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(54) **LETTUCE VARIETY 41-177 RZ**

(71) Applicant: **RIJK ZWAAN ZAADTEELT EN ZAADHANDEL B.V.**, De Lier (NL)

(72) Inventor: **Adrianus Martinus Jozef Ammerlaan**, Aramon (FR)

(73) Assignee: **RIJK ZWAAN ZAADTEELT EN ZAADHANDEL B.V.**, De Lier (NL)

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C12Q 1/68 (2006.01)

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(58) **Field of Classification Search**
None
See application file for complete search history.

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Primary Examiner — Eileen B O Hara
(74) *Attorney, Agent, or Firm* — Vedder Price P.C.; Thomas J. Kowalski; Deborah L. Lu

(57) **ABSTRACT**

The present invention relates to a *Lactuca sativa* seed designated 41-177 RZ, which exhibits a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves. The present invention also relates to a *Lactuca sativa* plant produced by growing the 41-177 RZ seed. The invention further relates to methods for producing the lettuce cultivar, represented by lettuce variety 41-177 RZ.

34 Claims, 3 Drawing Sheets

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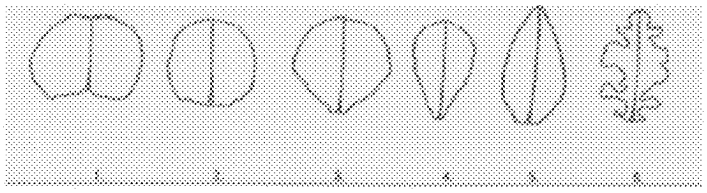

FIG. 1

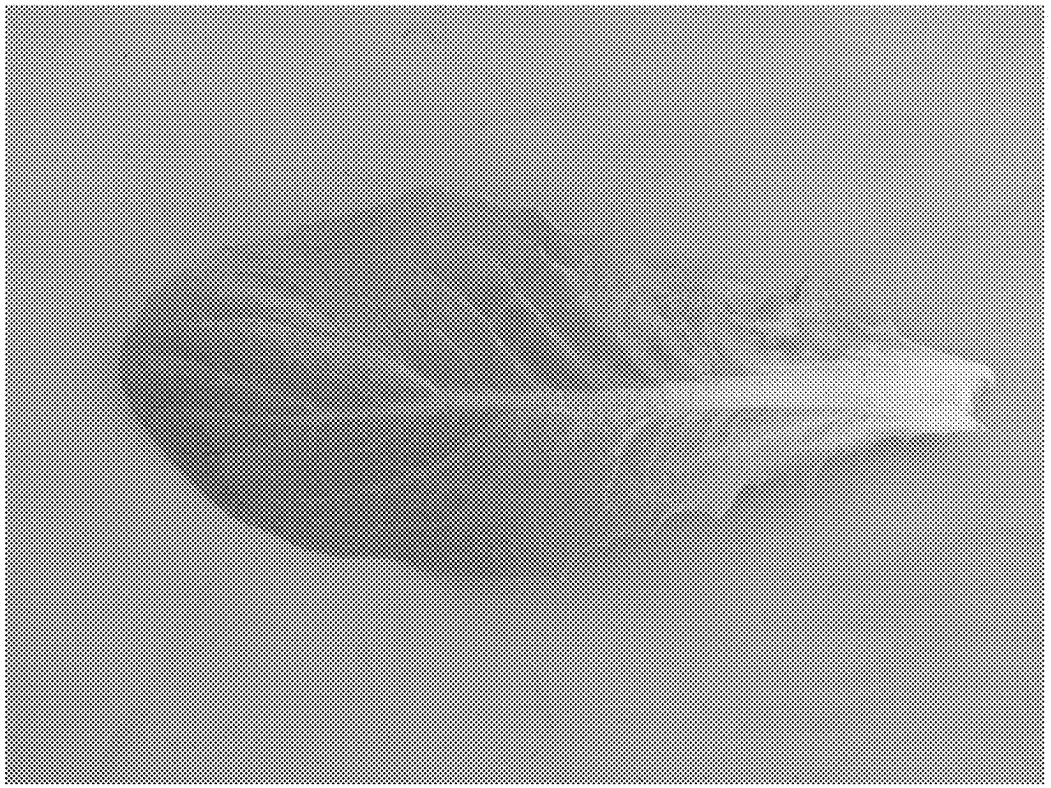

FIG. 2

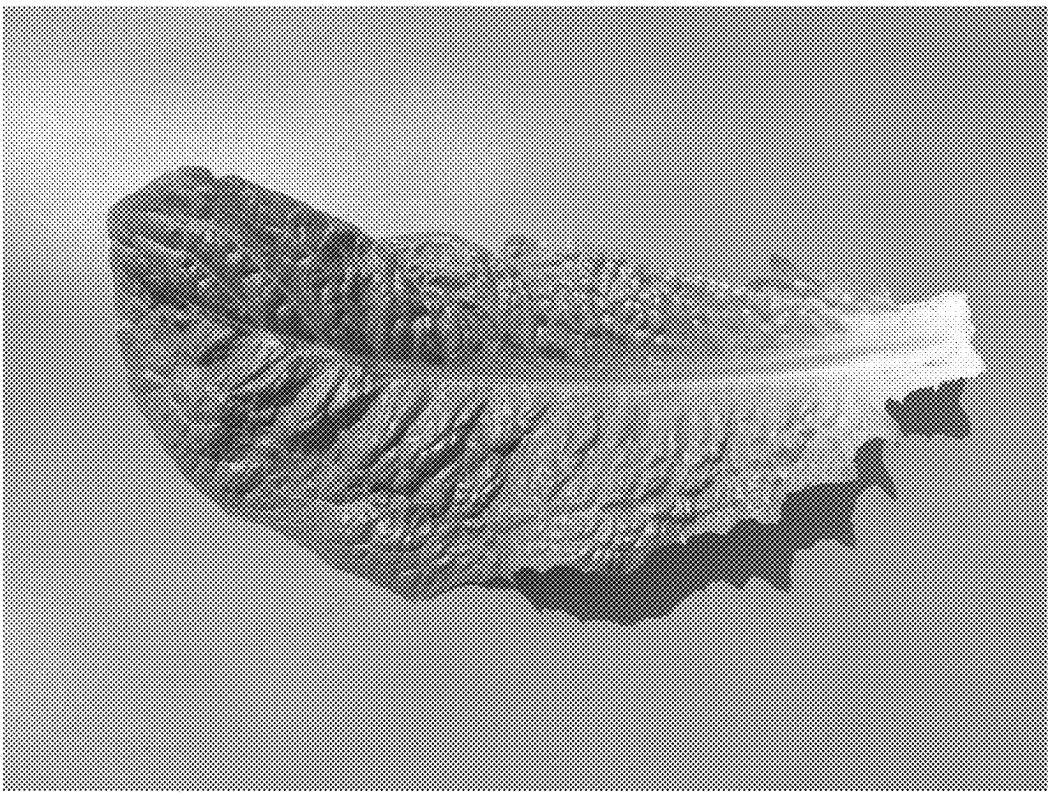

FIG. 3

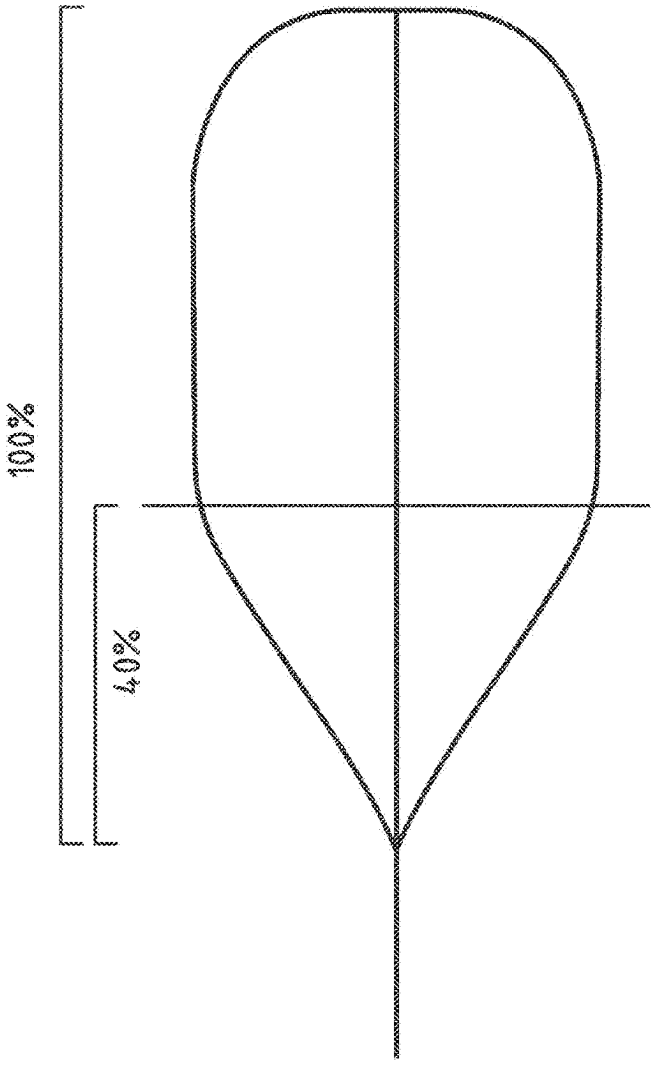

FIG. 4

LETTUCE VARIETY 41-177 RZ

INCORPORATION BY REFERENCE

All documents cited or referenced herein ("herein cited documents"), and all documents cited or referenced in herein cited documents, together with any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated herein by reference, are hereby incorporated herein by reference, and may be employed in the practice of the invention.

FIELD OF THE INVENTION

The present invention relates to a new romaine lettuce (*Lactuca sativa*) variety which exhibits a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and to corky root rot (*Sphingomonas suberifaciens*), and which is suitable for mechanical harvest.

BACKGROUND OF THE INVENTION

All cultivated forms of lettuce belong to the highly polymorphic species, *Lactuca sativa*, which is grown for its edible head and leaves. As a crop, lettuces are grown commercially wherever environmental conditions permit the production of an economically viable yield.

Lactuca sativa is in the Cichoreae tribe of the Asteraceae (Compositae) family. Lettuce is related to chicory, sunflower, aster, scorzonera, dandelion, artichoke and chrysanthemum. *Sativa* is one of about 300 species in the genus *Lactuca.*

Lettuce cultivars are susceptible to a number of pests and diseases such as downy mildew (*Bremia lactucae*). Every year this disease leads to millions of dollars of lost lettuce crop throughout the world. Downy mildew (*Bremia lactucae*) is highly destructive of lettuce grown at relatively low temperature and high humidity. Downy mildew is caused by a fungus, *Bremia lactucae*, which can be one of the following strains: NL1, NL2, NL4, NL5, NL6, NL7, NL10, NL12, NL13, NL14, NL15, NL16, Bl:17, Bl:18, Bl:20, Bl:21, Bl:22, Bl:23, Bl:24, Bl:25, Bl:26, Bl:27, Bl:28 (Van Ettekoven, K. et al., "Identification and denomination of 'new' races of *Bremia lactucae*," In: Lebeda, A. and Kristkova, E (eds.), Eucarpia Leafy Vegetables, 1999, Palacky University, Olomouc, Czech Republic, pp. 171-175; Van der Arend et al. "Identification and denomination of "new" races of *Bremia lactucae* in Europe by IBEB until 2002." In: Van Hintum, Th et al. (eds.), Eucarpia Leafy Vegetables Conference 2003, Centre for Genetic Resources, Wageningen, The Netherlands, p. 151; Plantum NL (Dutch association for breeding, tissue culture, production and trade of seeds and young plants), Van der Arend et al. "Identification and denomination of "new" races of *Bremia lactucae* in Europe by IBEB until 2002." In: Van Hintum, Th et al. (eds.), Eucarpia Leafy Vegetables Conference 2003, Centre for Genetic Resources, Wageningen, The Netherlands, p. 151; Plantum NL (Dutch association for breeding, tissue culture, production and trade of seeds and young plants); IBEB press release "New race of *Bremia lactucae* Bl:27 identified and nominated", May 2010; Plantum NL (Dutch association for breeding, tissue culture, production and trade of seeds and young plants), "New race of *Bremia lactucae* Bl:28 identified and nominated", March 2011), Ca-I, Ca-IIA, Ca-IIB, Ca-III, Ca-IV (Schettini, T. M., Legg, E. J., Michelmore, R. W., 1991. Insensitivity to metalaxyl in California populations of *Bremia lactucae* and resistance of California lettuce cultivars to downy mildew. Phytopathology 81(1). p. 64-70), and Ca-V, Ca-VI, Ca-VII, Ca-VIII (Michelmore R. & Ochoa. O. "Breeding Crisphead Lettuce." In: California Lettuce Research Board, Annual Report 2005-2006, 2006, Salinas, Calif., pp. 55-68).

Downy mildew causes pale, angular, yellow areas bounded by veins on the upper leaf surfaces. Sporulation occurs on the opposite surface of the leaves. The lesions eventually turn brown, and they may enlarge and coalesce. These symptoms typically occur first on the lower leaves of the lettuce, but under ideal conditions may move into the upper leaves of the head. When the fungus progresses to this degree, the head cannot be harvested. Less severe damage requires the removal of more leaves than usual, especially when the lettuce reaches its final destination.

Of the various species of aphids that feed on lettuce, the currant-lettuce aphid (*Nasonovia ribisnigri*) is the most destructive species because it feeds both on the leaves of the lettuce as well as the heart of the lettuce, making it difficult to control with conventional insecticides. The lettuce aphid feeds by sucking sap from the lettuce leaves. Although direct damage to the lettuce may be limited, its infestation has serious consequences because the presence of aphids makes lettuce unacceptable to consumers.

Corky root rot is caused by a soil-borne bacterium, *Sphingomonas suberifaciens*, which is prevalent in most coastal lettuce growing areas. Corky root rot affects both leaf and head lettuce varieties. The disease typically is more severe when soil temperatures are warmer and in fields where lettuce is grown consecutively. High soil nitrate levels also increase disease severity. Early symptoms of corky root rot are yellow bands on tap and lateral roots of lettuce seedlings. The yellow areas gradually expand, taking on a greenish-brown color and developing cracks and rough areas on the surface of the root. As disease severity increases, the entire tap root may become brown, severely cracked, and nonfunctional; the feeder root system also may be reduced and damaged. At this point, roots are very brittle and easily break off when examined. Corky root also may cause internal discoloration of the root. When the root is severely diseased, aboveground symptoms consist of wilting during warm temperatures, stunting of plants, and general poor and uneven growth.

Lettuce mosaic virus (LMV) mainly infects lettuce seeds, which is the primary way that the virus is introduced to lettuce in the fields, but can also infect numerous crops and weeds, thereby creating reservoirs of the virus. Additionally, LMV can be vectored by aphids, which spread the virus within a lettuce field and introduce it into lettuce fields from infected weeds and crops outside the field.

Symptoms of LMV vary greatly. Leaves of plants that are infected at a young stage are stunted, deformed and (in some varieties) show a mosaic or mottling pattern. Such plants rarely grow to full size; head lettuce varieties infected early fail to form heads. Plants that are infected later in the growth cycle show a different set of symptoms. These plants may reach full size, but the older outer leaves turn yellow, twisted, and otherwise are deformed. On head lettuce, the wrapper leaves often will curve back away from the head and developing heads may be deformed. In some cases, brown necrotic flecks occur on the wrapper leaves.

Mature romaine lettuce is mainly harvested by hand. This harvesting process is labor intensive, and relatively laborer-unfriendly. As it is getting more and more costly to hire laborers that want to work outdoor in the field, close to the ground and under all kinds of adverse weather conditions, there is strong interest of the lettuce industry to mechanize

this manual harvesting process. A major constraint is caused by the use of mechanically-driven horizontal knives that easily damage the hollow main veins of romaine leaves and cut halfway through the prostrate, round-shaped, lower outer leaves, which results in wide cut surfaces. Cutting damage on a leaf with a hollow main vein is often not restricted to the cut surface but bruising extends into the leaf along the main vein.

Citation or identification of any document in this application is not an admission that such document is available as prior art to the present invention.

SUMMARY OF THE INVENTION

There exists a need, therefore, for a new type of romaine, or cos lettuce (*Lactuca sativa* L. var. *longifolia* Lam) variety, which is suitable for mechanical harvest with a simple horizontal knife and which exhibits a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*).

The present invention addresses this need by providing a new type of lettuce (*Lactuca sativa*) variety, designated 41-177 RZ. Lettuce cultivar 41-177 RZ exhibits a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves.

The present invention provides seeds of lettuce cultivar 41-177 RZ, which have been deposited with the National Collections of Industrial, Marine and Food Bacteria (NCIMB) in Bucksburn, Aberdeen AB21 9YA, Scotland, UK and have been assigned NCIMB Accession No. 42094.

In one embodiment, the invention provides a lettuce plant which may exhibit a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves, representative seed of which have been deposited under NCIMB Accession No. 42094.

In one embodiment, the invention provides a lettuce plant which may exhibit a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), solid main veins, narrow base leaves, and a moderately to prominently raised midrib, representative seed of which have been deposited under NCIMB Accession No. 42094.

In one embodiment, the invention provides a lettuce plant designated 41-177 RZ, representative seed of which have been deposited under NCIMB Accession No. 42094.

In an embodiment of the present invention, there also is provided parts of a lettuce plant of the invention, which may include parts of a lettuce plant exhibiting a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves, or parts of a lettuce plant having any of the aforementioned resistance(s) and a combination of traits including one or more morphological or physiological characteristics tabulated herein, including parts of lettuce variety 41-177 RZ, wherein the plant parts are involved in sexual reproduction, which include, without limitation, microspores, pollen, ovaries, ovules, embryo sacs or egg cells and/or wherein the plant parts are suitable for vegetative reproduction, which include, without limitation, cuttings, roots, stems, cells or protoplasts and/or wherein the plant parts are tissue culture of regenerable cells in which the cells or protoplasts of the tissue culture are derived from a tissue such as, for example and without limitation, leaves, pollen, embryos, cotyledon, hypocotyls, meristematic cells, roots, root tips, anthers, flowers, seeds or stems. The plants of the invention from which such parts may come from include those wherein representative seed of which has been deposited under NCIMB Accession No. 42094 or lettuce variety or cultivar designated 41-177 RZ, as well as seed from such a plant, plant parts of such a plant (such as those mentioned herein) and plants from such seed and/or progeny of such a plant, advantageously progeny exhibiting such combination of such traits, each of which, is within the scope of the invention; and such combination of traits.

In another embodiment there is a plant grown from seeds, representative seed of which having been deposited under NCIMB Accession No. 42094. In a further embodiment there is a plant regenerated from the above-described plant parts or regenerated from the above-described tissue culture. Advantageously such a plant may have morphological and/or physiological characteristics of lettuce variety 41-177 RZ and/or of a plant grown from seed, representative seed of which having been deposited under NCIMB Accession No. NCIMB 42094—including without limitation such plants having all of the morphological and physiological characteristics of lettuce variety 41-177 RZ and/or of plant grown from seed, representative seed of which having been deposited under NCIMB Accession No. NCIMB 42094. Advantageously, such a plant demonstrates the traits of resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves. Accordingly, in still a further embodiment, there is provided a lettuce plant having all of the morphological and physiological characteristics of lettuce variety 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. 42094. Such a plant may be grown from the seeds, regenerated from the above-described plant parts, or regenerated from the above-described tissue culture. A lettuce plant having any of the aforementioned resistance(s), and one or more morphological or physiological characteristics recited or tabulated herein, and a lettuce plant advantageously having all of the aforementioned resistances and the characteristics recited and tabulated herein, are preferred. Parts of such plants—such as those plant parts above-mentioned—are encompassed by the invention.

In one embodiment, there is provided progeny of lettuce cultivar 41-177 RZ produced by sexual or vegetative reproduction, grown from seeds, regenerated from the above-described plant parts, or regenerated from the above-described tissue culture of the lettuce cultivar or a progeny plant thereof, representative seed of which having been deposited under NCIMB Accession No. 42094. The progeny may have any of the aforementioned resistance(s), and one or more morphological or physiological characteristics recited or tabulated herein, and a progeny plant advantageously having all of the aforementioned resistances and the characteristics recited and tabulated herein, are preferred. Advantageously, the progeny demonstrate the traits of resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0,

lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves.

Progeny of the lettuce variety 41-177 RZ may be modified in one or more other characteristics, in which the modification is a result of, for example and without limitation, mutagenesis or transformation with a transgene.

In still another embodiment, the present invention provides progeny of lettuce cultivar 41-177 RZ produced by sexual or vegetative reproduction, grown from seeds, regenerated from the above-described plant parts, or regenerated from the above-described tissue culture of the lettuce cultivar or a progeny plant thereof, in which the regenerated plant shows a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves.

In still a further embodiment, the invention may comprise a method of producing a hybrid lettuce seed which may comprise crossing a first parent lettuce plant with a second parent lettuce plant and harvesting the resultant hybrid lettuce seed, in which the first parent lettuce plant or the second parent lettuce plant may be a lettuce plant of the invention, e.g. a lettuce plant having a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves and one or more morphological or physiological characteristics tabulated herein, including a lettuce plant of lettuce cultivar 41-177 RZ, representative seed of which having been deposited under NCIMB 42094.

In another embodiment, the invention may comprise producing a lettuce plant having a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves which may comprise: crossing a mother lettuce plant with a father lettuce plant to produce a hybrid seed; growing said hybrid seed to produce a hybrid plant; selfing said hybrid plant to produce F2 progeny seed; growing said F2 progeny seed to produce F2-plants; selecting said F2-plants for exhibiting a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves.

Advantageously the selfing and selection may be repeated; for example at least once, or at least twice, thrice, four times, five times, six times or more, to produce F3 or F4 or F5 or F6 or subsequent progeny, especially as progeny from F2 may exhibit the aforementioned combination of traits, and may be desirable.

In still a further embodiment, the invention may comprise a method of producing a lettuce cultivar containing a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves.

The invention even further relates to a method of producing lettuce which may comprise: (a) cultivating to the vegetative plant stage a plant of lettuce variety 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. NCIMB 42094, and (b) harvesting lettuce leaves or heads from the plant. The invention further comprehends packaging the lettuce plants, heads or leaves.

Accordingly, it is an object of the invention to not encompass within the invention any previously known product, process of making the product, or method of using the product such that Applicants reserve the right and hereby disclose a disclaimer of any previously known product, process, or method. It is further noted that the invention does not intend to encompass within the scope of the invention any product, process, or making of the product or method of using the product, which does not meet the written description and enablement requirements of the USPTO (35 U.S.C. §112, first paragraph) or the EPO (Article 83 of the EPC), such that Applicants reserve the right and hereby disclose a disclaimer of any previously described product, process of making the product, or method of using the product.

It is noted that in this disclosure and particularly in the claims, terms such as "comprises", "comprised", and "comprising" and the like (e.g., "includes", "included", "including", "contains", "contained", "containing", "has", "had", "having", etc.) can have the meaning ascribed to them in US Patent law, i.e., they are open ended terms. For example, any method that "comprises," "has" or "includes" one or more steps is not limited to possessing only those one or more steps and also covers other unlisted steps. Similarly, any plant that "comprises," "has" or "includes" one or more traits is not limited to possessing only those one or more traits and covers other unlisted traits. Similarly, the terms "consists essentially of" and "consisting essentially of" have the meaning ascribed to them in US Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention. See also MPEP §2111.03. In addition, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

These and other embodiments are disclosed or are obvious from and encompassed by the following Detailed Description.

DEPOSIT

The Deposit with NCIMB Ltd, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB21 9YA, UK, on Jan. 11, 2013, under deposit accession number NCIMB 42094 was made pursuant to the terms of the Budapest Treaty. Upon issuance of a patent, all restrictions upon the deposit will be removed, and the deposit is intended to meet the requirements of 37 CFR §1.801-1.809. The deposit will be maintained in the depository for a period of 30 years, or 5 years after the last request, or for the effective life of the patent, whichever is longer, and will be replaced if necessary during that period.

BRIEF DESCRIPTION OF THE DRAWINGS

The following detailed description, given by way of example, but not intended to limit the invention solely to the specific embodiments described, may best be understood in conjunction with the accompanying drawing, in which:

FIG. **1** is an illustration of six different shapes of the fourth leaf from a 20-day old seedling grown under optimal conditions.

FIG. **2** is a picture of the fourth leaf of 41-177 RZ.

FIG. **3** is a picture of a mature leaf of 41-177 RZ.

FIG. **4**. Transverse section at 40% of leaf lamina length to observe solidness of main vein.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides methods and compositions relating to plants, seeds and derivatives of a new lettuce variety herein referred to as lettuce variety 41-177 RZ. Lettuce variety 41-177 RZ is a uniform and stable line, distinct from other such lines.

In a preferred embodiment, the specific type of breeding method employed for developing a lettuce cultivar is pedigree selection, where both single plant selection and mass selection practices are employed. Pedigree selection, also known as the "Vilmorin system of selection," is described in Fehr, W., Principles of Cultivar Development, Volume I, MacMillan Publishing Co., which is hereby incorporated by reference.

When pedigree selection is applied, in general selection is first practiced among F_2 plants. In the next season, the most desirable F_3 lines are first identified, and then desirable F_3 plants within each line are selected. The following season and in all subsequent generations of inbreeding, the most desirable families are identified first, then desirable lines within the selected families are chosen, and finally desirable plants within selected lines are harvested individually. A family refers to lines that were derived from plants selected from the same progeny from the preceding generation.

Using this pedigree method, two parents may be crossed using an emasculated female and a pollen donor (male) to produce F_1 offspring. Lettuce is an obligate self-pollination species, which means that pollen is shed before stigma emergence, assuring 100% self-fertilization. Therefore, in order to optimize crossing, a method of misting may be used to wash the pollen off prior to fertilization to assure crossing or hybridization.

Parental varieties are selected from commercial varieties that individually exhibit one or more desired phenotypes. Additionally, any breeding method involving selection of plants for the desired phenotype may be used in the method of the present invention.

The F_1 may be self-pollinated to produce a segregating F_2 generation. Individual plants may then be selected which represent the desired phenotype in each generation (F_3, F_4, F_5, etc.) until the traits are homozygous or fixed within a breeding population.

Lettuce variety 41-177 RZ was developed by crossing a plant of line 07A.26117 with a plant of line 07A.26150 under greenhouse conditions in Aramon, France, in 2007. In 2008 F1 plants were grown for the production of F2 seeds, which also took place also in Aramon, France. In 2009, F2 plants were selected under field conditions in Valencia, Spain, followed by F3 seed production. In 2010, F3 plants were selected under field conditions in Fijnaart, The Netherlands, followed by F4 seed production. Subsequently in 2011, F4 plants were grown under field conditions in Le Thor, France, followed by F5 seed production. In 2011-2012, a uniform F5 line was established. The F5 line was then multiplied under plastic tunnel conditions in Daylesford (Australia). This seedlot was again multiplied in Aramon, France, in 2012 and was designated 12R.3295. A representative sample of seed from seedlot 12R.3295 designated variety 41-177 RZ was deposited with the NCIMB under deposit no. 42094.

In one embodiment, a plant of the invention has all the morphological and physiological characteristics of lettuce variety 41-177 RZ. These characteristics of a lettuce plant of the invention, e.g. variety 41-177 RZ, are summarized in Tables 1 and 2.

Next to the morphological and physiological characteristics mentioned in Tables 1 and 2, a plant of the invention also exhibits resistance to downy mildew (*Bremia lactucae* Regel.), currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*).

As used herein resistance against *Bremia lactucae* Regel. is defined as the ability of a plant to resist infection by each of the various strains Bl:1 to Bl:28, and Ca-I to Ca-VIII of *Bremia lactucae* Regel. in all stages between the seedling stage and the harvestable plant stage. Bl:1-28 means strains NL1, NL2, NL4, NL5, NL6, NL7, NL10, NL12, NL13, NL14, NL15, NL16, Bl:17, Bl:18, Bl:20, Bl:21, Bl:22, Bl:23, Bl:24, Bl:25, Bl:26, Bl:27, Bl:28 (Van Ettekoven K, Van der Arend A J M, 1999. identification and denomination of 'new' races of *Bremia lactucae*. In: Lebeda A, Kristkova E (eds.) Eucarpia leafy vegetables '99. Palacky University, Olomouc, Czech Republic, 1999: 171-175; Van der Arend, A. J. M., Gautier, J., Guenard, M., Michel, H., Moreau, B., de Ruijter, J., Schut, J. W. and de Witte, I. (2003). Identification and denomination of 'new' races of *Bremia lactucae* in Europe by IBEB until 2002. In: Eucarpia leafy vegetables 2003. Proceedings of the Eucarpia Meeting on leafy vegetables genetics and breeding. Noorwijkerhout, The Netherlands. Eds. Van Hintum T., Lebeda A., Pink D., Schut J. pp 151-160; Van der Arend A J M, Gautier J, Grimault V, Kraan P, Van der Laan R, Mazet J, Michel H, Schut J W, Smilde D, De Witte I (2006) Identification and denomination of "new" races of *Bremia lactucae* in Europe by IBEB until 2006; incorporated herein by reference; Plantum NL (Dutch association for breeding, tissue culture, production and trade of seeds and young plants), IBEB press release, "New race of *Bremia lactucae* Bl:27 identified and nominated", May 2010; Plantum NL (Dutch association for breeding, tissue culture, production and trade of seeds and young plants), IBEB press release, "New race of *Bremia lactucae* Bl:28 identified and nominated", March 2011). Ca-I to Ca-VIII means Ca-I, Ca-IIA, Ca-IIB, Ca-III, Ca-IV (Schettini, T. M., Legg, E. J., Michelmore, R. W., 1991. Insensitivity to metalaxyl in California populations of *Bremia lactucae* and resistance of California lettuce cultivars to downy mildew, Phytopathology 81(1). p. 64-'70), and Ca-V, Ca-VI, Ca-VII, Ca-VIII (Michelmore R. & Ochoa. O. "Breeding Crisphead Lettuce." In: California Lettuce Research Board, Annual Report 2005-2006, 2006, Salinas, Calif., pp. 55-68).

Resistance typically is tested by two interchangeable methods, described by Bonnier, F. J. M. et al. (Euphytica, 61(3): 203-211, 1992; incorporated herein by reference). One method involves inoculating 7-day old seedlings, and observing sporulation 10 to 14 days later. The other method involves inoculating leaf discs with a diameter of 18 mm obtained from a non-senescent, fully grown true leaf and observing sporulation 10 days later.

As used herein, resistance against *Nasonovia ribisnigri* (Mosley) biotype Nr:0, or currant-lettuce aphid, is defined as the plant characteristic which results in a non-feeding response of the aphid on the leaves of the plant in all stages between 5 true-leaf stage and harvestable plant stage (U.S. Pat. No. 5,977,443 to Jansen, J. P. A., "Aphid Resistance in Composites," p. 12, 1999; incorporated herein by reference).

Resistance is tested by spreading at least ten aphids of biotype Nr:0 on a plant in a plant stage between 5 true leaves and harvestable stage, and observing the density of the aphid population on the plant as well as the growth reduction after

14 days in a greenhouse, with temperature settings of 23 degrees Celsius in daytime and 21 degrees Celsius at night. Day length is kept at 18 hours by assimilation lights.

As used herein, resistance against Lettuce Mosaic Virus (LMV) is defined as the ability of the plant to grow normally after LMV infection and to inhibit the virus transmission via seed. Resistance is tested by mechanical inoculation of young plants in a climate cell or a greenhouse, as described by Pink, D. A. C. et al. (Plant Pathology, 41(1):5-12, 1992), incorporated herein by reference. Inoculated resistant plants grow just as well as uninoculated plants and show no chlorosis or mosaic symptoms. The LMV isolate which is used for testing is Ls-1 (International Union for the Protection of New Varieties of Plants [UPOV]), Guidelines for the conduct of tests for distinctness, uniformity and stability; lettuce (*Lactuca sativa* L.), 2002, p. 35; incorporated herein by reference).

As used herein, resistance against *Sphingomonas suberifaciens*, also known as corky root rot or *Rhizomonas suberifaciens*, is defined as the ability of the plant to grow relatively normally in a corky root-infected soil (Yabuuchi, E, et al., Microbiol. Immunol., 43; 339-349, 1997). As the resistance of the lettuce plant to the bacterium is incomplete, a susceptible and a resistant standard variety, for example "Parris Island" (susceptible) and "Tall Guzmaine" (resistant), should be used for comparison. Resistance is tested with a young plant in a greenhouse using the CA1-strain of the bacterium, as described by Brown, P. R. et al. (Phytopathology, 78:1145-1150, 1988), incorporated herein by reference.

As used herein, a solid main vein is the main vein of a fully-grown tenth to fifteenth leaf, which is characterized by the fact that the vein is not hollow, which is observed by visual inspection of a transverse section of the leaf. The transverse section should be made at 40% of the total leaf length, starting from the leaf base (see FIG. **4**). For comparison two standard varieties may be used: Maximus, with hollow veins, and Tiberius, with solid veins.

As used herein, a narrow base leaf is characterized by a length/width-ratio of 1.5 or higher. A mature romaine lettuce plant has got narrow base leaves if the average length/width ratio of the fully-grown tenth to the fifteenth leaf is 1.5 or higher. For comparison two standard varieties may be used: Maximus with broad base leaves, and Chilim with narrow base leaves.

Embodiments of the inventions advantageously have one or more, and most advantageously all, of these characteristics.

In Table 1, the seed color, cotyledon shape and characteristics of the fourth leaf of "41-177 RZ" is compared with "Auvona RZ" and "Verde degli ortolani".

TABLE 1

Character	"41-177 RZ"	"Auvona RZ" (41-122 RZ)	"Verde degli ortolani"
Plant Type	Cos/Romaine	Cos/Romain	Cos/Romaine
Seed Color	White	White	White
Cotyledon Shape	Intermediate	Intermediate to spatulate	Spatulate to intermediate
Shape of Fourth Leaf	Elongated	Elongated	Elongated
Rolling of Fourth Leaf	Present	Present	Present
Cupping of Fourth Leaf	Uncupped	Uncupped	Uncupped
Fourth Leaf Apical Margin	Entire	Entire	Entire
Fourth Leaf Basal Margin	Coarsely dentate	Coarsely dentate	Coarsely dentate
Undulation	Flat	Flat	Flat
Green color	Dark green	Medium to dark green	Medium to dark green
Anthocyanin distribution	Absent	Absent	Absent

In Table 2, the mature leaf and head characteristics of "41-177 RZ" is compared with "Auvona RZ" and "Verde degli ortolani".

TABLE 2

Character	"41-177 RZ"	Auvona RZ	Verde
Leaf Color	Dark green	Dark green to very dark green	Medium to dark green
Anthocyanin Distribution	Absent	Absent	Absent
Margin Incision Depth	Absent/shallow	Absent/shallow	Absent/shallow
Margin Indentation	Entire	Entire	Entire
Undulations of the Apical Margin	Absent/slight	Absent/slight	Absent/slight
Leaf Size	Medium	Medium	Medium to large
Leaf Glossiness	Glossy	Glossy	Medium
Leaf Blistering	Strong	Strong	Strong to moderate
Leaf Thickness	Intermediate to thick	Intermediate to thick	Intermediate to thick
Trichomes	Absent	Absent	Absent
Head Shape	Elongate	Elongate	Elongate
Butt Shape	Rounded	Rounded	Rounded
Midrib	Moderately to prominently raised	Prominently raised	Prominently raised

In an embodiment, the invention relates to lettuce plants that has all the morphological and physiological characteristics of the invention and have acquired said characteristics by introduction of the genetic information that is responsible for the characteristics from a suitable source, 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 an (agricultural) trait, from the crop plant itself or from a sexually compatible donor plant. Transgenesis is genetic modification of a plant with a gene from a non-crossable species or a synthetic gene.

Just as useful traits that may be introduced by backcrossing, useful traits may be introduced directly into the plant of the invention, being a plant of lettuce variety 41-177 RZ, by genetic transformation techniques; and, such plants of lettuce variety 41-177 RZ that have additional genetic information introduced into the genome or that express additional traits by having the DNA coding there for introduced into the genome via transformation techniques, are within the ambit of the invention, as well as uses of such plants, and the making of such plants.

Genetic transformation may therefore be used to insert a selected transgene into the plant of the invention, being a plant of lettuce variety 41-177 RZ or may, alternatively, be used for the preparation of transgenes which may be introduced by backcrossing. Methods for the transformation of plants, including lettuce, are well known to those of skill in the art.

Vectors used for the transformation of lettuce cells are not limited so long as the vector may express an inserted DNA in the cells. For example, vectors which may comprise promoters for constitutive gene expression in lettuce cells (e.g.,

cauliflower mosaic virus 35S promoter) and promoters inducible by exogenous stimuli may be used. Examples of suitable vectors include pBI binary vector. The "lettuce cell" into which the vector is to be introduced includes various forms of lettuce cells, such as cultured cell suspensions, protoplasts, leaf sections, and callus. A vector may be introduced into lettuce cells by known methods, such as the polyethylene glycol method, polycation method, electroporation, *Agrobacterium*-mediated transfer, particle bombardment and direct DNA uptake by protoplasts. To effect transformation by electroporation, one may employ either friable tissues, such as a suspension culture of cells or embryogenic callus or alternatively one may transform immature embryos or other organized tissue directly. In this technique, one would partially degrade the cell walls of the chosen cells by exposing them to pectin-degrading enzymes (pectolyases) or mechanically wound tissues in a controlled manner.

A particularly efficient method for delivering transforming DNA segments to plant cells is microprojectile bombardment. In this method, particles are coated with nucleic acids and delivered into cells by a propelling force. Exemplary particles include those comprised of tungsten, platinum, and preferably, gold. For the bombardment, cells in suspension are concentrated on filters or solid culture medium. Alternatively, immature embryos or other target cells may be arranged on solid culture medium. The cells to be bombarded are positioned at an appropriate distance below the macroprojectile stopping plate. An illustrative embodiment of a method for delivering DNA into plant cells by acceleration is the Biolistics Particle Delivery System, which may be used to propel particles coated with DNA or cells through a screen, such as a stainless steel or Nytex screen, onto a surface covered with target lettuce cells. The screen disperses the particles so that they are not delivered to the recipient cells in large aggregates. It is believed that a screen intervening between the projectile apparatus and the cells to be bombarded reduces the size of projectiles aggregate and may contribute to a higher frequency of transformation by reducing the damage inflicted on the recipient cells by projectiles that are too large. Microprojectile bombardment techniques are widely applicable, and may be used to transform virtually any plant species, including a plant of lettuce variety 41-177 RZ.

Agrobacterium-mediated transfer is another widely applicable system for introducing gene loci into plant cells. An advantage of the technique is that DNA may be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations. Moreover, advances in vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate the construction of vectors capable of expressing various polypeptide coding genes. The vectors have convenient multi-linker regions flanked by a promoter and a polyadenylation site for direct expression of inserted polypeptide coding genes. Additionally, *Agrobacterium* containing both armed and disarmed Ti genes may be used for transformation. In those plant strains where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene locus transfer. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells, including lettuce plant cells, is well known in the art (See, e.g., U.S. Pat. Nos. 7,250,560 and 5,563,055).

Transformation of plant protoplasts also may be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments.

A number of promoters have utility for plant gene expression for any gene of interest including but not limited to selectable markers, scoreable markers, genes for pest tolerance, disease resistance, nutritional enhancements and any other gene of agronomic interest. Examples of constitutive promoters useful for lettuce plant gene expression include, but are not limited to, the cauliflower mosaic virus (CaMV) P-35S promoter, a tandemly duplicated version of the CaMV 35S promoter, the enhanced 35S promoter (P-e35S), the nopaline synthase promoter, the octopine synthase promoter, the figwort mosaic virus (P-FMV) promoter (see U.S. Pat. No. 5,378,619), an enhanced version of the FMV promoter (P-eFMV) where the promoter sequence of P-FMV is duplicated in tandem, the cauliflower mosaic virus 19S promoter, a sugarcane bacilliform virus promoter, a commelina yellow mottle virus promoter, the promoter for the thylakoid membrane proteins from lettuce (psaD, psaF, psaE, PC, FNR, atpC, atpD, cab, rbcS) (see U.S. Pat. No. 7,161,061), the CAB-1 promoter from lettuce (see U.S. Pat. No. 7,663,027), the promoter from maize prolamin seed storage protein (see U.S. Pat. No. 7,119,255), and other plant DNA virus promoters known to express in plant cells. A variety of plant gene promoters that are regulated in response to environmental, hormonal, chemical, and/or developmental signals may be used for expression of an operably linked gene in plant cells, including promoters regulated by (1) heat, (2) light (e.g., pea rbcS-3A promoter, maize rbcS promoter, or chlorophyll a/b-binding protein promoter), (3) hormones, such as abscisic acid, (4) wounding (e.g., wunl, or (5) chemicals such as methyl jasmonate, salicylic acid, or Safener. It may also be advantageous to employ organ-specific promoters.

Exemplary nucleic acids which may be introduced to the lettuce variety of this invention include, for example, DNA sequences or genes from another species, or even genes or sequences which originate with or are present in lettuce species, but are incorporated into recipient cells by genetic engineering methods rather than classical reproduction or breeding techniques. However, the term "exogenous" is also intended to refer to genes that are not normally present in the cell being transformed, or perhaps simply not present in the form, structure, etc., as found in the transforming DNA segment or gene, or genes which are normally present and that one desires to express in a manner that differs from the natural expression pattern, e.g., to over-express. Thus, the term "exogenous" gene or DNA is intended to refer to any gene or DNA segment that is introduced into a recipient cell, regardless of whether a similar gene may already be present in such a cell. The type of DNA included in the exogenous DNA may include DNA which is already present in the plant cell, DNA from another plant, DNA from a different organism, or a DNA generated externally, such as a DNA sequence containing an antisense message of a gene, or a DNA sequence encoding a synthetic or modified version of a gene.

Many hundreds if not thousands of different genes are known and could potentially be introduced into a plant of lettuce variety 41-177 RZ. Non-limiting examples of particular genes and corresponding phenotypes one may choose to introduce into a lettuce plant include one or more genes for insect tolerance, pest tolerance such as genes for fungal disease control, herbicide tolerance, and genes for quality improvements such as yield, nutritional enhancements, envi-

ronmental or stress tolerances, or any desirable changes in plant physiology, growth, development, morphology or plant product(s).

Alternatively, the DNA coding sequences may affect these phenotypes by encoding a non-translatable RNA molecule that causes the targeted inhibition of expression of an endogenous gene, for example via antisense- or cosuppression-mediated mechanisms. The RNA could also be a catalytic RNA molecule (i.e., a ribozyme) engineered to cleave a desired endogenous mRNA product. Thus, any gene which produces a protein or mRNA which expresses a phenotype or morphology change of interest is useful for the practice of the present invention. (See also U.S. Pat. No. 7,576,262, "Modified gene-silencing RNA and uses thereof.")

U.S. Pat. Nos. 7,230,158, 7,122,720, 7,081,363, 6,734,341, 6,503,732, 6,392,121, 6,087,560, 5,981,181, 5,977,060, 5,608,146, 5,516,667, each of which, and all documents cited therein are hereby incorporated herein by reference, consistent with the above INCORPORATION BY REFERENCE section, are additionally cited as examples of U.S. patents that may concern transformed lettuce and/or methods of transforming lettuce or lettuce plant cells, and techniques from these US patents, as well as promoters, vectors, etc., may be employed in the practice of this invention to introduce exogenous nucleic acid sequence(s) into a plant of lettuce variety 41-177 RZ (or cells thereof), and exemplify some exogenous nucleic acid sequence(s) which may be introduced into a plant of lettuce variety 41-177 RZ (or cells thereof) of the invention, as well as techniques, promoters, vectors etc., to thereby obtain further plants of lettuce variety 41-177 RZ, plant parts and cells, seeds, other propagation material harvestable parts of these plants, etc. of the invention, e.g. tissue culture, including a cell or protoplast, such as an embryo, meristem, cotyledon, pollen, leaf, anther, root, root tip, pistil, flower, seed or stalk.

The invention further relates to propagation material for producing plants of the invention. Such propagation material may comprise inter alia seeds of the claimed plant and parts of the plant that are involved in sexual reproduction. Such parts are for example selected from the group consisting of seeds, microspores, pollen, ovaries, ovules, embryo sacs and egg cells. In addition, the invention relates to propagation material which may comprise parts of the plant that are suitable for vegetative reproduction, for example cuttings, roots, stems, cells, protoplasts.

According to a further aspect thereof the propagation material of the invention may comprise a tissue culture of the claimed plant. The tissue culture may comprise regenerable cells. Such tissue culture may be derived from leaves, pollen, embryos, cotyledon, hypocotyls, meristematic cells, roots, root tips, anthers, flowers, seeds and stems. (See generally U.S. Pat. No. 7,041,876 on lettuce being recognized as a plant that may be regenerated from cultured cells or tissue).

Also, the invention comprehends methods for producing a seed of a "41-177 RZ"-derived lettuce plant which may comprise (a) crossing a plant of lettuce variety 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. NCIMB 42094, with a second lettuce plant, and (b) whereby seed of a 41-177 RZ-derived lettuce plant form. Such a method may further comprise (c) crossing a plant grown from 41-177 RZ-derived lettuce seed with itself or with a second lettuce plant to yield additional 41-177 RZ-derived lettuce seed, (d) growing the additional 41-177 RZ-derived lettuce seed of step (c) to yield additional 41-177 RZ-derived lettuce plants, and (e) repeating the crossing and growing of steps (c) and (d) for an additional 3-10 generations to further generate 41-177 RZ-derived lettuce plants.

The invention further relates to the above methods that further comprise selecting at steps b), d), and e), a 41-177 RZ-derived lettuce plant, exhibiting a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves. In particular, the invention relates to methods for producing a seed of a 41-177 RZ-derived lettuce plant which may comprise (a) crossing a plant of lettuce variety 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. NCIMB 42094, with a second lettuce plant and (b) whereby seed of a 41-177 RZ-derived lettuce plant forms, wherein such a method may further comprise (c) crossing a plant grown from 41-177 RZ-derived lettuce seed with itself or with a second lettuce plant to yield additional 41-177 RZ-derived lettuce seed, (d) growing the additional 41-177 RZ-derived lettuce seed of step (c) to yield additional 41-177 RZ-derived lettuce plants and selecting plants exhibiting a combination of the traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves, and (e) repeating the crossing and growing of steps (c) and (d) for an additional 3-10 generations to further generate 41-177 RZ-derived lettuce plants that exhibit a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves.

The invention additionally provides a method of introducing a desired trait into a plant of lettuce variety 41-177 RZ which may comprise: (a) crossing a plant of lettuce variety 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. NCIMB 42094, with a second lettuce plant that may comprise a desired trait to produce F1 progeny; (b) selecting an F1 progeny that may comprise the desired trait; (c) crossing the selected F1 progeny with a plant of lettuce variety 41-177 RZ, to produce backcross progeny; (d) selecting backcross progeny which may comprise the desired trait and the physiological and morphological characteristic of a plant of lettuce variety 41-177 RZ; and, optionally, (e) repeating steps (c) and (d) one or more times in succession to produce selected fourth or higher backcross progeny that comprise the desired trait and all of the physiological and morphological characteristics of a plant of lettuce variety 41-177 RZ, when grown in the same environmental conditions. The invention, of course, includes a lettuce plant produced by this method.

Backcrossing may also be used to improve an inbred plant. Backcrossing transfers a specific desirable trait from one inbred or non-inbred source to an inbred that lacks that trait. This may be accomplished, for example, by first crossing a superior inbred (A) (recurrent parent) to a donor inbred (non-recurrent parent), which carries the appropriate locus or loci for the trait in question. The progeny of this cross are then mated back to the superior recurrent parent (A) followed by selection in the resultant progeny for the desired trait to be transferred from the non-recurrent parent. After five or more backcross generations with selection for the desired trait, the progeny are heterozygous for loci controlling the characteristic being transferred, but are like the superior parent for most or almost all other loci. The last backcross generation

would be selfed to give pure breeding progeny for the trait being transferred. When a plant of lettuce variety 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. NCIMB 42094, is used in backcrossing, offspring retaining the combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves are progeny within the ambit of the invention. Backcrossing methods may be used with the present invention to improve or introduce a characteristic into a plant of the invention, being a plant of lettuce variety 41-177 RZ. See, e.g., U.S. Pat. No. 7,705,206 (incorporated herein by reference consistent with the above INCORPORATION BY REFERENCE section), for a general discussion relating to backcrossing.

The invention further involves a method of determining the genotype of a plant of lettuce variety 41-177 RZ, representative seed of which has been deposited under NCIMB Accession No. NCIMB 42094, or a first generation progeny thereof, which may comprise obtaining a sample of nucleic acids from said plant and detecting in said nucleic acids a plurality of polymorphisms. This method may additionally comprise the step of storing the results of detecting the plurality of polymorphisms on a computer readable medium. The plurality of polymorphisms are indicative of and/or give rise to the expression of the morphological and physiological characteristics of lettuce variety 41-177 RZ.

There are various ways of obtaining genotype data from a nucleic acid sample. Genotype data may be gathered which is specific for certain phenotypic traits (e.g. gene sequences), but also patterns of random genetic variation may be obtained to construct a so-called DNA fingerprint. Depending on the technique used a fingerprint may be obtained that is unique for lettuce variety 41-177 RZ. Obtaining a unique DNA fingerprint depends on the genetic variation present in a variety and the sensitivity of the fingerprinting technique. A technique known in the art to provide a good fingerprint profile is called AFLP fingerprinting technique (See generally U.S. Pat. No. 5,874,215), but there are many other marker based techniques, such as RFLP (or Restriction fragment length polymorphism), SSLP (or Simple sequence length polymorphism), RAPD (or Random amplification of polymorphic DNA) VNTR (or Variable number tandem repeat), Microsatellite polymorphism, SSR (or Simple sequence repeat), STR (or Short tandem repeat), SFP (or Single feature polymorphism) DArT (or Diversity Arrays Technology), RAD markers (or Restriction site associated DNA markers) (e.g. Baird et al. PloS One Vol. 3 e3376, 2008; Semagn et al. African Journal of Biotechnology Vol. 5 number 25 pp. 2540-2568, 29 Dec., 2006). Nowadays, sequence-based methods are utilizing Single Nucleotide Polymorphisms (SNPs) that are randomly distributed across genomes, as a common tool for genotyping (e.g. Elshire et al. PloS One Vol. 6: e19379, 2011; Poland et al. PloS One Vol. 7: e32253; Truong et al. PLoS One Vol. 7 number 5: e37565, 2012).

With any of the aforementioned genotyping techniques, polymorphisms may be detected when the genotype and/or sequence of the plant of interest is compared to the genotype and/or sequence of one or more reference plants. As used herein, the genotype and/or sequence of a reference plant may be derived from, but is not limited to, any one of the following: parental lines, closely related plant varieties or species, complete genome sequence of a related plant variety or species, or the de novo assembled genome sequence of one or more related plant varieties or species. It is possible, for example, to detect polymorphisms for the characteristic of "solid main vein" by comparing the genotype and/or the sequence of lettuce variety 41-177 RZ with the genotype and/or the sequence of one or more reference plants. The reference plant(s) used for comparison in this example may be any of the comparison varieties "Auvona RZ" or "Verde degli ortolani". It is also possible for example, to detect polymorphisms for any of the resistances e.g. the LMV or Corky root rot resistances by comparing the genotype and/or the sequence of lettuce variety 41-177 RZ with the genotype and/or the sequence of one or more reference plants. The reference plant(s) used for comparison may for example be, but is not limited to, any of the comparison varieties "Auvona RZ" or "Verde degli ortolani".

The polymorphism revealed by these techniques may be used to establish links between genotype and phenotype. The polymorphisms may thus be used to predict or identify certain phenotypic characteristics, individuals, or even species. The polymorphisms are generally called markers. It is common practice for the skilled artisan to apply molecular DNA techniques for generating polymorphisms and creating markers.

The polymorphisms of this invention may be provided in a variety of mediums to facilitate use, e.g. a database or computer readable medium, which may also contain descriptive annotations in a form that allows a skilled artisan to examine or query the polymorphisms and obtain useful information.

As used herein "database" refers to any representation of retrievable collected data including computer files such as text files, database files, spreadsheet files and image files, printed tabulations and graphical representations and combinations of digital and image data collections. In a preferred aspect of the invention, "database" refers to a memory system that may store computer searchable information.

As used herein, "computer readable media" refers to any medium that may be read and accessed directly by a computer. Such media include, but are not limited to: magnetic storage media, such as floppy discs, hard disc, storage medium and magnetic tape; optical storage media such as CD-ROM; electrical storage media such as RAM, DRAM, SRAM, SDRAM, ROM; and PROMs (EPROM, EEPROM, Flash EPROM), and hybrids of these categories such as magnetic/optical storage media. A skilled artisan may readily appreciate how any of the presently known computer readable mediums may be used to create a manufacture which may comprise computer readable medium having recorded thereon a polymorphism of the present invention.

As used herein, "recorded" refers to the result of a process for storing information in a retrievable database or computer readable medium. For instance, a skilled artisan may readily adopt any of the presently known methods for recording information on computer readable medium to generate media which may comprise the polymorphisms of the present invention. A variety of data storage structures are available to a skilled artisan for creating a computer readable medium where the choice of the data storage structure will generally be based on the means chosen to access the stored information. In addition, a variety of data processor programs and formats may be used to store the polymorphisms of the present invention on computer readable medium.

The present invention further provides systems, particularly computer-based systems, which contain the polymorphisms described herein. Such systems are designed to identify the polymorphisms of this invention. As used herein, "a computer-based system" refers to the hardware, software and memory used to analyze the polymorphisms. A skilled artisan

may readily appreciate that any one of the currently available computer-based system are suitable for use in the present invention.

Lettuce leaves are sold in packaged form, including without limitation as pre-packaged lettuce salad or as lettuce heads. Mention is made of U.S. Pat. No. 5,523,136, incorporated herein by reference consistent with the above INCORPORATION BY REFERENCE section, which provides packaging film, and packages from such packaging film, including such packaging containing leafy produce, and methods for making and using such packaging film and packages, which are suitable for use with the lettuce leaves of the invention. Thus, the invention comprehends the use of and methods for making and using the leaves of the lettuce plant of the invention, as well as leaves of lettuce plants derived from the invention. The invention further relates to a container which may comprise one or more plants of the invention, or one or more lettuce plants derived from a plant of the invention, in a growth substrate for harvest of leaves from the plant in a domestic environment. This way the consumer may pick very fresh leaves for use in salads. More generally, the invention includes one or more plants of the invention or one or more plants derived from lettuce of the invention, wherein the plant is in a ready-to-harvest condition, including with the consumer picking his own, and further including a container which may comprise one or more of these plants.

The invention is further described by the following numbered paragraphs:

1. Lettuce plant exhibiting a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves, representative seed of which having been deposited under NCIMB Accession No. 42094.
2. Lettuce plant of paragraph 1 wherein the midrib is moderately to prominently raised.
3. Lettuce plant designated 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. 42094.
4. A seed of the plant of paragraph 1.
5. Parts of the plant of paragraph 1 or paragraph 2 or paragraph 3, wherein said parts of the plant are suitable for sexual reproduction.
6. Parts of the plant as described in paragraph 5, wherein said parts are selected from the group consisting of microspores, pollen, ovaries, ovules, embryo sacs and egg cells.
7. Parts of the plant of paragraph 1 or paragraph 2 or paragraph 3, wherein said parts of the plant are suitable for vegetative reproduction.
8. Parts as described in paragraph 7, wherein said parts are selected from the group consisting of cuttings, roots, stems, cells and protoplasts.
9. A tissue culture of regenerable cells from the lettuce plant of paragraph 1.
10. A tissue culture as described in paragraph 9, wherein said cells or protoplasts of the tissue culture which are derived from a tissue selected from the group consisting of leaves, pollen, embryos, cotyledon, hypocotyls, meristematic cells, roots, root tips, anthers, flowers, seeds and stems.
11. Progeny of a lettuce plant of paragraph 1 or paragraph 2 or paragraph 3.
12. Progeny as described in paragraph 11, wherein said progeny is produced by sexual or vegetative reproduction of said lettuce plant, and wherein said progeny exhibits a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves.
13. Progeny of a lettuce plant of paragraph 3, having all the morphological and physiological characteristics of the lettuce plant of paragraph 3, representative seed of which having been deposited under NCIMB Accession No. 42094 wherein the morphological and physiological characteristics are as found in lettuce variety 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. 42094.
14. Progeny of a lettuce plant of paragraph 1 or paragraph 2 or paragraph 3, representative seed of which having been deposited under NCIMB Accession No. 42094, and is modified in one or more other characteristics.
15. Progeny as described in paragraph 14, wherein the modification is effected by mutagenesis.
16. Progeny as described in paragraph 14, wherein the modification is effected by transformation with a transgene.
17. A method of producing a hybrid lettuce seed comprising crossing a first parent lettuce plant with a second parent lettuce plant and harvesting the resultant hybrid lettuce seed, wherein said first parent lettuce plant or said second parent lettuce plant is the lettuce plant of paragraph 1.
18. A hybrid lettuce plant produced by the method of paragraph 17.
19. A method of producing a lettuce cultivar containing a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves, comprising: crossing a mother lettuce plant with a father lettuce plant to produce a hybrid seed; growing said hybrid seed to produce a hybrid plant; selfing said hybrid plant to produce F2 progeny seed; growing said F2 progeny seed to produce F2-plants; selecting said F2-plants for exhibiting resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves, and, selfing said selected F2-plants to produce F3 progeny seed; growing said F3 progeny seed to produce F3-plants; selecting F3-plants for exhibiting resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves, optionally followed by more selfing and selection steps.
20. A lettuce cultivar produced by the method of paragraph 19.
21. A method for producing lettuce leaves as a fresh vegetable comprising packaging leaves of a plant of paragraph 1.
22. A method for producing lettuce leaves as a processed food comprising processing leaves of a plant of paragraph 1.

23. One or more lettuce plants of paragraph 1, in a container, for harvest of leaves.
24. Lettuce plant having morphological and/or physiological characteristics of a lettuce plant, representative seed of which having been deposited under NCIMB Accession No. 42094.
25. Lettuce plant of paragraph 23 having all the morphological and physiological characteristics of the lettuce plant, representative seed of which having been deposited under NCIMB Accession No. 42094.
26. A method of introducing a desired trait into a plant of lettuce variety 41-177 RZ comprising: (a) crossing a plant of lettuce variety 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. NCIMB 42094, with a second lettuce plant that comprises the desired trait to produce F1 progeny; (b) selecting an F1 progeny that comprises the desired trait; (c) crossing the selected F1 progeny with a plant of lettuce variety 41-177 RZ, to produce backcross progeny and (d) selecting backcross progeny comprising the desired trait and the physiological and morphological characteristic of a plant of lettuce variety 41-177 RZ, when grown in the same environmental conditions.
27. The method of paragraph 26 further comprising (e) repeating steps (c) and (d) one or more times in succession to produce selected fourth or higher backcross progeny that comprise the desired trait and all of the physiological and morphological characteristics of a plant of lettuce variety 41-177 RZ, when grown in the same environmental conditions.
28. A lettuce plant produced by the method of paragraph 26 or paragraph 27.
29. A method for producing a seed of a 41-177 RZ-derived lettuce plant comprising (a) crossing a plant of lettuce variety 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. NCIMB 42094, with a second lettuce plant, and (b) whereby seed of a 41-177 RZ-derived lettuce plant form.
30. The method of paragraph 29 further comprising (c) crossing a plant grown from 41-177 RZ-derived lettuce seed with itself or with a second lettuce plant to yield additional 41-177 RZ-derived lettuce seed, (d) growing the additional 41-177 RZ-derived lettuce seed of step (c) to yield additional 41-177 RZ-derived lettuce plants, and (e) repeating the crossing and growing of steps (c) and (d) for an additional 3-10 generations to generate further 41-177 RZ-derived lettuce plants.
31. The method of paragraph 29 or 30 further comprising selecting at steps b), d), and e), a 41-177 RZ-derived lettuce plant, exhibiting a combination of traits including resistance against downy mildew (*Bremia lactucae*) races Bl:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves.
32. Seed produced by the method of paragraphs 29, or paragraph 30, or paragraph 31.
33. A method of determining the genotype of a plant of lettuce variety 41-177 RZ, representative seed of which has been deposited under NCIMB Accession No. NCIMB 42094, or a first generation progeny thereof, comprising obtaining a sample of nucleic acids from said plant and comparing said nucleic acids to a sample of nucleic acids obtained from a reference plant, and detecting a plurality of polymorphisms between the two nucleic acid samples, wherein the plurality of polymorphisms are indicative of lettuce variety 41-177 RZ and/or give rise to the expression of any one or more, or all, of the morphological and physiological characteristics of lettuce variety 41-177 RZ as described in paragraph 1.
34. The method of paragraph 33 additionally comprising the step of storing the results of detecting the plurality of polymorphisms on a computer readable medium, or transmitting the results of detecting the plurality of polymorphisms.
35. The computer readable medium of paragraph 34.

Having thus described in detail preferred embodiments of the present invention, it is to be understood that the invention is not to be limited to particular details set forth in the above description as many apparent variations thereof are possible without departing from the spirit or scope of the present invention.

What is claimed is:

1. A lettuce plant exhibiting a combination of traits including resistance against downy mildew (*Bremia lactucae*) races B1:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves, representative seed of which having been deposited under NCIMB Accession No. 42094.

2. The lettuce plant of claim **1** wherein the midrib is moderately to prominently raised.

3. A lettuce plant designated 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. 42094.

4. A seed of the plant of claim **1**.

5. A part of the plant of claim **1**, wherein said part of the plant is suitable for sexual reproduction.

6. The part of the plant as claimed in claim **5**, wherein said part comprises a microspore, pollen, ovary, ovule, embryo sac or egg cell.

7. A part of the plant of claim **1**, wherein said part of the plant is suitable for vegetative reproduction.

8. The part as claimed in claim **7**, wherein said part comprises a cutting, root, stem, cell or protoplast.

9. A tissue culture of regenerable cells or protoplasts from the lettuce plant of claim **1**.

10. The tissue culture as claimed in claim **9**, wherein said cells or protoplasts of the tissue culture are derived from a tissue comprising a leaf, pollen, embryo, cotyledon, hypocotyl, meristematic cell, root, root tip, anther, flower, seed or stem.

11. A progeny of a lettuce plant of claim **1**, wherein the progeny exhibits the combination of traits of the lettuce plant of claim **1**.

12. The progeny as claimed in claim **11**, wherein said progeny is produced by sexual or vegetative reproduction of said lettuce plant, and wherein said progeny exhibits a combination of traits including resistance against downy mildew (*Bremia lactucae*) races B1:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves.

13. A progeny of a lettuce plant of claim **3**, having all the morphological and physiological characteristics of the lettuce plant of claim **3**, wherein the morphological and physiological characteristics are as found in lettuce variety 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. 42094.

14. A progeny of a lettuce plant of claim **1**, representative seed of which having been deposited under NCIMB Acces-

sion No. 42094, wherein the progeny exhibits the combination of traits of the lettuce plant of claim **1** and wherein the progeny is further modified in one or more other characteristics.

15. The progeny as claimed in claim **14**, wherein the modification is effected by mutagenesis.

16. The progeny as claimed in claim **14**, wherein the modification is effected by transformation with a transgene.

17. A method of producing a hybrid lettuce seed comprising crossing a first parent lettuce plant with a second parent lettuce plant and harvesting the resultant hybrid lettuce seed, wherein said first parent lettuce plant or said second parent lettuce plant is the lettuce plant of claim **1**.

18. A hybrid lettuce plant grown from the seed produced by the method of claim **17**.

19. A method of producing a lettuce cultivar containing a combination of traits including resistance against downy mildew (*Bremia lactucae*) races B1:1-28and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves, representative seed of which having been deposited under NCIMB Accession No. 42094, comprising: crossing a mother lettuce plant with a father lettuce plant to produce a hybrid seed; growing said hybrid seed to produce a hybrid plant; selfing said hybrid seed to produce F2 progeny seed; growing said F2 progeny seed to produce F2-plants; selecting said F2-plants for exhibiting resistance against downy mildew (*Bremia lactucae*) races B1:1-28and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves, and, selfing said selected F2-plants to produce F3 progeny seed; growing said F3 progeny seed to produce F3-plants; selecting F3-plants for exhibiting resistance against downy mildew (*Bremia lactucae*) races B1:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves, representative seed of which having been deposited under NCIMB Accession No. 42094, optionally followed by more selfing and selection steps.

20. A lettuce cultivar produced by the method of claim **19**.

21. A method for producing lettuce leaves as a fresh vegetable comprising packaging leaves of a plant of claim **1**.

22. A method for producing lettuce leaves as a processed food comprising processing leaves of a plant of claim **1**.

23. A lettuce plant having the combination of traits of the lettuce plant of claim **1** and other morphological and/or physiological characteristics of a lettuce plant, representative seed of which having been deposited under NCIMB Accession No. 42094.

24. The lettuce plant of claim **23** having all the morphological and physiological characteristics of the lettuce plant, representative seed of which having been deposited under NCIMB Accession No. 42094.

25. A method of introducing a desired trait into a plant of lettuce variety 41-177 RZ comprising: (a) crossing a plant of lettuce variety 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. NCIMB 42094, with a second lettuce plant that comprises the desired trait to produce F1 progeny; (b) selecting an F1 progeny that comprises the desired trait; (c) crossing the selected F1 progeny with a plant of lettuce variety 41-177 RZ, to produce backcross progeny and (d) selecting backcross progeny comprising the desired trait and the physiological and morphological characteristics of a plant of lettuce variety 41-177 RZ, when grown in the same environmental conditions.

26. The method of claim **25** further comprising (e) repeating steps (c) and (d) one or more times in succession to produce selected fourth or higher backcross progeny that comprise the desired trait and all of the physiological and morphological characteristics of a plant of lettuce variety 41-177 RZ, when grown in the same environmental conditions.

27. A lettuce plant produced by the method of claim **25** or claim **26**.

28. A method for producing a seed of a 41-177 RZ-derived lettuce plant comprising (a) crossing a plant of lettuce variety 41-177 RZ, representative seed of which having been deposited under NCIMB Accession No. NCIMB 42094, with a second lettuce plant, and (b) whereby seed of a 41-177 RZ-derived lettuce plant form.

29. The method of claim **28** further comprising (c) crossing a plant grown from 41-177 RZ-derived lettuce seed with itself or with a second lettuce plant to yield additional 41-177 RZ-derived lettuce seed, (d) growing the additional 41-177 RZ-derived lettuce seed of step (c) to yield additional 41-177 RZ-derived lettuce plants, and (e) repeating the crossing and growing of steps (c) and (d) for an additional 3-10 generations to generate further 41-177 RZ-derived lettuce plants.

30. The method of claim **28** further comprising selecting at steps b), d), and e), a 41-177 RZ-derived lettuce plant, exhibiting a combination of traits including resistance against downy mildew (*Bremia lactucae*) races B1:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves.

31. A seed produced by the method of claims claim **28**.

32. A method of determining the genotype of a plant of lettuce variety 41-177 RZ, representative seed of which has been deposited under NCIMB Accession No. NCIMB 42094, or a first generation progeny thereof, comprising obtaining a sample of nucleic acids from said plant and comparing said nucleic acids to a sample of nucleic acids obtained from a reference plant, and detecting a plurality of polymorphisms between the two nucleic acid samples, wherein the plurality of polymorphisms are indicative of lettuce variety 41-177 RZ and/or give rise to the expression of any one or more, or all, of the morphological and physiological characteristics of lettuce variety 41-177 RZ as claimed in claim **1**.

33. The method of claim **32** additionally comprising the step of storing the results of detecting the plurality of polymorphisms on a computer readable medium, or transmitting the results of detecting the plurality of polymorphisms.

34. A lettuce plant exhibiting a combination of traits including resistance against downy mildew (*Bremia lactucae*) races B1:1-28 and Ca-I to Ca-VIII, currant-lettuce aphid (*Nasonovia ribisnigri*) biotype Nr:0, lettuce mosaic virus (LMV), and corky root rot (*Sphingomonas suberifaciens*), as well as solid main veins and narrow base leaves, and having genetic information for so exhibiting the combination of traits, wherein the genetic information is as contained in a plant representative seed of which having been deposited under NCIMB Accession No. 42094.

* * * * *

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 9,161,510 B2
APPLICATION NO. : 13/792017
DATED : October 20, 2015
INVENTOR(S) : Adrianus Martinus Jozeph Ammerlaan

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

ON THE TITLE PAGE OF THE PATENT:

(72) Inventor: change "Adrianus Martinus Jozef Ammerlaan" to --Adrianus Martinus Jozeph Ammerlaan--

Signed and Sealed this
Seventeenth Day of May, 2016

Michelle K. Lee

Michelle K. Lee
Director of the United States Patent and Trademark Office