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(54) Title: LETTUCE THAT IS RESISTANT TO THE LETTUCE APHID NASONOVIA RIBISNIGRI BIOTYPE 1

(57) Abstract: The present invention relates to a lettuce (*Lactuca sativa*) plant, which has the resistance against *Nasonovia ribisnigri* biotype 1 (Nr: 1) as found in plants grown from seeds of *Lactuca serriola* 1OG.913571 representative seeds of which were deposited under NCIMB accession number 41775. Such a lettuce plant is obtainable by introgressing the resistance to *Nasonovia ribisnigri* Nr: 1 as found in *Lactuca serriola* 1OG.913571 representative seeds of which were deposited under NCIMB accession number 41775 into a plant that is not resistant.

**LETTUCE THAT IS RESISTANT TO THE LETTUCE APHID  
NASONOVIA RIBISNIGRI BIOTYPE 1**

The present invention relates to lettuce plants and  
5 heads that are resistant to *Nasonovia ribisnigri* biotype 1  
(also called herein Nr:1) as well as to progeny of the  
plants and propagation material for producing the plants.  
The invention further relates to a source of the resistance  
for use in breeding.

10           The lettuce aphid (*Nasonovia ribisnigri* (Mosley))  
is a major pest occurring in lettuce worldwide. The problem  
started to be severe for lettuce production in the 70s in  
North Western Europe and spread rapidly all across Europe.  
Then, in the 80s, the aphid was detected in Canada. Later  
15 on, the problem was reported in the USA (California and  
Arizona). More recently, the lettuce aphid was found in New  
Zealand and Australia.

Lettuce aphids can colonize lettuce plants at any  
plant stage and feed preferably from younger leaves. Large  
20 amount of aphids on the plant are able to reduce plant  
growth and deform the shape of the head so that the lettuce  
heads are then not marketable. The presence of high amounts  
of aphids in lettuce heads is a reason for retailers to  
refuse to buy lettuce from growers. At young plant stage, it  
25 is possible to control the lettuce aphid using insecticide.  
Several products were reported efficient to control aphid  
population. However, resistances to chemicals were reported  
in some aphid population. Moreover, at maturity, it is not  
possible to control aphids using insecticides as chemical  
30 products cannot enter into the lettuce head.

One of the most valuable strategies to control  
lettuce is genetic resistance. Extensive gene bank screening  
was performed and some *Lactuca virosa* accessions were found

completely resistant to *Nasonovia ribisnigri* (Eenink and Dieleman, *Euphytica* 32(3), 691-695 (1982)). However, *Lactuca virosa* is in the third gene pool of the *Lactuca* germplasm according to the definition of Harlan. Therefore, these interspecific crosses are sterile, and the use of bridge species (as *L. serriola*) was necessary to transfer the resistance into *L. sativa*. Genetic analyses showed that the resistance to *Nasonovia ribisnigri* was controlled by a single dominant gene (*Nr* gene) in a *L. sativa* background.

10                However, breeders experienced that the release of varieties resistant to lettuce aphid was not straightforward. The *Nr*-resistance gene was found tightly linked to recessive genes conferring strong negative side-effects. Such plants showed a reduced growth, a pale green colour and a lack of fertility in seed set. Using large-sized progeny and molecular markers enabled lettuce breeders to find resistant recombinant plants without the negative side-effect phenotype (see EP-0 921 720). These resistant plants served as the source of the resistance gene that was not linked to the negative side-effect phenotype.

                  After this finding, the release of varieties resistant to *Nasonovia* became more and more important. The resistance became a major requirement for outdoor lettuce production for processing and also for fresh market.

25                In 2007, populations of lettuce aphids able to infect varieties resistant to *Nasonovia ribisnigri* were found in four distinct areas in Europe (France and Germany but also in Belgium and Austria). Four isolates (two from France and two from Germany) were analysed further by the Netherlands Inspection Service for Horticulture (also known as Naktuinbouw). They concluded the existence of a new *Nasonovia ribisnigri* biotype. This biotype is officially named *Nr:1* and is able to overcome the *Nr* resistance gene.

The Nr:0 biotype of the aphid can, however, still be efficiently controlled by the *Nr* gene.

The presence of Nr:1 *Nasonovia ribisnigri* organisms on lettuce has the same disadvantages as described above for  
5 Nr:0.

It is therefore the object of the present invention to provide a new source of *Nasonovia ribisnigri* resistance that is active against Nr:1.

According to the present invention a source of Nr:1  
10 resistance is provided in the form of *Lactuca serriola* plants of which representative seed was deposited with the NCIMB and given accession number NCIMB 41776. The plants are further identified herein with the reference number 10G.913571. Plants carrying this number are grown from seed  
15 lot 10G.913571 of which a part was deposited under the above accession number.

The invention thus relates to a cultivated lettuce (*Lactuca sativa*) plant, which has the resistance against *Nasonovia ribisnigri* biotype 1 (Nr:1) as found in plants  
20 grown from seeds of *Lactuca serriola* 10G.913571, representative seeds of which were deposited on 16 November 2010 with NCIMB Ltd, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB21 9YA, UK under deposit accession number NCIMB 41776.

25 A lettuce plant of the invention is obtainable by introgressing the resistance to *Nasonovia ribisnigri* Nr:1 as found in *Lactuca serriola* 10G.913571, representative seeds of which were deposited with the NCIMB under NCIMB accession number 41776, into a plant that is not resistant.  
30 "Introgression" as used herein is intended to mean introduction of a Nr:1 resistance into a plant not carrying the Nr:1 resistance by means of crossing and selection. Introgression is not limited to one cross only but

encompasses the minimum number of generations needed for the plant to become resistant, preferably stably resistant.

A lettuce plant of the invention, which is resistant to *Nasonovia ribisnigri* biotype 1 (Nr:1), is  
5 obtainable by crossing a lettuce plant with a plant grown from seeds of *Lactuca serriola* 10G.913571, representative seeds of which were deposited with the NCIMB under NCIMB accession number 41776, and selecting in the progeny of the cross for plants showing the resistance. In one embodiment,  
10 selection is made in the F2 progeny that is obtained after selfing the F1 of the cross between the plant grown from deposited seed and the susceptible plant. Since the resistance is dominant, plants resistant against Nr:1 can also be found in the F1 progeny.

15 The invention further relates to seed of a lettuce plant as claimed and to progeny of the lettuce plant or the lettuce seeds.

According to a further aspect thereof, the invention relates to propagation material suitable for  
20 producing a plant as claimed. In one embodiment, such propagation material is formed by seeds.

In one embodiment, the propagation material is formed by parts of the plant that are suitable for sexual reproduction, in particular microspores, pollen, ovaries,  
25 ovules, embryo sacs and egg cells.

In one embodiment, the propagation material is formed by parts of the plant that are suitable for vegetative reproduction, in particular cuttings, roots, stems, cells and protoplasts, tissue cultures of regenerable  
30 cells.

In one embodiment, the propagation material is formed by parts of the plant that are suitable for preparing tissue cultures, in particular leaves, pollen, embryos,

cotyledons, hypocotyls, meristematic cells, roots, root tips, anthers, flowers, seeds and stems.

Suitably, the plant produced from the propagation material shows the resistance to *Nasonovia ribisnigri* biotype 1 (Nr:1) according to the invention, in particular the resistance as found in *Lactuca serriola* 10G.913571, representative seeds of which were deposited with the NCIMB under NCIMB accession number 41776.

The invention further relates to the head of a lettuce plant as claimed. The invention further relates to a food product, comprising the lettuce head or parts thereof. In particular, the food product comprises leaves of the lettuce plant or parts thereof. The food product is for example a salad or a salad mixture comprising leaves of the lettuce plant of the invention.

According to a further aspect thereof the invention relates to the use of *Lactuca serriola* 10G.913571, representative seeds of which were deposited under NCIMB accession number 41776, in breeding to confer resistance against *Nasonovia ribisnigri* biotype 1 (Nr:1) to plants that are susceptible to *Nasonovia ribisnigri* biotype 1 (Nr:1).

The invention also relates to *Lactuca serriola* 10G.913571, representative seeds of which were deposited under NCIMB accession number 41776.

In one embodiment, the invention relates to lettuce plants of the species *Lactuca sativa* obtainable by crossing a first lettuce parent plant with a second lettuce parent plant that has the Nr:1 resistance as found in plants of which representative seed was deposited under deposit accession number NCIMB 41776 to obtain an F1 and selecting plants from the F2 that have the Nr:1 resistance as plants of the invention. In one embodiment, plants of the F1 are subsequently selfed to obtain an F2 and selecting plants

from the F2 that have the Nr:1 resistance as plants of the invention. The Nr:1 resistance is transferred in a pattern consistent with dominant inheritance.

In one embodiment, the second lettuce parent plant is a plant grown from the deposited seeds. In one embodiment the second lettuce parent plant is a progeny plant of a plant grown from the deposited seeds. In one embodiment the second lettuce parent plant is a plant having the genetic information that encodes the Nr:1 resistance. In one embodiment this genetic information is substantially identical to the genetic information that encodes the Nr:1 resistance as found in plants of which representative seeds were deposited under accession number NCIMB 41776, in particular plants grown from seeds as deposited under deposit accession number NCIMB 41776.

In one embodiment, the invention thus relates to a lettuce plant, showing the Nr:1 resistance of the invention, which plant is obtainable by:

- a) growing plants representative seed of which was deposited under accession number NCIMB 41776;
- b) crossing a plant from step a) with a lettuce plant that does not have the Nr:1 resistance to obtain an F1 population;
- c) selfing plants from the F1 to obtain a F2 population; and
- d) identifying plants showing the Nr:1 resistance in the F2 population as Nr:1 resistant lettuce plants.

In the method described above steps c) and d) can be repeated one or more times by selfing an F<sub>n</sub> population to obtain an F<sub>n+1</sub> population and identifying plants showing the Nr:1 resistance in the F<sub>n+1</sub> population as Nr:1 resistant lettuce plants.

The Nr:1 resistance is phenotypically visible because plants will not be attacked by aphids of the Nr:1 biotype. The Nr:1 resistance is however caused by the plant's genotype. The genotype as far as it concerns the  
5 Nr:1 resistance is the same or a similar genotype as found in the deposited seeds. The part of the genotype of a lettuce plant that causes the Nr:1 resistance will be called herein the "genetic information that encodes the Nr:1  
10 resistance". Presence of this genetic information is phenotypically visible in a resistance test and plants having this genetic information can thus be selected on the basis of this phenotypic expression of the underlying gene or genes. As used herein "genetic information" is intended to mean the gene or genes that are responsible for the  
15 resistance.

The presence of the genetic information that is responsible for the Nr:1 resistance of the invention in the genome of a resistant plant can be determined with the following test. The plant to be tested should be or should  
20 be made homozygous for the genetic information responsible for the Nr:1 resistance. The skilled person knows how to obtain a plant that is homozygous for the Nr:1 resistance to be tested.

This plant is then crossed with a tester plant that  
25 carries the genetic information that is responsible for the Nr:1 resistance of the invention in homozygous condition. If the plant to be tested has the resistance as a result of the same genetic information that is responsible for the resistance of the invention, all progeny plants of the first  
30 cross and successive generations will be resistant. If the resistance of the plant to be tested is the result of a different part of the genome, e.g. another gene or locus, segregation will occur from the F2 onward. The tester plant

can be any plant that carries the genetic information of the invention in homozygous condition, such as plants of which representative seed was deposited under accession number NCIMB 41776 or plants directly grown from the deposited seeds or progeny thereof that has retained the resistance.

The resistance to *Nasonovia ribisnigri* biotype 1 (Nr:1) of the invention is at a locus corresponding to the locus comprised in plants grown from seed of seed lot 10G.913571, a representative sample of which was deposited with NCIMB under accession number NCIMB 41776, and preferably co-segregates with the resistance present in plants grown from seed lot 10G.913571.

The locus conferring the resistance to *Nasonovia ribisnigri* biotype 1 (Nr:1) is obtainable from *Lactuca serriola* 10G.913571 plants, representative seeds of which have the accession number NCIMB 41776, or from a progeny or ancestor of plants of 10G.913571 plants comprising said resistance. In another preferred embodiment, the locus for the resistance is derived or obtained from *Lactuca serriola* 10G.913571, or from a progeny or ancestor of *Lactuca serriola* 10G.913571 comprising said resistance.

The present invention further relates to a lettuce plant comprising a resistance to *Nasonovia ribisnigri* biotype 1 (Nr:1), wherein when said plant is homozygous for said resistance and said plant homozygous for said resistance is crossed with a tester plant homozygous for a monogenic and dominant resistance to *Nasonovia ribisnigri* biotype 1 (Nr:1), the plants of the first generation progeny resulting from said cross show a 1:0 segregation for the resistance.

When said plants of said first generation progeny are self-pollinated, plants of the resulting second generation show a 3:1 segregation for the resistance.

In one embodiment, the tester plant is a plant derived from *Lactuca serriola* 10G.913571 plants, representative seed of which has accession number NCIMB 41776 and comprises the monogenic and dominant resistance to *Nasonovia ribisnigri* biotype 1 (Nr:1) of the invention, or a progeny or ancestor plant thereof that has the resistance locus. In one embodiment, the tester plant is derived from *Lactuca serriola* 10G.913571 or a progeny plant thereof by cell fusion.

10 The present invention further relates to a lettuce plant comprising a resistance to *Nasonovia ribisnigri* biotype 1 (Nr:1), wherein when said plant is heterozygous for said resistance and said plant heterozygous for said resistance is crossed with a tester plant heterozygous for a monogenic and dominant resistance to Nr:1, plants of the first generation progeny resulting from said cross show a 3:1 segregation for resistance to Nr:1.

In one embodiment, when said resistant plants of said first generation progeny are further crossed with said plant heterozygous for said resistance, plants of the resulting second generation progeny show a 5:1 segregation for resistance to Nr:1. In a further preferred embodiment, the tester plant is a *Lactuca serriola* 10G.913571 plant, representative seed of which has the accession number NCIMB 41776, or a progeny or ancestor plant thereof comprising the monogenic and dominant resistance to Nr:1 comprised in said *Lactuca serriola* 10G.913571 plants.

In one embodiment, when progenies of individual plants are scored, the resulting second generation progeny shows a 3:1, 1:0 or 1:1 segregation ratio for the resistance, depending on the genetic status of the individual plants in the first generation offspring

(heterozygous, homozygous resistant or homozygous susceptible respectively).

The resistance to Nr:1 of the invention is monogenic, preferably monogenic and dominant. In one  
5 embodiment, the *Lactuca sativa* plant is homozygous for the resistance. In another embodiment, the *Lactuca sativa* plant is heterozygous for the resistance.

The Nr:1 resistance is independent of other Nr:1 resistances of a lettuce plant. The resistance of the  
10 invention can thus occur in lettuce plants that are completely different from the deposited plant in all their other characteristics, for example in different lettuce varieties.

It is clear that a parent that provides the Nr:1  
15 resistance of the invention is not necessarily a plant grown directly from the deposited seeds. The parent can also be a progeny plant from the seed or a progeny plant from seeds that are identified to have or to have acquired the Nr:1 resistance of the invention by other means.

20 In one embodiment, the invention relates to lettuce plants that carry the resistance of the invention and have acquired said resistance by introduction of the genetic information that is responsible for the resistance from a suitable source, in particular *Lactuca serriola* 10G.913571,  
25 either by conventional breeding, or genetic modification, in particular by cisgenesis or transgenesis. Cisgenesis is genetic modification of plants with a natural gene, coding for Nr:1 resistance, from the crop plant itself or from a sexually compatible donor plant. Transgenesis is genetic  
30 modification of a plant with a gene from a non-crossable species or a synthetic gene.

In one embodiment, the source from which the genetic information is acquired is formed by plants grown

from the deposited seeds of NCIMB 41776 or sexual or vegetative descendants thereof.

According to another aspect of the invention *Lactuca sativa* plants are provided that have all of the morphological and physiological characteristics of Nr:1 resistant lettuce plants of the invention, representative seed of which have the accession number NCIMB 41776, which plants are grown from seeds of a plant of the invention or regenerated from parts thereof, or from a tissue culture. Plants of the invention should have the morphological and physiological characteristics that correspond with the Nr:1 resistance but do not necessarily have all the other characteristics of plants of the deposited seeds. The Nr:1 resistance is broadly transferrable over multiple lettuce types and varieties.

The invention also relates to progeny of the lettuce plants of the invention. Such progeny can be produced by sexual or vegetative reproduction of a plant of the invention or a progeny plant thereof. The regenerated progeny plant shows the resistance in the same or a similar way as the plant, of which representative seed was deposited (NCIMB 41776). This means that such progeny has the same characteristics as claimed for the lettuce plants of the invention. In addition to this, the plant may be modified in one or more other characteristics. Such additional modifications are for example effected by mutagenesis or by transformation with a transgene or cisgene. Alternatively, modifications in characteristics other than the Nr:1 resistance can be introduced by introducing the Nr:1 resistance in a different background. In the first case the resistant plant is taken as the original plant and modified. In the latter case the resistance is transferred to another plant, in particular a susceptible plant.

As used herein the word "progeny" is intended to mean the offspring or the first and all further descendants from a cross with a plant of the invention that is Nr:1 resistant. Progeny of the invention are descendants of any cross with a plant of the invention that carries the Nr:1 resistance. Progeny plants are preferably also resistant.

"Progeny" also encompasses plants that carry the Nr:1 resistance of the invention which are obtained from other plants of the invention by vegetative propagation or multiplication.

The invention further relates to cells of Nr:1 resistant plants as described herein. The cells comprise the genetic information, i.e. gene(s) or locus/loci, that leads to the Nr:1 resistance as described herein. Suitably, this genetic information is substantially identical, preferably completely identical to the genetic information encoding the Nr:1 resistance of plants that have all of the morphological and physiological characteristics pertaining to the Nr:1 resistance of lettuce plants of the invention, of which representative seed has the accession number NCIMB 41776. Preferably, the cell of the invention is part of a plant or plant part, but the cell may also be in isolated form.

In one embodiment the plants of the invention are plants grown from seeds having the deposit accession number NCIMB 41776.

In one embodiment the plants of the invention are progeny plants of plants grown from seeds having the deposit accession number NCIMB 41776 that carry the Nr:1 resistance.

In one embodiment the plants of the invention are plants that carry in their genome the genetic information that is responsible for the Nr:1 resistance.

The invention, furthermore, relates to hybrid seed and to a method of producing hybrid seed comprising crossing

a first parent plant with a second parent plant and harvesting the resultant hybrid seed. In order for the hybrid seed to express the Nr:1 resistance of the invention, at least one of the parent plants need to be homozygous for the Nr:1 resistance but is not necessarily uniform for other characteristics.

The invention also relates to the germplasm of plants of the invention. The germplasm is constituted by all inherited characteristics of an organism and according to the invention encompasses at least the Nr:1 resistance of the invention.

In this specification the term "Nr:1 resistance" is intended to mean having the genetic information (in particular a gene, genes, locus, loci, allele or alleles) that in heterozygous or homozygous state leads to the plant no longer being susceptible to *Nasonovia ribisnigri* biotype 1 (Nr:1) and which resistance is as found in plants of which representative seeds were deposited under accession number NCIMB 41776, in particular in plants grown from the deposited seeds.

The invention will be further illustrated in the following example.

#### **EXAMPLE**

*Protocol of the young-plant test*

In order to test whether a plant is resistant to Nr:1 the following test is performed.

Seeds are sown in potting compost blocks. Twenty plants per line were evaluated. The young plants are inoculated 2 weeks after sowing by pouring aphids of the Nr:1 biotype on the top of the plants. One week after inoculation, the test is scored. The second and final score occurs two weeks after inoculation.

*Nasonovia ribisnigri* isolates

The Nr:1 isolate used in this example was sampled in 2007 from Köningen (Germany) on a Nr:0 resistant variety Estelle (Nunhems): sample 279. The aphid isolate is maintained on the variety Kitare (Nr:0 resistant variety, Rijk Zwaan).

*Introduction of the Nr:1 resistance into cultivated lettuce*

Nr:1 resistant *L. serriola* 10G.913571 plants (called herein "the donors") were used to introgress the Nr:1 resistance into cultivated lettuce (*L. sativa*).

These donors were crossed to a cultivated lettuce variety. Cultivated varieties belonging to different lettuce types are used. Many F1 seeds were obtained. The F1s obtained were then crossed with the different varieties used in the original F1 (also called the "recurrent parents"). The BC1F1 population is propagated into BC1F2 by one cycle of inbreeding. Then, the different BC1F2 populations are placed in a Nr:1 disease resistance test as described above. At the end of the test, Nr:1 resistant plantlet are selected.

These selected plants are crossed again to the cultivated lettuce varieties (recurrent parents). The F1s obtained are propagated into F2 generation and the F2 populations are again placed in the Nr:1 disease test.

Resistant F2 plants are selected and crossed again to the recurrent parent. By performing this recurrent breeding process (also named backcrossing) the agronomic properties of the breeding material is improved and in the end the Nr:1 resistance in a cultivated lettuce is obtained.

**CLAIMS**

1. Lettuce (*Lactuca sativa*) plant, which has the resistance against *Nasonovia ribisnigri* biotype 1 (Nr:1) as  
5 found in plants grown from seeds of *Lactuca serriola* 10G.913571 representative seeds of which were deposited under NCIMB accession number 41776.

2. Lettuce plant as claimed in claim 1, obtainable by introgressing the resistance to *Nasonovia ribisnigri*  
10 Nr:1 as found in *Lactuca serriola* 10G.913571 representative seeds of which were deposited under NCIMB accession number 41776 into a plant that is not resistant.

3. Lettuce plant as claimed in claim 1 or 2, which is resistant to *Nasonovia ribisnigri* biotype 1 (Nr:1), and  
15 which plant is obtainable by crossing a lettuce plant with a plant grown from seeds of *L. serriola* 10G.913571 representative seeds of which were deposited with the NCIMB under NCIMB accession number 41776 and selecting in the F2 progeny of the cross that is obtained after selfing the F1  
20 for plants showing the resistance.

4. Seed of a lettuce plant as claimed in any one of the claims 1-3.

5. Seed as claimed in claim 4, wherein the plant that can be grown from the seed is resistant to *Nasonovia*  
25 *ribisnigri* biotype 1 (Nr:1).

6. Progeny of a lettuce plant or lettuce seeds as claimed in any one of the claims 1 to 5, wherein the progeny is resistant to *Nasonovia ribisnigri* biotype 1 (Nr:1).

7. Propagation material suitable for producing a  
30 plant as claimed in any one of the claims 1-6.

8. Propagation material as claimed in claim 7, which propagation material is formed by seeds.

9. Propagation material as claimed in claim 7, which propagation material is formed by parts of the plant that are suitable for sexual reproduction, in particular microspores, pollen, ovaries, ovules, embryo sacs and egg  
5 cells.

10. Propagation material as claimed in claim 7, which propagation material is formed by parts of the plant that are suitable for vegetative reproduction, in particular cuttings, roots, stems, cells and protoplasts, tissue  
10 cultures of regenerable cells.

11. Propagation material as claimed in claim 7, which propagation material is formed by parts of the plant that are suitable for preparing tissue cultures, in particular leaves, pollen, embryos, cotyledons, hypocotyls,  
15 meristematic cells, roots, root tips, anthers, flowers, seeds and stems.

12. Propagation material as claimed in any one of the claims 7-11, wherein the plant produced from the propagation material is resistant to *Nasonovia ribisnigri*  
20 biotype 1 (Nr:1)

13. Head of a lettuce plant as claimed in any one of the claims 1-6.

14. Food product, comprising the lettuce head of claim 13 or parts thereof.

25 15. Food product as claimed in claim 14, wherein the parts are leaves or parts thereof.

16. Use of *Lactuca serriola* 10G.913571, representative seeds of which were deposited under NCIMB accession number 41776, in breeding to confer resistance  
30 against *Nasonovia ribisnigri* biotype 1 (Nr:1) to plants that are susceptible to *Nasonovia ribisnigri* biotype 1 (Nr:1).

17. *Lactuca serriola* 10G.913571 representative seeds of which were deposited under NCIMB accession number 41776.