds and their anticarcinogenic properties were known at the time of filing of the opposed patent. Based on these attacks, it is clear that

elevating the levels of each of these two glucosinolates individually does not confer any inventive step.

Consequently, claim 7 and 8 lack inventive step over the combinations of references cited against the inventive step of claim 1 to 6.

#### 7.8. Claims 9-11 lack inventive step

Claims 9-11 depend on claims 1 to 6, which have already been shown to lack inventive step. The fact that claims 9-11 refer to an "edible Brassica plant", an "edible portion of a broccoli plant" or to a "seed of a broccoli plant" do not confer any inventive step to these claims, as these features are well known parts of e.g. broccoli plants.

Consequently, claims 9-11 lack inventive step over the combinations of references cited against claims 1 to 6 for the same reasons as those used against these claims.

#### 7.9. Claim 12 lacks inventive step

Claim 12, which depends on claim 6, further recites DNA probes.

However, the opposed patent itself acknowledges that these DNA probes "are available from Dr. Tom Osborne" and are described in D10 and D11. It has therefore to be assumed that the particular probes set forth in claim 12 were publicly available at the effective date of the opposed patent.

Consequently, these DNA probes cannot confer any inventive step to claim 6, and the claim lacks inventive step for the same reasons as for claim 6.

#### 7.10. Claim 13 lacks inventive step

##### 7.10.1. Claim 13 lacks inventive step over D3 in combination with D2

D3 can be considered as the closest prior art as it relates to glucosinolates in *B. oleracea* and their anticancer activities.

D3 discloses a cross between *B. drepanensis* and a hybrid of *B. drepanensis* and *B. atlantica* (paragraph bridging pages 1007 and 1008). A selected hybrid resulting from this cross is shown to have levels of 3-methylsulfinylpropyl elevated above that of the hybrid of *B. drepanensis* and *B. atlantica* (page 1008, 2<sup>nd</sup> column, lines 12-13, and Figure 4 A and C).

The hybrid of D3 is not a broccoli plant and one of its parents is not a broccoli breeding line.

Starting from D3, the objective problem can therefore be formulated as providing alternative starting materials to obtain *B.oleracea* with elevated levels of glucosinolates.

D3 also discloses *B.oleracea* breeding lines and their use in the crosses reported in D3 (cultivated forms of *B.oleracea*, page 1010, 1<sup>st</sup> column, lines 27-30). The skilled person would therefore not need any inventive activity to substitute the hybrid of *B.drepanensis* and *B.atlantica* in the cross exemplified in D3 with *B.oleracea* breeding lines. Moreover, based on the teachings of D2, the skilled person would know that hybrids between wild and cultivated forms of *B.oleracea* are at least partially fertile (D2 page 1969, 1<sup>st</sup> column, lines 6-8). This would further invite the person skilled in the art to carry out such crosses. D2 also discloses a Cv. Green Duke known from D6 to be a broccoli cultivar, leading the skilled person to use a broccoli breeding line to arrive at the subject-matter of claim 13 without any inventive step.

Consequently, claim 13 lacks inventive step over D3 in combination with D2 under Art.56 EPC.

#### 7.10.2. Claim 13 lacks inventive step over D5 in combination with D3

D5 can be considered as the closest prior art as it relates to levels of glucosinolates in *Brassicás*.

D5 discloses crosses between *B.oleracea* breeding lines (Brussel sprouts parent lines, known as *B.oleracea* L. *gemmifera*, from D6, page 173, second line of abstract) to produce hybrids progeny possessing a more desirable glucosinolate combination (D5, page 223, lines 12-14). Elevated levels of glucosinolates in the hybrid progeny compared to the parent lines are disclosed (maintaining (or even increasing) the amount of those glucosinolates, D5, page 223, lines 19-20).

D5 suggests that the selection of suitable parent lines is important to produce a desirable pattern of glucosinolate in hybrids (page 223, abstract, lines 7-8) and states that the relative amounts or patterns of glucosinolates in the F1 hybrids generally match the mean value of both parents (page 223, abstract, lines 5-7).

D5 also mentions the anticarcinogenic properties of glucosinolates (D5, bridging paragraph between pages 219 and 220).

D5 is silent about the use of wild *Brassica* species and does not specifically mention 3-methylsulfinylpropyl or 4-methylsulfinylbutyl.

Starting from D5, the objective problem to be solved may be formulated as improving the anticarcinogenic properties of *Brassicás*. This problem is addressed in the opposed patent at page 4, section [0018], lines 51-53.

D3 also addresses the problem of improved nutritional properties of glucosinolates in Brassicas, for example anticancer activity (D3, page 1006, abstract, lines 12-15, and 2<sup>nd</sup> column, lines 21-24).

D3 discloses wild *Brassica* species *B. drepanensis* having high levels of 3-methylsulfinylpropyl (D3 page 1008, 1<sup>st</sup> column, first sentence of the Results and discussion section). D3 shows that elevated levels of 3-methylsulfinylpropyl can be transferred to *B. drepanensis* progeny (Figure 4 A and C).

Consequently, the skilled person would apply the teachings of D3 to D5 and substitute one of the parent lines of D5 for *B. drepanensis* in the crosses to produce *Brassica* hybrids with elevated levels of 3-methylsulfinylpropyl.

Based on the teachings of D2, the skilled person would know that hybrids between wild and cultivated forms of *B. oleracea* are at least partially fertile (D2 page 1969, 1<sup>st</sup> column, lines 6-8). The skilled person would also know from D5 that the relative amounts or patterns of glucosinolates in the F1 hybrids generally match the mean value of both parents (page 223, abstract, lines 5-7).

As D5 suggests that its teaching may apply to other *Brassica* species (page 223, penultimate paragraph), and as D3 refers to vegetables (page 1010, 1<sup>st</sup> column, line 23), the skilled person would not need any inventive step to use broccoli breeding lines in the cross.

Therefore, by combining the teachings of D5 and D3, the skilled person would arrive at the subject-matter of claim 13 without any inventive step.

Consequently, claim 13 lacks inventive step in view of D5 combined with D3 under Art. 56 EPC.

#### 7.10.3. Claim 13 lacks inventive step over D2 in combination with D3 or D4

D2 can be considered as the closest prior art as it relates to glucosinolate levels in wild and cultivated forms of *B. oleracea* (D2, page 1969, column 1, lines 6-8).

D2 discloses *B. drepanensis* having high levels of 3-methylsulfinylpropyl and *B. incana* having high levels of 4-methylsulfinylbutyl (D2, Tables 1 and 2). D2 further discloses broccoli breeding lines (cultivated Green Duke, known from D6 to be a broccoli cultivar, see D2, page 1970, Table 2). The glucosinolate content of these lines is also disclosed in D2 (Tables 1 and 2). D2 also further discloses that crosses between wild and cultivated forms of *B. oleracea* are at least partially fertile (D2, page 1969, 1<sup>st</sup> column, lines 6-8).

D2 is silent about crossing *Brassica* wild species and breeding lines and selecting hybrids with elevated levels of glucosinolates.

Starting from D2, the objective problem to be solved can be formulated as increasing levels of anticarcinogenic glucosinolate derivatives in broccoli. This problem is addressed in opposed patent at page 1, section [0001], lines 1-2.

Although D2 mentions that high levels of glucosinolates within wild species may deter plant breeders from using them, D2 also recognizes that "it may be possible to reduce undesirable glucosinolates" (page 1969, 2<sup>nd</sup> column, lines 31-34). D2 also recognizes that wild Brassica species may be sources of genes to use in breeding programs (D2, page 1969, column 2, lines 17-18). Therefore, D2 does not teach away from transferring other genes from wild species to cultivated forms of *B. oleracea*.

The skilled person would know the anticarcinogenic effects of 3-methylsulfinylpropyl from D3 (D3, page 1006, 2<sup>nd</sup> column, lines 24-26), and of 4-methylsulfinylbutyl from D4 (D4, abstract, page 10367, lines 25-29).

The skilled person would also recognize that the beneficial glucosinolates set forth in D3 and D4 correspond to those mentioned in wild species of D2 (Table 2). The skilled person would also know from D2 that crosses between wild and cultivated forms of *B. oleracea* are at least partially fertile (page 1969, 1<sup>st</sup> column, lines 6-8). Therefore, in front of the combined teachings of D2 and D3 or D4, the skilled person would be led to cross for example *B. drepanensis* and Green Duke, and arrive at the subject-matter of claim 13 without any inventive step.

Therefore, claim 13 lacks inventive step over D2 in combination with D3 or D4.

#### 7.10.4. Claim 13 lacks inventive step over D1 in combination with D3

D1 can be considered as the closest prior art as it relates to variations of glucosinolate contents in *Brassica oleracea* (D1, lines 13-15) and to the development of *Brassica* leading to enhanced cancer chemoprevention (D1, lines 20-22).

D1 discloses a breeding program in brassicas, in particular in *B. oleracea* and vegetables cultivars (lines 12 and 14 of D1) aimed at developing germplasm with enhanced cancer chemoprotection (last sentence of D1).

D1 refers for example to sulforaphane, known as the chemoprotective metabolite of glucoraphanin (see D4, page 10367, column 2, 3<sup>rd</sup> paragraph). Glucoraphanin is the common chemical name for the glucosinolate 4-methylsulfinylbutyl (see D2, page 1970, Table 1). The inter-relation between sulforaphane and 4-methylsulfinylbutyl regarding cancer chemoprotective or anticarcinogenic activity is clearly acknowledged in the opposed patent at page 4, section [0016], lines 22-25.

The person skilled in the art knows that a breeding program involves crossing and selecting plants (see for example D7). D1 also refers to crosses (line 17) and to assays for chemical

content (line 18). It is also well known in the art that breeding lines are used in a breeding program and that a cross between two plants results in a hybrid.

Therefore, D1 discloses all the features of claim 13 with the exception of the use of wild *Brassica oleracea* species and broccoli breeding lines as parents.

Starting from D1, the objective problem can thus be formulated as providing novel compositions of matter to increase levels of glucosinolates in *Brassica* to provide enhanced cancer chemoprotection. This problem is addressed in the opposed patent at page 3, section [0012], lines 49-51.

D3 also relates to the manipulation of *Brassica oleracea* (see title and abstract, lines 12-15) and to the anticancer activity of glucosinolates (D3, page 1006, 2<sup>nd</sup> column, lines 24-26). D3 discloses wild *Brassica* species, such as *B. drepanensis* and *B. atlantica*, and plants having *B. drepanensis* as a parent with elevated level of 3-methylsulfinylpropyl compared to the other parent (see for example Figure 4 A and C, peak 1).

Therefore, the skilled person faced with the problem at stake would use a wild *Brassica* such as *B. drepanensis* disclosed in D3 in a breeding program as set out in D1. It would also be mere routine activity to select broccoli as *B. oleracea* vegetable for the breeding program. The skilled person would therefore arrive at the subject-matter of claim 13 without any inventive step.

Consequently, claim 13 lacks inventive step over D1 in combination with D3 under Art. 56 EPC.

#### 7.11. Claim 14 lacks inventive step

Claim 14, which depends on claim 13, further recites that the concentration of 3-methylsulfinylpropyl glucosinolates, or 4-methylsulfinylbutyl glucosinolates, or both, is between 10 and 100  $\mu$ moles per gram of dry weight.

D3 discloses contents of glucosinolates varying from 26.2 to 118.5  $\mu$ moles per gram of dry weight (page 1008, 2<sup>nd</sup> column, lines 6-8). In particular, D3 discloses a phenotype with high levels of 3-methylsulfinylpropyl glucosinolates (page 1008, 2<sup>nd</sup> column, lines 12-13). This high level of 3-methylsulfinylpropyl glucosinolates inevitably falls within the range of 10 to 100  $\mu$ moles per gram of dry weight recited in claim 14, and anticipates this additional feature of the claim.

Consequently, claim 14 lacks inventive step over the combinations of references used to attack claim 13, further combined with D3.



### 7.12. Claims 15-16 lack inventive step

The only difference between claims 15-16 and claims 13-14 is that claims 15-16 are drawn to "broccoli inflorescence" instead of "broccoli plant".

However, inflorescences are known parts of a plant and this feature cannot confer any inventive step to the claim. Consequently, claims 15-16 lack inventive step for the same reasons as claims 13-14 (see sections 7.10. and 7.11. above).

### 7.13. Claims 17-18 lack inventive step

#### 7.13.1. Claims 17-18 lack inventive step for the same reasons as claims 13-14

The only difference between claims 17-18 and claims 13-14 is that claims 17-18 are drawn to "*Brassica* plant cell" instead of "broccoli plant".

However, *Brassica* plant cell are known from a plant and this feature cannot confer any inventive step to the claim. Consequently, claims 17-18 lack inventive step for the same reasons as claims 13-14 (see sections 7.10. and 7.11. above).

#### 7.13.2. Claims 17-18 lack inventive step in view of D12

D12 can be used as prior art against claims 17-18, as claims 17-18 are not entitled to the date of priority of the opposed patent.

Claim 17 recites a *Brassica* plant having elevated levels of 3-methylsulfinylpropyl glucosinolates, or 4-methylsulfinylbutyl glucosinolates, or both (Abstract of D12, page 605, lines 11-15). The *Brassica* plant is obtained from a hybrid produced by crossing a broccoli breeding line with a wild species (Abstract of D12, lines 13-14). The levels of glucosinolates are elevated above that initially found in broccoli breeding lines (10-fold increase, D2, Abstract, lines 11-12).

D12 does not specifically refer to a plant cell, but it is obvious to the skilled person that cells are present in the *Brassica* plants of D12. Therefore, claim 17 lacks inventive step over D12. The same applies to claim 18, which refers to an inflorescence cell.

## 8. Summary

In view of the above, it is respectfully requested that the patent be revoked in its entirety.

In the name of Syngenta Participations AG



Walter Smolders  
Professional Representative, GA No. 42789

Enclosures: Copy of this Notice of Opposition for Patentee  
References D1 to D12, in duplicate  
Debit order ("Abbuchungsauftrag")

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European Patent Application: 99915886.8  
European Patent: 1069819-B1  
Case: S-E7000

Dear Sirs,

The following fees for the above-mentioned European patent application or the above-mentioned European patent are to be debited from the current account no. 28110232 of the company Syngenta Crop Protection AG at the European Patent Organisation in Munich upon receipt of this payment form:

Type of fee	Amount (EUR)
<b>Opposition Fee</b>	<b>610.--</b>

In the case of incorrect calculation of fees, you are authorized to debit underpaid amounts in the same way, or to credit overpayment of fees.

Yours faithfully

On behalf of Syngenta Crop Protection AG

Walter Smolders  
Professional Representative, GA No. 42789