TITLE: METHOD and/or COMPOSITIONS FOR LETTUCE (LACTUCA SATIVA) BREEDING and/or VARIETIES DEVELOPED THEREBY

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to provisional application U.S. Serial No. 62/706,580, filed August 26, 2020, which is incorporated herein by reference in its entirety.

FIELD

The present invention relates to the field of plant breeding and molecular biology. In particular, this invention relates to specialty Lettuce (Lactuca sativa) plants, cultivars and varieties, including methods for making and using said Lettuce plants and compositions derived thereof. Genetic markers and novel genes associated with valuable traits in Lettuce are also disclosed.

BACKGROUND

There are numerous steps in the development of any novel, desirable plant germplasm. Plant breeding preferably begins with the analysis and definition of problems and weaknesses of the current germplasm, the establishment of program goals, and the definition of specific breeding objectives. The next step is preferable selection of germplasm that possess the traits to meet the program goals. The goal is to combine in a single variety or hybrid an improved combination of desirable traits from the parental germplasm.

There is also a need in lettuce breeding to identify lettuce germplasm that provides valuable traits including, for example, canopy diameter, plant height, number of leaves and overall leaf area. There is also a need to develop polymorphic markers for monitoring and introgressing alleles associated with these traits, and to further develop agronomically elite lettuce lines comprising these traits for enhancing plant productivity

SUMMARY

Provided herein, are novel agronomically elite lettuce cultivars with unique and superior traits including one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) when compared to traditional and closely related cultivars grown in the same environment. In some examples, this disclosure thus relates to the seeds of the Lettuce cultivars of the disclosure, to plants of the Lettuce cultivars of the disclosure, to plant parts of the Lettuce cultivars of the disclosure, to methods for

producing a Lettuce cultivars produced by crossing the Lettuce cultivars of the disclosure with other Lettuce cultivars, and to methods for producing Lettuce cultivars containing in their genetic material one or more backcross conversion traits or transgenes and to the backcross conversion Lettuce cultivars, plants and plant parts produced by those methods.

This disclosure also relates to Lettuce cultivars and plant parts derived from the Lettuce cultivars of the disclosure, to methods for producing other Lettuce cultivars derived from Lettuce cultivars of the disclosure and to the Lettuce cultivars and their parts derived by the use of those methods. This disclosure further relates to Lettuce cultivar seeds, plants and plant parts produced by crossing the Lettuce cultivars of the disclosure or a backcross conversion of the cultivars of the disclosure with another Lettuce cultivar.

The disclosure further relates to products and compositions produced or purified from plants of the disclosure including the leaves, head, stalk, stem, bolts, flowers, seeds, and the like. Products produced form the cultivar of the disclosure include primarily harvested leaves for consumption but other uses for plant parts are contemplated, such as animal feed, extracts and the like.

The present disclosure also provides single nucleotide polymorphism (SNP) markers associated with valuable traits described herein. Breeding for Lettuce plants with these traits can be greatly facilitated by the use of marker-assisted selection. The present disclosure provides and includes a method for screening and selecting a Lettuce plant for use in breeding to develop etlie varieties with these traits.

The present disclosure provides a method of introgressing an allele into a Lettuce plant for development of lettuce varieties comprising (a) crossing at least one Lettuce plant having an allele associated with one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) as depicted in one or more of SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 11, or 12 with at least one second Lettuce plant in order to form a population, (b) genotyping with at least one Lettuce plant in the formed population with respect to said Lettuce genomic nucleic acid marker, and (c) selecting from the population at least one Lettuce plant comprising at least one genotype corresponding to a Lettuce plant having one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2). In certain examples, the selected plants are used for further breeding. In certain examples of the methods, the population formed, genotyped, and selected from can be a segregating population. The disclosure further provides a Lettuce plant produced by such methods. More specifically the markers and genomic sequences incorporated into the plants include one or more sequences of SEQ ID NOS 1-12. In some

examples the plants incorporating the same include a SNP from wild-type of a G at position 51 of SEQ ID NO:1, a G at position 51 of SEQ ID NO:2, a G at position 49 of SEQ ID NO:3, a G at position 49 of SEQ ID NO:4, a G at position 50 of SEQ ID NO:5, a G at position 50 of SEQ ID NO:6, a G at position 48 of SEQ ID NO;7, a T at position 50 of SEQ ID NO:8, A G at position 50 of SEQ ID NO:9, a G at position 51 of SEQ ID NO:10, a G at position 50 of SEQ ID NO: 11, or a C at position 49 of SEQ ID NO:12 as annotated to wildtype reference sequence contigs disclosed herein. Plant cultivars incorporating these marker positions or sequences are also contemplated herein.

The disclosure further provides a method of introgressing an allele associated with one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) that is significantly different from typical wild type lettuce into a Lettuce plant comprising: (a) crossing at least one Lettuce plant having with at least one Lettuce plant having no such traits to form a population; (b) screening the population with at least one nucleic acid marker to determine if one or more Lettuce plants from the population contains said allele, wherein the allele is selected from the group of SEQ ID NOs: 1-12. In certain examples of this method, the population formed, genotyped, and selected from can be a segregating population. The disclosure provides a Lettuce plant obtained by such methods, the Lettuce plant or variety comprising a nucleic acid molecule selected from the group of SEQ ID NOs: 1-12.

The disclosure provides a substantially purified nucleic acid molecule for the detection of loci related to plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2) comprising a nucleic acid molecule selected from the group of SEQ ID NOs: 1-12 and complements thereof. The disclosure further provides assays for detecting one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) loci in a Lettuce plant.

Methods of identifying Lettuce plants comprising at least one allele associated with one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) are also provided. In certain examples of these methods of identifying a Lettuce plant comprising at least one allele associated with one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) in a Lettuce plant, the methods comprise: (a) genotyping at least one Lettuce plant with at least one Lettuce genomic nucleic acid marker selected from the group of SEQ ID NOs: 1-12, and (b) selecting at least one Lettuce plant comprising an allele of at least one of the nucleic acid markers that is associated with one or more of plant (canopy) diameter (cm), plant height

(cm), number of leaves, and overall leaf area (cm^2). In certain examples, the at least one Lettuce plant genotyped in step (a) and/or the at least one Lettuce plant selected in step (b) is a Lettuce plant from a population generated by a cross. In certain examples, the selected one or more Lettuce plants exhibit one or more of superior plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2). In examples where the population is generated by a cross, the cross can be of at least one Lettuce plant having one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) with at least Lettuce plant having no superior plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2). In still other examples, the methods can further comprise the step (c) of assaying the selected Lettuce plant for one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2). In still other examples, the methods can further comprise the step of crossing the Lettuce plant selected in step (b) to another Lettuce plant. In still other examples, the methods can further comprise the step of obtaining seed from the Lettuce plant selected in step (b).

In one aspect, the present disclosure refers to a method of identifying a Lettuce plant for use in a plant breeding program comprising at least one allele associated with plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2) in a Lettuce plant comprising: a) genotyping at least one Lettuce plant with at least one nucleic acid marker selected from the group of SEQ ID NOs: 1-12; and b) selecting based upon said genotyping at least one Lettuce plant comprising an allele of at least one of said nucleic acid markers that is associated with plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2) for breeding.

Also provided herein are Lettuce plants obtained by any of these methods of identifying Lettuce plants comprising at least one allele associated with one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2). In certain examples, Lettuce plants obtained by these methods can comprise an allele of at least one nucleic acid molecule selected from the group of SEQ ID NOs: 1-12 that is associated with one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2), and wherein the Lettuce plant exhibits one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2). In certain examples, Lettuce plants obtained by these methods are elite Lettuce plants.

In one aspect, the present disclosure refers to a Lettuce plant obtained by the method disclosed herein, wherein said Lettuce plant comprises an allele of at least one nucleic acid molecule selected from SEQ ID NOs: 1-12 that is associated with plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2), and produces the same traits.

Methods of introgressing a one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) locus into a Lettuce plant are also provided. In certain examples, these methods of introgressing a one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) locus into a Lettuce plant comprise: (a) screening a population with at least one nucleic acid marker to determine if one or more Lettuce plants from the population contains a one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) locus, and (b) selecting from the population at least one Lettuce plant comprising an allele of the marker associated with the one or more of plant (canopy) diameter (cm), plant

height (cm), number of leaves, and overall leaf area (cm^2) locus. In certain examples of these methods, at least one of the markers is as provided in Table 5. In certain examples of these methods, at least one of the markers is located within 5 cM, 2 cM, or 1 cM of the one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2). In certain examples of these methods, at least one of the markers is located within 100 Kb of the one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) locus. In other examples, at least one of the markers is located within 1 Mb, or 1 Kb of the one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2).

In one aspect, the present disclosure refers to a method of introgressing a plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2) locus allele into a Lettuce plant, the method comprising the steps of: a) crossing at least one first Lettuce plant comprising the plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2) locus allele, wherein the allele comprises one or more of SEQ ID NOs: 1-12, with at least one second Lettuce plant in order to form a segregating population; b) screening said segregating population with one or more nucleic acid markers to determine if one or more Lettuce plants contain the plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2) locus allele comprising one or more of SEQ ID NOs: 1-12; and c) selecting said plants based upon said screening from said segregating population one or more Lettuce plants comprising said plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2) locus allele for further breeding.

In one aspect, the present disclosure refers to a Lettuce plant obtained by the method disclosed herein.

In certain examples of these methods, the population is a segregating population. In certain examples of these methods, at least one of the markers exhibits a LOD score of greater than 2.0 with the one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) locus. In other examples, at least one of the markers exhibits a LOD score of greater than 3.0 or greater than 4.0 with the one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) locus. In certain examples of these methods, at least one of the markers is selected from the group of SEQ ID NOs: 1-12.

In one aspect, the present disclosure refers to a method of creating a population of Lettuce plants each comprising at least one allele associated with plant (canopy) diameter

(cm), plant height (cm), number of leaves, and overall leaf area (cm2), the method comprising the steps of: a) genotyping a first population of Lettuce plants, said population or said plants containing at least one allele associated with plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2), the at least one allele associated with plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2) comprising one or more of SEQ ID NOs: 1-12; b) selecting from said first population of Lettuce plants based upon said genotyping one or more Lettuce plants containing said at least one allele associated with plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2); and c) producing from said selected one or more Lettuce plants a second population of Lettuce plants comprising at least one allele associated with plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2) comprising one or more of SEQ ID NO: 1-12.

In one aspect, the present disclosure refers to a population of Lettuce plants obtained by the method disclosed herein, wherein the Lettuce plants produce superior plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm2).

Also provided herein are Lettuce plants obtained by any of these methods of introgressing a one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) locus into a Lettuce plant. In certain examples, a Lettuce plant obtained by these methods can comprise an allele of at least one of nucleic acid marker selected from the group of SEQ ID NOs: 1-12 that is associated with one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2). In certain examples, a Lettuce plant obtained by these methods can exhibit one or more of plant (canopy) diameter (cm), plant height (cm), number of leaves, and overall leaf area (cm^2) In certain examples, the progeny of a Lettuce plant obtained by these methods.

Also provided are isolated nucleic acid molecules for detecting a molecular marker representing a polymorphism in Lettuce DNA, wherein the nucleic acid molecule comprises at least 15 nucleotides that include or are adjacent to the polymorphism, wherein the nucleic acid molecule is at least 70%, 80%, 90%, 95%, 98%, or 99% identical to a sequence of the same number of consecutive nucleotides in either strand of DNA that include or are adjacent to the polymorphism, and wherein the molecular marker is selected from the group of SEQ ID NOs: 1-12. In some examples, the polymorphism is a G at position 51 of SEQ ID

NO:1, a G at position 51 of SEQ ID NO:2, a G at position 49 of SEQ ID NO:3, a G at position 49 of SEQ ID NO:4, a G at position 50 of SEQ ID NO:5, a G at position 50 of SEQ ID NO:6, a G at position 48 of SEQ ID NO;7, a T at position 50 of SEQ ID NO:8, A G at position 50 of SEQ ID NO:9, a G at position 51 of SEQ ID NO:10, a G at position 50 of SEQ ID NO: 11, or a C at position 49 of SEQ ID NO:12 as annotated to wildtype reference sequence contigs disclosed herein. In some examples, isolated nucleic acid molecules comprising SEQ ID NO: 1-12, or a nucleotide sequence having at least 70%, 80%, 90%, 95%, 98%, or 99% sequence identity to SEQ ID NO: 1-12 that include a G at position 51 of SEQ ID NO:1, a G at position 51 of SEQ ID NO:2, a G at position 49 of SEQ ID NO:3, a G at position 49 of SEQ ID NO:4, a G at position 50 of SEQ ID NO:5, a G at position 50 of SEQ ID NO:6, a G at position 48 of SEQ ID NO;7, a T at position 50 of SEQ ID NO:8, A G at position 50 of SEQ ID NO:9, a G at position 51 of SEQ ID NO:10, a G at position 50 of SEQ ID NO: 11, or a C at position 49 of SEQ ID NO:12 as annotated to wildtype reference sequence contigs disclosed herein are provided. In at least some examples the nucleic acid includes one or more base changes so that the sequence is not the naturally occurring sequence. In certain examples, the nucleic acids can further comprise a detectable label or provide for incorporation of a detectable label. In certain examples, the nucleic acid molecule hybridizes to at least one allele of the molecular marker under stringent hybridization conditions.

In one aspect, the present disclosure refers to an isolated nucleic acid molecule comprising SEQ ID NO: 1-12, or a nucleic acid sequence having at least 90% sequence identity to SEQ ID NO: 1-12 that includes G at position 51 of SEQ ID NO:1, a G at position 51 of SEQ ID NO:2, a G at position 49 of SEQ ID NO:3, a G at position 49 of SEQ ID NO:4, a G at position 50 of SEQ ID NO:5, a G at position 50 of SEQ ID NO:6, a G at position 48 of SEQ ID NO;7, a T at position 50 of SEQ ID NO:8, A G at position 50 of SEQ ID NO:9, a G at position 51 of SEQ ID NO:10, a G at position 50 of SEQ ID NO: 11, or a C at position 49 of SEQ ID NO:12.

In one aspect, the present disclosure refers to an isolated nucleic acid molecule for detecting a molecular marker representing a polymorphism in Lettuce DNA, wherein said nucleic acid molecule comprises at least 15 nucleotides that include or are immediately adjacent to said polymorphism, wherein said nucleic acid molecule is at least 90% identical to a sequence of the same number of consecutive nucleotides in either strand of DNA that include or are immediately adjacent to said polymorphism, and wherein said molecular marker is selected from the group of SEQ ID NOs: 1-12.

In one aspect, the present disclosure refers to an isolated nucleic acid molecule for detecting a molecular marker representing a polymorphism in Lettuce DNA, wherein said nucleic acid molecule comprises at least 15 nucleotides that include or are immediately adjacent to said polymorphism, wherein said nucleic acid molecule is at least 90% identical to a sequence of the same number of consecutive nucleotides in either strand of DNA that include or are immediately adjacent to said polymorphism, and wherein said molecular marker is selected from the group of SEQ ID NOs: 1-12.

DESCRIPTION OF THE FIGURES

Figure 1. Comparison of F_4 derivedRecombinant Inbred Lines (Rlls) from three cross to the corresponding Fl hybrids(•), maternal parents{@) and paternalparents (o) for number of leaves (A), leaf length (B), plant diameter(C), plant height (D), and plant area (E).

DETAILED DESCRIPTION

Definitions

In the description and tables which follow, a number of terms are used. In order to provide a clear and consistent understanding of the present invention, the following definitions are provided:

The invention provides Lettuce plants. As used herein, the term "plant" refers to plants in the genus of Lettuce and plants derived thereof. Such as Lettuce plants produced via asexual reproduction and via seed production.

The invention provides plant parts. As used herein, the term "plant part" refers to