

(19)



(11)

EP 0 942 643 B2

(12)

NEW EUROPEAN PATENT SPECIFICATION

After opposition procedure

(45) Date of publication and mention
of the opposition decision:
02.11.2016 Bulletin 2016/44

(45) Mention of the grant of the patent:
15.10.2008 Bulletin 2008/42

(21) Application number: **98935398.2**

(22) Date of filing: **15.07.1998**

(51) Int Cl.:
A01H 5/12 (2006.01)

(86) International application number:
PCT/NL1998/000412

(87) International publication number:
WO 1999/003329 (28.01.1999 Gazette 1999/04)

(54) **MULTILEAF LETTUCE**

MEHRBLATTSALATPFLANZE

LAITUE A FEUILLES TRES NOMBREUSES

(84) Designated Contracting States:
AT BE CH DE ES FR GB IT LI NL PT

(30) Priority: **15.07.1997 NL 1006588**

(43) Date of publication of application:
22.09.1999 Bulletin 1999/38

(73) Proprietor: **Rijk Zwaan Zaadteelt en Zaadhandel
B.V.
2678 KX De Lier (NL)**

(72) Inventors:
• **MOOR, Cornelis, Marinus
NL-2681 AN Monster (NL)**
• **BERG, Jurjen, Johannes
NL-4851 TC Ulvenhout (NL)**

(74) Representative: **van Someren, Petronella F. H. M.
et al
Arnold & Siedsma
Bezuidenhoutseweg 57
2594 AC The Hague (NL)**

EP 0 942 643 B2

Description

[0001] The present invention relates to a new head form characteristic in lettuce, to use of the new characteristic in the development of new types of lettuce plant which differ morphologically from the lettuce plants known heretofore, and to heads of lettuce which contain this characteristic and thereby differ morphologically from known heads of lettuce.

[0002] A great diversity of lettuce varieties exists nowadays. At present more than a thousand cultivars are known which can be divided roughly into the groups butterhead lettuce, iceberg lettuce, batavia lettuce, cos lettuce or romaine lettuce, oakleaf lettuce and lollo lettuce. This classification is based on the morphology of the leaf and the degree of head forming. However, other classifications are used in addition to this one. The UPOV list of descriptions of lettuce varieties thus refers for instance to the following six groups: butterhead lettuce, crisp lettuce, cos lettuce, "Grasse" lettuce, cutting lettuce and stem lettuce.

[0003] The known head lettuce, also known as butterhead lettuce, and the iceberg lettuce have the drawback that in these types of lettuce the outer leaves of a head of lettuce are clearly much larger than the inner leaves. For applications wherein cut or peeled whole leaves are used, such as in sandwiches, on hamburgers and the like, leaves of the most uniform possible size are preferred. In the case of heads of lettuce with a clear heterogeneity in leaf size, such as the head-forming lettuce types butterhead lettuce or head lettuce, iceberg lettuce, batavia lettuce, cos lettuce or romaine lettuce, and "Grasse" lettuce, the outer large leaves will either be unusable or will always have to be reduced in size before use by means of cutting or tearing. Browning occurs quite quickly on the cut or torn edges whereby the lettuce loses its attractive appearance and rotting can also occur more rapidly. Similar problems occur in automatic processing into cut lettuce.

[0004] It is the object of the invention to provide a new type of lettuce with which the above described problems can be avoided.

[0005] According to the invention a type of lettuce is now provided according to claim 1 which, in comparison with the known types of lettuce, comprises significantly more leaves of substantially equal dimensions at the ready-to-harvest stage. "Of substantially equal dimensions" is understood to mean that the leaves of this so-called multileaf type have on average a shorter length and lower weight relative to a normal lettuce plant with a comparable leaf morphology and comparable plant weight.

[0006] In the case of butterhead lettuce of the new type, due to the significantly higher number of leaves and the average shorter leaf length, a large number in absolute sense, and a large part in relative sense, of the harvestable leaves falls within the size of 4 to 12 cm leaf length (see Table 1).

[0007] "Significantly more leaves" is understood to mean in this case at least 1½ times as many as a type with a comparable leaf morphology at equal (or comparable) plant weight, preferably at least twice as many, more preferably at least 2½ times as many, most preferably at least 200 leaves of substantially equal dimensions.

[0008] The lettuce provided according to the invention is per se a new type but can have different leaf morphologies which in turn originate from other types of lettuce. The type of lettuce according to the invention is however always "multileaf" lettuce.

[0009] The characteristic "significantly more leaves of substantially equal dimensions" is a new head form characteristic and will be further designated "multileaf characteristic and likewise form part of the invention. The presence of the multileaf characteristic in a lettuce plant can be determined simply by comparing the total number of leaves of a ready-to-harvest lettuce plant suspected of possessing the multileaf characteristic with the total number of leaves of a ready-to-harvest lettuce plant which is comparable in respect of leaf morphology and plant weight but which certainly does not possess the multileaf characteristic (control plant). When the suspected multileaf lettuce plant has at least 1½ times as many leaves as the control plant, it can be stated that the multileaf characteristic is present. In such plants use is in that case made of the characteristic according to the invention. Use has also been made of the characteristic in the manufacture of such plants.

[0010] The multileaf characteristic means in fact that plants have a genetic constitution which enhances the occurrence of fasciation at a determined stage of the vegetative growth. Fasciation in butterhead lettuce occurs at a relatively early stage of the vegetative growth.

[0011] Fasciation, also referred to as band formation, is a per se undesired phenomenon which can occur in many types of vascular plants. It was already described in 1948 by White in "fascination", Bot. Rev. 14, 319-358 (1948). Fasciation in lettuce is characterized by the apex of a stem becoming increasingly wide when it begins to lengthen. A cone-shaped apex normally results. In the case of fasciation however, a flat, wide and comb-like (grooved) band of meristematic tissue is formed. It is known that fasciation in lettuce has a genetic basis and is inherited recessively (Haque & Godward, Genetica-Iberica 38, 139-155 (1986)) or additively (Eenink & Garretsen, Euphytica 29, 653-660 (1980)). In addition, influences of environmental conditions on the expression of fasciation have also been described (Eenink & Garretsen, *supra*).

[0012] Fasciation in lettuce is a phenomenon which has been known for some time and which was long considered mainly as disadvantageous, because lines submitted for registration under the UPOV Treaty often did not react uniformly

to fasciation and because fasciation hinders seed production. Fasciation does however also have a positive side, since fasciated plants are slow-bolting, which is a particular advantage for greenhouse varieties in the summer period.

[0013] According to the invention the phenomenon of fasciation has now been used to arrive at an entirely new type of lettuce. By specific selection it has been possible to increase, respectively advance the degree and the time of fasciation, whereby a fasciated apex is already formed, for instance in butterhead lettuce, at a very early stage of the vegetative growth. The final lettuce plant hereby acquires a rosette-like head which is built up of a very large number of leaves of substantially equal size on a greatly enlarged stem base.

[0014] The advantage of the new lettuce plants according to the invention is that a very large number of leaves of substantially equal dimensions can be cut from one head of lettuce. Moreover, owing to the greatly increased circumference of the stem, the leaves can be cut from the stem at the location of their petiole more easily than in lettuce types with a comparable leaf morphology. Only a limited cut surface hereby results and the leaves themselves remain intact.

[0015] Owing to their substantially equal dimensions, the leaves are particularly suitable for use on for instance sandwiches or hamburgers, or in pre-cut salads.

[0016] Described and shown in the accompanying examples and figures as illustration of the invention are the obtaining (example 1), the degree of expression and inheritance (example 2) and the appearance (figures) of a head of lettuce with a leaf morphology of butterhead lettuce.

[0017] The multileaf characteristic can however also be combined with characteristics of other types of lettuce, such as leaf shape, leaf thickness, serration, leaf colour and leaf consistency (for instance crispiness). These characteristics can be transferred from other lettuce types to the type described herein. This transfer can take place by means of recombinant DNA techniques and/or by conventional crossing-in. The variants which result in this manner likewise fall within the scope of the present invention.

[0018] "Leaf morphology" is understood to mean the whole of the phenotypical characteristics which provide a leaf with the appearance characteristic of the associated lettuce type.

[0019] Figures 1-3 show respectively the top, bottom and cross-section of a head of multileaf butterhead lettuce according to the invention. By way of comparison, figures 4 and 5 show top and bottom views of a head of butterhead lettuce. On the underside of the head of multileaf lettuce is clearly visible that the head possesses a larger number of leaves of smaller dimensions than the head of butterhead lettuce. Figure 6 shows a typical head of multileaf lettuce from which the stem base has been removed. Figure 7 shows a comparison between the removed leaves of a head of butterhead lettuce (top) and a head of multileaf butterhead lettuce according to the invention (below).

[0020] The present invention will be illustrated with reference to the accompanying examples.

EXAMPLE 1

Obtaining butterhead lettuce plants with multileaf characteristic

[0021] A hybridization was made (designated with the number 5358) between two lettuce plants of the butterhead lettuce type. This was a hybridization between two selection lines of applicant. The mother had code 16487 and the father had F4 [1366 OPG x F2 (15336 x Troppo x 1600 x Bizet)].

[0022] In the F2 of this hybridization a plant was found a year later with features such as the plant of the invention. Seed was recovered from this plant and this seed was sown the following year in order to assess the external features on a larger number of progeny plants. Of - these F3 plants a number of plants were selected once again on the basis of the combination of (new) phenotype according to the invention and other quality-determining features (leaf thickness, leaf shape, leaf colour, head shape, head structure, yield, resistance to downy mildew, etc.).

[0023] This process of line selection was continued in the following 10 years, and a pure line was thus obtained (designated with the number 5338). This line is a good combination of a new phenotype according to the invention and the other quality-determining features.

[0024] For a further improvement of the phenotype of the plants of the invention, this line was crossed two years later with the variety S0256. In the progeny of this hybridization a rigorous selection was once again made from the F2 for plants with a very large number of leaves of substantially equal dimensions, combined with attractive leaf and head features and other features desirable for the culture of lettuce. After plant selection in the F2 and line selection from the F3 generation, a sufficiently pure line was finally obtained three years later, which is designated with the number RZ 97.41561, and seed of which was deposited on 1 April 1997 at The National Collections of Industrial and Marine Bacteria Limited (23 St. Machar Drive, Aberdeen AB2 1RY, Scotland, UK) under number NCIMB 40877 in order to illustrate the invention.

EXAMPLE 2

Degree of expression and inheritance of the multileaf characteristic

5 **[0025]** Plants of several standard varieties of the butterhead lettuce type for greenhouse culture (Floris RZ, Vegas RZ and Limit RZ), of two multileaf lines (No 5338 and RZ 97.41561) and of an F2 between a normal butterhead lettuce line and the multileaf line No 5338 were sown and further cultivated. The cultivation took place under a standard cultivation regime for greenhouse lettuce. The observations are an average of 4 plants per variety or line. The plants were prepared as for auction before assessment, i.e. old leaves with deposits or yellowed leaves were removed.

10

Table 1. Average head weight in grams (\pm SE), number of leaves (N) with a leaf length greater than 4 cm distributed over a number of length classes (\pm SE), and the quotient of the average head weight and the total number of leaves longer than 4 cm (\pm SE) of three butterhead lettuce varieties (not multileaf) and two multileaf lines.

15

20

25

30

Variety/ line	Multileaf ?	WEIGHT (grams)	N 4-8cm	N 8-12cm	N 12-16cm	N >16cm	Ntotal (>4 cm)	% in size 4-12 cm	WEIGHT/ Ntotal
Floris RZ	no	383 \pm 33	7.8	9.8	16.5	0.0	34.0 \pm 3.1	52	11.3 \pm 0.33
Vegas RZ	no	464 \pm 80	8.8	8.5	21.5	5.0	43.7 \pm 4.6	40	10.6 \pm 0.94
Limit RZ	no	410 \pm 34	19.8	10.5	23.3	5.8	59.2 \pm 5.6	51	6.9 \pm 0.84
No 5338	yes	419 \pm 39	44.8	43.5	50.5	0.0	138.8 \pm 25.3	68	3.0 \pm 0.25
RZ 97.41561	yes	430 \pm 29	168.0	87.8	19.5	0.0	275.3 \pm 35.2	93	1.6 \pm 0.18

35

[0026] Table 1 illustrates the differences which can be found in commercial (non-multileaf) butter lettuce varieties for the number of leaves longer than 4 cm: Floris RZ has relatively few leaves, the variety Limit RZ has almost twice as many in the category longer than 4 cm. The variety Limit RZ was included in this experiment because it was known that this variety forms relatively many leaves. However, the multileaf line No 5338 has more than twice as many leaves in the measured category than Limit RZ, and the line RZ 97.41561 even has four times as many. This increase in the number of leaves is accompanied by a sharp increase (both absolutely and in percentage terms) of leaves in the length category of 4-12 cm and a sharp decrease in the average weight per leaf.

40

[0027] 76 plants of the F2 (butterhead lettuce x No 5338) were assessed. The butterhead lettuce which was used for crossing closely resembled Vegas RZ in respect of morphology. Table 2 gives the frequency distribution for the number of leaves longer than 4 cm per head of lettuce. Table 3 shows the frequency distribution for the quotient of the head weight and the number of leaves longer than 4 cm.

45

Table 2. Frequency distribution for the number of leaves longer than 4 cm of 76 plants of an F2 population from a hybridization between a butterhead lettuce line (not multileaf) and No 5338 (multileaf).

50

55

Class: number of leaves longer than 4 cm	Number of plants
< 40	0
40-50	7
50-60	24
60-70	20
70-80	6
80-90	2
90-100	5

EP 0 942 643 B2

(continued)

Class: number of leaves longer than 4 cm	Number of plants
100-110	1
110-120	2
120-130	2
130-140	1
140-150	2
150-160	2
160-170	1
> 170	1
> Total	76

Table 3. Frequency distribution of the quotient of the head weight and the number of leaves longer than 4 cm of 76 plants of an F2 population from a hybridization between a butterhead lettuce line (not multileaf) and No 5338 (multileaf).

Class: Head weight/Number of leaves > 4 cm	Number of plants
< 3.0	0
3.0-3.5	8
3.5-4.0	2
4.0-4.5	5
4.5-5.0	2
5.0-5.5	2
5.5-6.0	2
6.0-6.5	0
6.5-7.0	1
7.0-7.5	5
7.5-8.0	11
8.0-8.5	16
8.5-9.0	12
9.0-9.5	8
9.5-10.0	2
> 10	0
Total	76

[0028] Table 2 shows that the number of leaves larger than 4 cm per plant has a continuous distribution with a peak at 50-60 leaves larger than 4 cm per plant and with 17 plants having more than 90 leaves larger than 4 cm. These latter 17 plants were all of the multileaf type. The fact that no discontinuous segregation of the multileaf characteristic was obtained may be because the plants differed mutually in plant size and weight. The average head weight of the F2 plants varied from 350 to 655 g. In contrast to the number of leaves per plant, the quotient of head weight and number of leaves does give a discontinuous distribution, with 21 plants having a quotient smaller than 6.0 (all of the multileaf type) and 55 plants having a quotient greater than 6.5 (all of the normal type). The segregation corresponds with a monogenic recessive inheritance of the multileaf characteristic.

[0029] Within the group of plants of the multileaf type a further segregation was found, which indicates that the degree to which a plant has the multileaf characteristic is also determined by other genes. This is also supported by the fact

that it was possible, starting from line No 5338 (an average of 139 leaves longer than 4 cm, see Table 1), to obtain a line via hybridization and specific selection having an average of 275 leaves longer than 4 cm (RZ 97.41561, see Table 1).

5 **Claims**

1. Multileaf lettuce plant of the genus *Lactuca* of a particular type having the heritable monogenic recessive multileaf characteristic, which plant is obtainable by conventional crossing-in using a plant of line RZ 97.41561 having the deposit accession number NCIMB 40877 and a second plant, said multileaf plant having at least 1.5 times as many leaves at the ready-to-harvest stage as a lettuce plant of the same type and equal plant weight.
2. Lettuce plant as claimed in claim 1 having at least twice as many leaves.
3. Lettuce plant as claimed in claim 1 or 2 having at least 2.5 times as many leaves.
4. Lettuce plant as claimed in any one of the claims 1-3 having at least 200 leaves.
5. Lettuce plant as claimed in any one of the claims 1-4 which is of the type butterhead lettuce.
6. Multileaf lettuce seed of a lettuce plant as claimed in any one of the claims 1-5, which seed is able to grow into a plant having at least 1.5 times as many leaves at the ready-to harvest stage and a determined head weight as a non-multileaf lettuce plant of the same type, which is caused by the heritable monogenic recessive multileaf characteristic.
7. Head of a multileaf lettuce plant as claimed in any one of the claims 1-5 or as grown from seed as claimed in claim 6.
8. Head of lettuce as claimed in claim 7, **characterized in that** the lettuce type is butterhead lettuce.

30 **Patentansprüche**

1. Mehrblattsalatpflanze der Gattung *Lactuca* eines speziellen Typs, der eine vererbare monogene rezessive Mehrblattcharakteristik aufweist, wobei die Pflanze durch konventionelles Einkreuzen unter Verwendung einer Pflanze der Linie RZ 97.41561 mit der Hinterlegungsnummer NCIMB 40877 und einer zweiten Pflanze erhältlich ist, wobei die Mehrblattpflanze im erntebereiten Stadium zumindest 1,5 Mal so viele Blätter hat wie eine Salatpflanze desselben Typs und gleichen Pflanzengewichts.
2. Salatpflanze nach Anspruch 1, die zumindest doppelt so viele Blätter hat.
3. Salatpflanze nach Anspruch 1 oder 2, die zumindest 2,5 Mal so viele Blätter hat.
4. Salatpflanze nach irgendeinem der Ansprüche 1 bis 3, die zumindest 200 Blätter hat.
5. Salatpflanze nach irgendeinem der Ansprüche 1 bis 4, die vom Typ Kopfsalat ist.
6. Mehrblattsalatsaat einer Salatpflanze wie beansprucht in irgendeinem der Ansprüche 1 bis 5, wobei die Saat in der Lage ist, zu einer Pflanze auszuwachsen, die zumindest 1,5 Mal so viele Blätter im erntebereiten Stadium und ein bestimmtes Kopfgewicht wie eine nicht mehrblättrige Salatpflanze desselben Typs hat, hervorgerufen durch die vererbare monogene rezessive Mehrblattcharakteristik.
7. Kopf einer Mehrblattsalatpflanze wie beansprucht in irgendeinem der Ansprüche 1 bis 5 oder gezogen aus Saat wie beansprucht in Anspruch 6.
8. Kopf eines Salats wie beansprucht in Anspruch 7, **dadurch gekennzeichnet, dass** der Salattyp Kopfsalat ist.

Revendications

- 5
1. Plant de laitue à multifeuilles du genre *Lactuca* d'un type particulier, ayant la caractéristique multifeuilles à récessivité monogénique héréditaire, lequel est susceptible d'être obtenue par croisement conventionnel en utilisant un plant de la lignée RZ 97.41561 ayant le numéro d'accès au dépôt NCIMB 40877 et un second plant, ledit plant à multifeuilles comprenant au moins 1,5 fois plus de feuilles au stade de récolte qu'un plant de laitue du même type présentant le même poids de plant.

10

 2. Plant de laitue selon la revendication 1, comprenant au moins 2 fois plus de feuilles.
 3. Plant de laitue selon la revendication 1 ou 2, comprenant au moins 2,5 fois plus de feuilles.
 4. Plant de laitue selon l'une quelconque des revendications 1 à 3, comprenant au moins 200 feuilles.

15

 5. Plant de laitue selon l'une quelconque des revendications 1 à 4 laquelle est du type laitue beurre.
 6. Graine de laitue à multifeuilles d'un plant de laitue selon l'une quelconque des revendications 1 à 5, laquelle est capable de donner un plant comprenant au moins 1,5 fois plus de feuilles au stade de récolte et a un poids de tête déterminé comme un plant de laitue non-multifeuilles du même type, ceux qui sont donnés par la caractéristique multifeuilles à récessivité monogénique héréditaire.

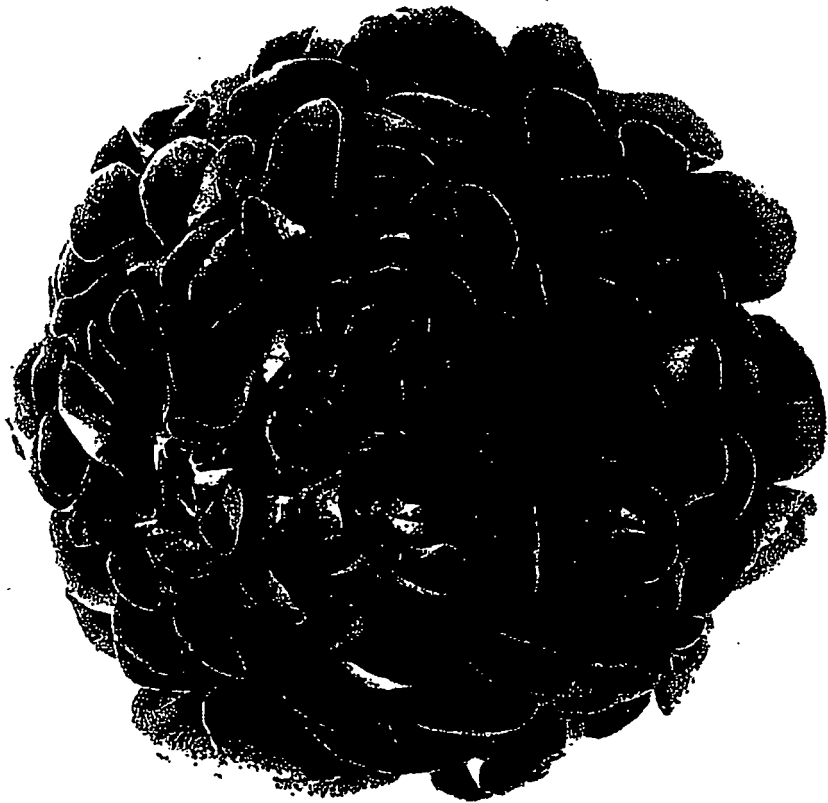
20

 7. Tête de plant de laitue multifeuilles selon l'une quelconque des revendications 1 à 5 ou tel qu'obtenue à partir de la graine selon la revendication 6.

25

 8. Tête de laitue selon la revendication 7, **caractérisée en ce que** le type de laitue est la laitue beurre.
- 30
- 35
- 40
- 45
- 50
- 55

FIG.1



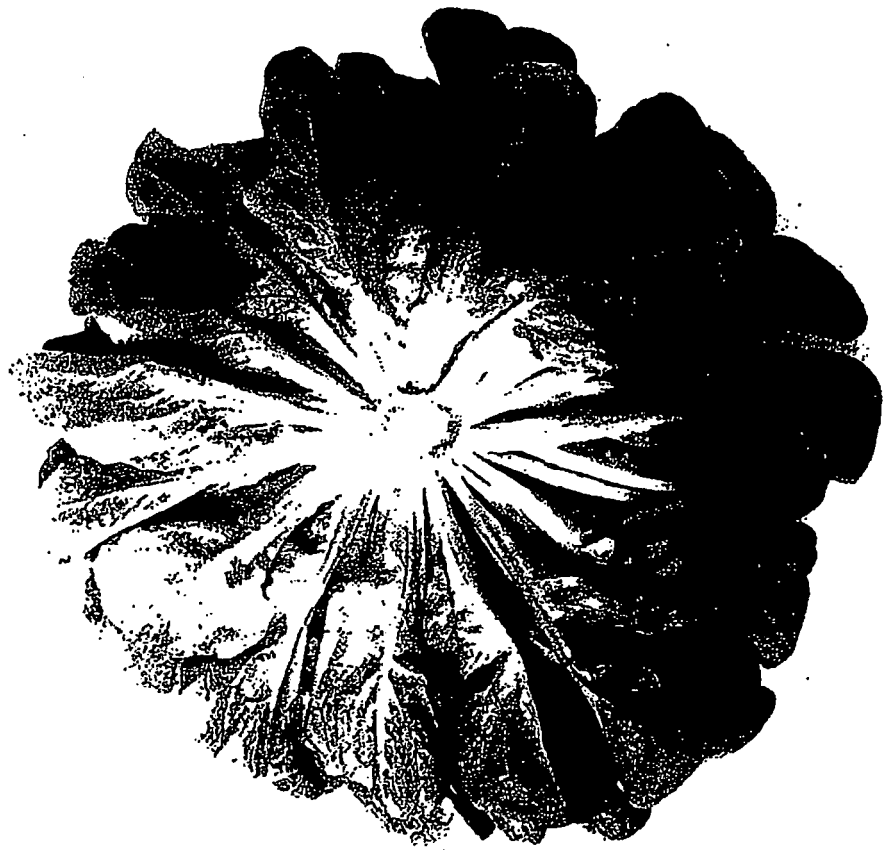


FIG.2



FIG. 3

FIG.4



FIG.5



FIG.6



FIG. 7



REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Non-patent literature cited in the description

- **WHITE**. *fascination. Bot. Rev.*, 1948, vol. 14, 319-358 [0011]
- **HAQUE ; GODWARD**. *Genetica-Iberica*, 1986, vol. 38, 139-155 [0011]
- **EENINK ; GARRETSEN**. *Euphytica*, 1980, vol. 29, 653-660 [0011]