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(54) Title: STALK-LENGTH-RELATED MARKER OF PLANT OF THE GENUS SACCHARUM AND THE USE THEREOF

(57) Abstract: According to the present invention, a novel sugarcane-stalk-length-related marker linked to a sugarcane quantitative trait such as an increase in stalk length is provided. Such marker is a sugarcane-stalk-length-related marker, which comprises a continuous nucleic acid region existing in a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 1 and the nucleotide sequence shown in SEQ ID NO: 13, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 14 and the nucleotide sequence shown in SEQ ID NO: 22, or a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 23 and the nucleotide sequence shown in SEQ ID NO: 29 of a sugarcane chromosome.



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Description

Title of Invention: STALK-LENGTH-RELATED MARKER OF PLANT OF THE GENUS SACCHARUM AND THE USE THEREOF

Technical Field

[0001] The present invention relates to a stalk-length-related marker whereby a plant line of the genus *Saccharum* characterized by an increase in stalk length can be selected, and a method for use thereof.

Background Art

[0002] Sugarcane has been cultivated as a raw material for sugar, liquor, and the like for edible use. In addition, sugarcane has been used as, for example, a raw material for biofuel in a variety of industrial fields. Under such circumstances, there is a need to develop novel sugarcane varieties having desirable characteristics (e.g., sugar content, enhanced vegetative capacity, sprouting capacity, disease resistance, insect resistance, cold resistance, an increase in leaf-blade-length, an increase in leaf area, and an increase in stalk length).

In general, the following three ways may be used for identification of a plant variety/line: "characteristics comparison" for comparison of characteristics data, "comparison during cultivation" for comparison of plants cultivated under the same conditions, and "DNA assay" for DNA analysis. There are many problems in line identification with characteristics comparison or comparison during cultivation, including reduction of precision due to differences in cultivation conditions, lengthy duration of field research that requires a number of steps, and the like. In particular, since sugarcane plants are much larger than other graminaceous crops such as rice and maize, it has been difficult to conduct line identification based on field research. In addition, in order to identify a line having morphologically distinct characteristics in terms of leaf-blade-length, leaf area, stalk length, and the like, it is necessary to collect such characteristic data after long-term cultivation of sugarcane. In addition, even after long-term cultivation of sugarcane, it is difficult to identify such line with high accuracy because morphological characteristics are environmentally susceptible.

Further, for creation of a novel sugarcane variety, first, tens of thousands of seedlings are created via crossing, followed by seedling selection and stepwise selection of excellent lines. Eventually, 2 or 3 types of novel varieties having desired characteristics can be obtained. As described above, for creation of a novel sugarcane variety, it is necessary to cultivate and evaluate an enormous number of lines, and it is also necessary to prepare a large-scale field and make highly time-consuming efforts.

Therefore, it has been required to develop a method for identifying a sugarcane line having desired characteristics with the use of markers present in the sugarcane genome. In particular, upon creation of a novel sugarcane variety, if excellent markers could be used to examine a variety of characteristics, the above problems particular to sugarcane would be resolved, and the markers would be able to serve as very effective tools. However, since sugarcane plants have a large number of chromosomes (approximately 100 to 130) due to higher polyploidy, the development of marker technology has been slow. In the case of sugarcane, although the USDA reported genotyping with the use of SSR markers (Non-Patent Document 1), the precision of genotyping is low because of the small numbers of markers and polymorphisms in each marker. In addition, the above genotyping is available only for American/Australian varieties, and therefore it cannot be used for identification of the major varieties cultivated in Japan, Taiwan, India, and other countries or lines that serve as useful genetic resources.

In addition, Non-Patent Document 2 suggests the possibility that a sugarcane genetic map can be created by increasing the number of markers, comparing individual markers in terms of a characteristic relationship, and verifying the results. However, in Non-Patent Document 2, an insufficient number of markers are disclosed and markers linked to desired characteristics have not been found.

Citation List

Non Patent Literature

[0003] NPL 1: Maydica 48(2003)319-329 "Molecular genotyping of sugarcane clones with microsatellite DNA markers"

NPL 2: Nathalie Piperidis et al., Molecular Breeding, 2008, Vol. 21, 233-247

Summary of Invention

Technical Problem

[0004] In view of the above, an object of the present invention is to provide a marker related to stalk length, which is a quantitative trait of sugarcane plants.

Solution to Problem

[0005] In order to achieve the object, the present inventors conducted intensive studies. The present inventors prepared many sugarcane plant markers and carried out linkage analysis of quantitative traits along with sugarcane plant markers for hybrid progeny lines. Accordingly, the present inventors found markers linked to quantitative traits such as an increase in stalk length. This has led to the completion of the present invention.

The present invention encompasses the following.

(1) A sugarcane-stalk-length-related marker, which comprises a continuous nucleic

acid region existing in a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 1 and the nucleotide sequence shown in SEQ ID NO: 13, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 14 and the nucleotide sequence shown in SEQ ID NO: 22, or a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 23 and the nucleotide sequence shown in SEQ ID NO: 29 of a sugarcane chromosome.

(2) The sugarcane-stalk-length-related marker according to (1), wherein the continuous nucleic acid region comprises any nucleotide sequence selected from the group consisting of the nucleotide sequences shown in SEQ ID NOS: 1 to 29.

(3) The sugarcane-stalk-length-related marker according to (1), wherein the continuous nucleic acid region is located at a position in a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 6 and the nucleotide sequence shown in SEQ ID NO: 8, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 18 and the nucleotide sequence shown in SEQ ID NO: 19, or a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 24 and the nucleotide sequence shown in SEQ ID NO: 28 of a sugarcane chromosome.

(4) A method for producing a sugarcane line having an increased stalk length comprising: a step of extracting a chromosome of a progeny plant obtained from parent plants, at least one of which is a sugarcane plant; and a step of determining the presence or absence of the sugarcane-stalk-length-related marker according to any one of (1) to (3) in the obtained sugarcane chromosome.

(5) The method for producing a sugarcane line according to (4), wherein a DNA chip provided with probes each serving as the sugarcane-stalk-length-related marker is used in the determination step.

(6) The method for producing a sugarcane line according to (4), wherein the progeny plant is in the form of seeds or a young seedling and the chromosome is extracted from the seeds or the young seedling.

Advantageous Effects of Invention

[0006] According to the present invention, a novel sugarcane-stalk-length-related marker linked to a sugarcane quantitative trait such as an increase in stalk length can be provided. With the use of the sugarcane-stalk-length-related marker of the present invention, the stalk length of a line obtained by crossing sugarcane lines can be identified. Thus, a sugarcane line characterized by an increase in stalk length can be identified at a very low cost.

Brief Description of Drawings

[0007] [fig.1] Fig. 1 schematically shows the process of production of a DNA microarray used for acquisition of sugarcane chromosome markers.

[fig.2]Fig. 2 schematically shows a step of signal detection with the use of a DNA microarray.

[fig.3]Fig. 3 is a characteristic chart showing stalk-length data for sugarcane variety/line groups used in the Examples.

[fig.4]Fig. 4 is a characteristic chart showing QTL analysis results regarding stalk length (the 18th linkage group).

[fig.5]Fig. 5 is a characteristic chart showing QTL analysis results regarding stalk length (the 23rd linkage group).

[fig.6]Fig. 6 is a characteristic chart showing QTL analysis results regarding stalk length (the 46th linkage group).

[fig.7]Fig. 7 is a characteristic chart showing N828509 signal levels for individual lines.

[fig.8]Fig. 8 is a characteristic chart showing N827910 signal levels for individual lines.

[fig.9]Fig. 9 is a characteristic chart showing N804812 signal levels for individual lines.

Description of Embodiments

[0008] The sugarcane-stalk-length-related marker and the method for using the same according to the present invention are described below. In particular, a method for producing a sugarcane line using a sugarcane-stalk-length-related marker is described.

<<Sugarcane-stalk-length-related markers>>

The sugarcane-stalk-length-related marker of the present invention corresponds to a specific region present on a sugarcane chromosome and is linked to causative genes (i.e., gene group) for a trait that causes an increase in sugarcane stalk length. Thus it can be used to identify a trait characterized by an increase in sugarcane stalk length. Specifically, it is possible to determine that a progeny line obtained using a known sugarcane variety/line is a line having a trait characterized by an increase in stalk length by confirming the presence of a sugarcane-stalk-length-related marker in such progeny line. The expression "increase in stalk length" used herein particularly indicates an increase in stalk length in the initial growth phase after germination. In other words, it corresponds to acceleration of stalk growth rate in the initial growth phase. In addition, the term "initial growth phase" refers to a period of approximately 5 months after germination.

The term "sugarcane" used herein refers to a plant belonging to the genus *Saccharum* of the family Poaceae. In addition, the term "sugarcane" includes both so-called noble cane (scientific name: *Saccharum officinarum*) and wild cane (scientific name: *Saccharum spontaneum*). The term "known sugarcane variety/line" is not particularly

limited. It includes any variety/line capable of being used in Japan and any variety/line used outside Japan. Examples of sugarcane varieties cultivated in Japan include, but are not limited to, Ni1, NiN2, NiF3, NiF4, NiF5, Ni6, NiN7, NiF8, Ni9, NiTn10, Ni11, Ni12, Ni14, Ni15, Ni16, Ni17, NiTn19, NiTn20, Ni22, and Ni23. Examples of main sugarcane varieties used in Japan described herein include, but are not limited to, NiF8, Ni9, NiTn10, and Ni15. In addition, examples of main sugarcane varieties that have been introduced into Japan include, but are not limited to, F177, NCo310, and F172. In addition, a progeny line may be a line obtained by crossing a mother plant and a father plant of the same species, each of which is a sugarcane variety/line, or it may be a hybrid line obtained from parent plants when one thereof is a sugarcane variety/line and the other is a closely related variety/line (*Erianthus arundinaceus*). In addition, a progeny line may be obtained via so-called backcrossing.

The sugarcane-stalk-length-related marker of the present invention has been newly identified by QTL (Quantitative Trait Loci) analysis using a genetic linkage map containing 3004 markers originally obtained from sugarcane chromosomes and sugarcane stalk length data. In addition, many genes are presumably associated with sugarcane stalk length, which is a quantitative trait characterized by a continuous distribution of stalk-length values. For QTL analysis, the QTL Cartographer gene analysis software (Wang S., C. J. Basten, and Z.-B. Zeng (2010); Windows QTL Cartographer 2.5. Department of Statistics, North Carolina State University, Raleigh, NC) is used, and the analysis is carried out by the composite interval mapping (CIM) method.

Specifically, peaks with LOD scores equivalent to or exceeding a given threshold (e.g., 2.5) have been found in 3 regions included in the above genetic linkage map by QTL analysis described above. That is, the following 3 regions have been specified: an approximately 39.9-cM (centimorgan) region, an approximately 21.1-cM region, and an approximately 15.2-cM region having such peaks. The term "morgan (M)" used herein refers to a unit representing the relative distance between genes on a chromosome, and it is expressed by the percentage of the crossover rate. In a case of a sugarcane chromosome, 1 cM corresponds to approximately 2000 kb. In addition, it is suggested that causative genes (i.e., gene group) for a trait that causes an increase in stalk length could be present at the peak positions or in the vicinity thereof.

The 39.9-cM region having the above peak is a region that comprises 13 types of markers listed in table 1 below in the order shown in table 1.

[0009]

[Table 1]

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO
	N826961	GCGGCGGTAGCCCCACAGGAAAAATGACTCCGGG ACGTTCTTTTTTTTCTT	2,500	SEQ ID NO: 1
	N816516	AGAGAGATTGAAGACAACGGCAAAAATGTCATT CACCCACCTTCTCTCTCT	1,000	SEQ ID NO: 2
	N827098	TAGGTCCAGCTTGGCTCAAGGAGTCAAGGAAGT AACTAAACTTATATGTAAACCTTCTATAAG	1,000	SEQ ID NO: 3
	N829001	CATGCTTCACTTGCAGAGGAGCTGCGGCGTCGG TACACATGCCCCGGCTG	750	SEQ ID NO: 4
	N828509	TTTTACAATAACATTTTTGCACTGCCACAAAAAG GTCAATATATACCGACATGATGTCACAAATGGGC TTCATA	1,500	SEQ ID NO: 5
	N810652	CAAACCGATCGATTTTGCTAAACCAAACCTCCAG AGTGACGCCCGCACT	1,000	SEQ ID NO: 6
18	N812117	GCAGCCGATTGAAGTCCGAAAAGTACTAAACTG CCCTTAACTTAATTAATAGCTGC	1,500	SEQ ID NO: 7
	N827138	CATTACATGAGCAAGGACCTACAGATGGGAACT AAACAAAACCTAACAAATCACATGATC	1,000	SEQ ID NO: 8
	N825829	GCGATACTTGACGAGCATCAGCACCTTCATCAG AGCTACCAAAGCTTATG	1,000	SEQ ID NO: 9
	N813742	AAACCGTTAGTTGCAATAAAGTCCGAAGTTGTGC CAAACCTTACTCAAATGAATTATCCACGCA	2,500	SEQ ID NO: 10
	N803417	TGATTGGTAATCCAGCAAATGGTTTATGAGCTTT GCATTTGAGCCATGCCCAT	1,500	SEQ ID NO: 11
	N810475	GGCAATGTATGAACCATACACAAATGACAGGATT CAATTGTTAGATTGATTGCTCCTATTACTTAAAT ATGAACA	1,000	SEQ ID NO: 12
	N825180	GCCATAGTGTTGGTATTCATTGCAAAGTTTCGAT AATGTTTTTATCAAGAGCACATTGCCATTAATAA ACTCG	1,000	SEQ ID NO: 13

The 21.1-cM region having the above peak is a region that comprises 9 types of markers listed in table 2 below in the order shown in table 2.

[0010] [Table 2]

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO
23	N827910	GCTGTOTTAACCAAATAACGTTTTCTTGCTGGAT ACTATAGTAAGTTAAGTCAACGGAAGATTGG	2,000	SEQ ID NO: 14
	N815704	ATGGTGGAGGCTTGGCTTGCTTGAAGGCTTTGGA ATCGGCTGATGAACAT	1,500	SEQ ID NO: 15
	N820994	TTGCAGACAAATATATATACAGAAGCGGTTTTCA GGTAGACACGGGCAGAAG	1,500	SEQ ID NO: 16
	N820544	TTGCAGACAAATATATATACAGAAGCGGTTTTCA GGTAGACACGGGCAGAAG	1,500	SEQ ID NO: 17
	N819412	TGCATCAAACACCCCTAAAGAAAAGTTAGAACACT ATATACAGATCATGGAATAAAATCAGAGATTAAC AAATA	2,000	SEQ ID NO: 18
	N829748	CTCGCTCGTCTCATCGGCGGCGCAGCAAGCGCA ACCGAGCGGCGGCATCA	1,000	SEQ ID NO: 19
	N824769	CGTGAGTGCGTAGGGCCCACTTGTGAGCGCTGT TCGCCAGAGCGGGAGGG	2,000	SEQ ID NO: 20
	N826558	GGTAGTGGATCTTTGATCGCATTATGAGTTTATG TGGAAGATTAAGCCGAACCTTAATTATAATGACTT CTAA	1,000	SEQ ID NO: 21
	N811685	TTTTCATTTGTACGGATAATTAGAAGAGATGCTC TTCAATATTTGACAGTGGTAATAACTGAATGGCA GAACG	1,000	SEQ ID NO: 22

The 15.2-cM region having the above peak is a region that comprises 7 types of markers listed in table 3 below in the order shown in table 3.

[0011]

[Table 3]

Linkage group	Marker name	Nucleotide sequence information	Signal threshold	SEQ ID NO
	N804812	GGTTCGTCAAGGCCAAGGCGGCGGCGCTGGTCG GAGCGCACAAGGTGAAG	1,500	SEQ ID NO: 23
	N826007	ATGTATAGATGACATAAGAAAGGATGTAATCACT TAACTGATGCTTACTAGATTATTAAGCAAATATA ATAGGTA	750	SEQ ID NO: 24
	N826674	TTCAGCACATTTCAGCACTAGTCATCAGTCACCAG CGAGTCAGTAGTATTTTT	1,000	SEQ ID NO: 25
46	N821523	GTCAATTTGOTTAATAAGAGTAGCGTCTGCTAAC ACCATATACAGAATGATTCCGGC	1,000	SEQ ID NO: 26
	N802816	TACCAAGACAGTTAGTGCAGAAGTATATGTCTTC GACTATAGCAAGCACCCATCTAA	750	SEQ ID NO: 27
	N829577	CCTGCACTTTGTGCGCAGAAGATGGCGTAATCACT ACOTTCAACGTCACGG	1,000	SEQ ID NO: 28
	N826565	GCCACCGCAACCGTCACTGGCAGGCCACAGCTA ATCAAGACTGAGGTAGA	1,500	SEQ ID NO: 29

In addition, in tables 1 to 3, "Linkage group" represents the number given to each group among a plurality of linkage groups specified by QTL analysis. In tables 1 to 3, "Marker name" represents the name given to each marker originally obtained in the present invention. In tables 1 to 3, "Signal threshold" represents a threshold used for determination of the presence or absence of a marker.

In addition, the peak contained in the 39.9-cM region is present in a region sandwiched between a marker (N810652) having the nucleotide sequence shown in SEQ ID NO: 6 and a marker (N827138) having the nucleotide sequence shown in SEQ ID NO: 8. The peak contained in the 21.1-cM region is present in a region sandwiched between a marker (N819412) having the nucleotide sequence shown in SEQ ID NO: 18 and a marker (N829748) having the nucleotide sequence shown in SEQ ID NO: 19. The peak contained in the 15.2-cM region is present in a region sandwiched between a marker (N826007) having the nucleotide sequence shown in SEQ ID NO: 24 and a marker (N829577) having the nucleotide sequence shown in SEQ ID NO: 28.

A continuous nucleic acid region existing in any of 3 regions containing markers shown in tables 1 to 3 can be used as a sugarcane-stalk-length-related marker. The term "nucleic acid region" used herein refers to a region having a nucleotide sequence

having 95% or less, preferably 90% or less, more preferably 80% or less, and most preferably 70% or less identity to a different region present on a sugarcane chromosome. If the identity of a nucleic acid region serving as a sugarcane-stalk-length-related marker to a different region falls within the above range, the nucleic acid region can be specifically detected according to a standard method. The identity level described herein can be calculated using default parameters and BLAST or a similar algorithm.

In addition, the base length of a nucleic acid region serving as a sugarcane-stalk-length-related marker can be at least 8 bases, preferably 15 bases or more, more preferably 20 bases or more, and most preferably 30 bases. If the base length of a nucleic acid region serving as a sugarcane-stalk-length-related marker falls within the above range, the nucleic acid region can be specifically detected according to a standard method.

In particular, among the 13 types of markers contained in the 39.9-cM region, a sugarcane-stalk-length-related marker is preferably designated as existing in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 6 and the nucleotide sequence shown in SEQ ID NO: 8. This is because the above peak is present in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 6 and the nucleotide sequence shown in SEQ ID NO: 8. In addition, among the 9 types of markers contained in the 21.1-cM region, a sugarcane-stalk-length-related marker is preferably designated as existing in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 18 and the nucleotide sequence shown in SEQ ID NO: 19. This is because the above peak is present in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 18 and the nucleotide sequence shown in SEQ ID NO: 19. Further, among the 7 types of markers contained in the 15.2-cM region, a sugarcane-stalk-length-related marker is preferably designated as existing in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 24 and the nucleotide sequence shown in SEQ ID NO: 28. This is because the above peak is present in the region sandwiched between the nucleotide sequence shown in SEQ ID NO: 24 and the nucleotide sequence shown in SEQ ID NO: 28.

In addition, a nucleic acid region containing a single marker selected from among the 29 types of markers shown in tables 1 to 3 can be used as a sugarcane-stalk-length-related marker. For example, it is preferable to use, as a sugarcane-stalk-length-related marker, a nucleic acid region containing a marker (N812117) comprising the nucleotide sequence shown in SEQ ID NO: 7 located closest to the peak position in the 39.9-cM region, a nucleic acid region containing a marker (N829748) comprising the nucleotide sequence shown in SEQ ID NO: 19 located closest to the peak position in the 21.1-cM region, or a nucleic acid region containing a

marker (N821523) comprising the nucleotide sequence shown in SEQ ID NO: 26 located closest to the peak position in the 15.2-cM region. In such case, the nucleotide sequence of a nucleic acid region containing the marker can be specified by inverse PCR using primers designed based on the nucleotide sequence of such marker. Further, as a sugarcane-stalk-length-related marker, any of the above 29 types of markers can be directly used. Specifically, one or more type(s) of markers selected from among the 29 types of such markers can be directly used as a sugarcane-stalk-length-related marker. For example, it is preferable to use, as a sugarcane-stalk-length-related marker, a marker (N812117) consisting of the nucleotide sequence shown in SEQ ID NO: 7 located closest to the peak position in the 39.9-cM region, a marker (N829748) consisting of the nucleotide sequence shown in SEQ ID NO: 19 located closest to the peak position in the 21.1-cM region, or a marker (N821523) consisting of the nucleotide sequence shown in SEQ ID NO: 26 closest to the peak position in the 15.2-cM region.

<<Sugarcane marker identification>>

As described above, sugarcane-stalk-length-related markers were identified from among 3004 markers originally obtained from sugarcane chromosomes in the present invention. These 3004 markers are described below. Upon identification of these markers, a DNA microarray can be used according to the method disclosed in JP Patent Application No. 2009-283430.

Specifically, 3004 markers originally obtained from sugarcane chromosomes are used with a DNA microarray having probes designed by the method disclosed in JP Patent Application No. 2009-283430. The method for designing probes as shown in fig. 1 is described below. First, genomic DNA is extracted from sugarcane (step 1a). Next, the extracted genomic DNA is digested with a single or a plurality of restriction enzyme(s) (step 1b). In addition, in the example shown in fig. 1, 2 types of restriction enzymes illustrated as restriction enzymes A and B are used (in the order of A first and then B) to digest genomic DNA. The restriction enzymes used herein are not particularly limited. However, examples of restriction enzymes that can be used include PstI, EcoRI, HindIII, BstNI, HpaII, and HaeIII. In particular, restriction enzymes can be adequately selected in consideration of the frequency of appearance of recognition sequences such that a genomic DNA fragment having a base length of 20 to 10000 can be obtained when genomic DNA is completely digested. In addition, when a plurality of restriction enzymes are used, it is preferable for a genomic DNA fragment obtained after the use of all restriction enzymes to have a base length of 200 to 6000. Further, when a plurality of restriction enzymes are used, the order in which restriction enzymes are subjected to treatment is not particularly limited. In addition, a plurality of restriction enzymes may be used in an identical reaction system if they are treated under identical

conditions (e.g., solution composition and temperature). Specifically, in the example shown in fig. 1, genomic DNA is digested using restriction enzymes A and B in such order. However, genomic DNA may be digested by simultaneously using restriction enzymes A and B in an identical reaction system. Alternatively, genomic DNA may be digested using restriction enzymes B and A in such order. Further, 3 or more restriction enzymes may be used.

Next, adapters are bound to a genomic DNA fragment subjected to restriction enzyme treatment (step 1c). The adapter used herein is not particularly limited as long as it can be bound to both ends of a genomic DNA fragment obtained via the above restriction enzyme treatment. For example, it is possible to use, as an adapter, an adapter having a single strand complementary to a protruding end (sticky end) formed at each end of genomic DNA via restriction enzyme treatment and a primer binding sequence to which a primer used upon amplification treatment as described in detail below can hybridize. In addition, it is also possible to use, as an adapter, an adapter having a single strand complementary to the above protruding end (sticky end) and a restriction enzyme recognition site that is incorporated into a vector upon cloning.

In addition, when genomic DNA is digested using a plurality of restriction enzymes, a plurality of adapters corresponding to the relevant restriction enzymes can be prepared and used. Specifically, it is possible to use a plurality of adapters having single strands complementary to different protruding ends formed upon digestion of genomic DNA with a plurality of restriction enzymes. Here, a plurality of adapters corresponding to a plurality of restriction enzymes each may have a common primer binding sequence such that a common primer can hybridize to each such adapter. Alternatively, they may have different primer binding sequences such that different primers can separately hybridize thereto.

Further, when genomic DNA is digested using a plurality of restriction enzymes, it is possible to use, as an adaptor, adapter(s) corresponding to one or some restriction enzyme(s) selected from among a plurality of the used restriction enzymes.

Next, a genomic DNA fragment to both ends of which adapters have been added is amplified (step 1d). When an adapter having a primer binding sequence is used, the genomic DNA fragment can be amplified using a primer that can hybridize to the primer binding sequence. Alternatively, a genomic DNA fragment to which an adapter has been added is cloned into a vector using the adapter sequence. The genomic DNA fragment can be amplified using primers that can hybridize to specific regions of the vector. In addition, as an example, PCR can be used for a genomic DNA fragment amplification reaction using primers.

In addition, when genomic DNA is digested using a plurality of restriction enzymes and a plurality of adapters corresponding to the relevant restriction enzymes are ligated

to genomic DNA fragments, the adapters are ligated to all genomic DNA fragments obtained via treatment with a plurality of restriction enzymes. In this case, all the obtained genomic DNA fragments can be amplified by carrying out a nucleic acid amplification reaction using primer binding sequences contained in adapters.

Alternatively, genomic DNA is digested using a plurality of restriction enzymes, followed by ligation of adapter(s) corresponding to one or more restriction enzyme(s) selected from among a plurality of the used restriction enzymes to genomic DNA fragments. In such case, among the obtained genomic DNA fragments, a genomic DNA fragment to both ends of which the selected restriction enzyme recognition sequences have been bound can be exclusively amplified.

Next, the nucleotide sequence of the amplified genomic DNA fragment is determined (step 1e). Then, at least one region, which has a base length shorter than the base length of the genomic DNA fragment and corresponds to at least a partial region of the genomic DNA fragment, is specified. Sugarcane probes are designed using at least one of the thus specified regions (step 1f). A method for determining the nucleotide sequence of a genomic DNA fragment is not particularly limited. A conventionally known method using a DNA sequencer applied to the Sanger method or the like can be used. For example, a region to be designed herein has a 20- to 100-base length, preferably a 30- to 90-base length, and more preferably a 50- to 75-base length as described above.

As described above, a DNA microarray can be produced by designing many probes using genomic DNA extracted from sugarcane and synthesizing an oligonucleotide having a desired nucleotide sequence on a support based on the nucleotide sequence of the designed probe. With the use of a DNA microarray prepared as described above, 3004 markers, including the above 29 types of sugarcane-stalk-length-related markers shown in SEQ ID NOS: 1 to 29, can be identified.

More specifically, the present inventors obtained signal data of known sugarcane varieties (NiF8 and Ni9) and a progeny line (line 191) obtained by crossing the varieties with the use of the DNA microarray described above. Then, genotype data were obtained based on the obtained signal data. Based on the obtained genotype data, chromosomal marker position information was obtained by calculation using the gene distance function (Kosambi) and the AntMap genetic map creation software (Iwata H, Ninomiya S (2006) AntMap: constructing genetic linkage maps using an ant colony optimization algorithm, *Breed Sci* 56: 371-378). Further, a genetic map datasheet was created based on the obtained marker position information using Mapmaker/EXP ver. 3.0 (A Whitehead Institute for Biomedical Research Technical Report, Third Edition, January, 1993) As a result, 3004 markers, including the aforementioned 29 types of sugarcane-stalk-length-related markers shown in SEQ ID NOS: 1 to 29, were

identified.

<<Use of sugarcane-stalk-length-related markers>>

The use of sugarcane-stalk-length-related markers makes it possible to determine whether a sugarcane progeny line or the like, which has a phenotype exhibiting unknown stalk length, is a line having a phenotype showing an increase in stalk length. The expression "the use of sugarcane-stalk-length-related markers" used herein indicates the use of a DNA microarray having probes corresponding to sugarcane-stalk-length-related markers in one embodiment. The expression "probes corresponding to sugarcane-stalk-length-related markers" indicates oligonucleotides that can specifically hybridize under stringent conditions to sugarcane-stalk-length-related markers defined as above. For instance, such oligonucleotides can be designed as partial or whole regions with base lengths of at least 10 continuous bases, 15 continuous bases, 20 continuous bases, 25 continuous bases, 30 continuous bases, 35 continuous bases, 40 continuous bases, 45 continuous bases, or 50 or more continuous bases of the nucleotide sequences or complementary strands thereof of sugarcane-stalk-length-related markers defined as above. In addition, a DNA microarray having such probes may be any type of microarray, such as a microarray having a planar substrate comprising glass, silicone, or the like, a bead array comprising microbeads as carriers, or a three-dimensional microarray having an inner wall comprising hollow fibers to which probes are fixed.

The use of a DNA microarray prepared as described above makes it possible to determine whether a sugarcane line such as a progeny line or the like, which has a phenotype exhibiting unknown stalk length, is a line having a phenotype showing an increase in stalk length. In addition, in the case of a method other than the above method involving the use of a DNA microarray, it is also possible to determine whether a sugarcane line, which has a phenotype exhibiting unknown stalk length, is a line having a trait characterized by an increase in stalk length by detecting the above sugarcane-stalk-length-related markers by a conventionally known method.

The method involving the use of a DNA microarray is described in more detail. As shown in fig. 2, first, genomic DNA is extracted from a sugarcane sample. In this case, a sugarcane sample is a sugarcane line such as a sugarcane progeny line, which has a phenotype exhibiting unknown stalk length, and thus which can be used as a subject to be determined whether to have a trait characterized by an increase in stalk length or not. Next, a plurality of genomic DNA fragments are prepared by digesting the extracted genomic DNA with restriction enzymes used for preparing the DNA microarray. Then, the obtained genomic DNA fragments are ligated to adapters used for preparation of the DNA microarray. Subsequently, the genomic DNA fragments, to both ends of which adapters have been added, are amplified using primers employed

for preparation of the DNA microarray. Accordingly, sugarcane-sample-derived genomic DNA fragments corresponding to the genomic DNA fragments amplified in step 1d upon preparation of the DNA microarray can be amplified.

In this step, among the genomic DNA fragments to which adapters have been added, specific genomic DNA fragments may be selectively amplified. For instance, in a case in which a plurality of adapters corresponding to a plurality of restriction enzymes are used, genomic DNA fragments to which specific adapters have been added can be selectively amplified. In addition, when genomic DNA is digested with a plurality of restriction enzymes, genomic DNA fragments to which adapters have been added can be selectively amplified by adding adapters only to genomic DNA fragments that have protruding ends corresponding to specific restriction enzymes among the obtained genomic DNA fragments. Thus, DNA fragment concentration can be increased by selectively amplifying specific genomic DNA fragments.

Thereafter, amplified genomic DNA fragments are labeled. Any conventionally known substance may be used as a labeling substance. Examples of a labeling substance that can be used include fluorescent molecules, dye molecules, and radioactive molecules. In addition, this step can be omitted using a labeled nucleotide in the step of amplifying genomic DNA fragments. This is because when genomic DNA fragments are amplified using a labeled nucleotide in the amplification step, amplified DNA fragments can be labeled.

Next, labeled genomic DNA fragments are allowed to come into contact with the DNA microarray under certain conditions such that probes fixed to the DNA microarray hybridize to the labeled genomic DNA fragments. At such time, preferably, highly stringent conditions are provided for hybridization. Under highly stringent conditions, it becomes possible to determine with high accuracy whether or not sugarcane-stalk-length-related markers are present in a sugarcane sample. In addition, stringent conditions can be adjusted based on reaction temperature and salt concentration. That is, an increase in temperature or a decrease in salt concentration results in more stringent conditions. For example, when a probe having a length of 50 to 75 bases is used, the following more stringent conditions can be provided as hybridization conditions: 40 degrees C to 44 degrees C; 0.21SDS; and 6 x SSC.

In addition, hybridization between labeled genomic DNA fragments and probes can be confirmed by detecting a labeling substance. Specifically, after the above hybridization reaction of labeled genomic DNA fragments and probes, unreacted genomic DNA fragments and the like are washed, and the labeling substance bound to each genomic DNA fragment specifically hybridizing to a probe is observed. For instance, in a case in which the labeling substance is a fluorescent material, the fluorescence wavelength is detected. In a case in which the labeling substance is a dye molecule, the dye

wavelength is detected. More specifically, apparatuses such as fluorescent detectors and image analyzers used for conventional DNA microarray analysis can be used. As described above, it is possible to determine whether or not a sugarcane sample has the above sugarcane-stalk-length-related marker(s) with the use of a DNA microarray. In particular, according to the method described above, it is not necessary to cultivate a sugarcane sample to such an extent that the actual stalk length thereof becomes measurable. For instance, seeds of a progeny line or a young seedling obtained as a result of germination of such seeds can be used. Therefore, the area of a field used for cultivation of a sugarcane sample and other factors such as cost of cultivation can be significantly reduced with the use of the sugarcane-stalk-length-related marker(s). In particular, when a novel sugarcane variety is created, it is preferable to produce several tens of thousands of hybrid varieties via crossing and then to identify a novel sugarcane variety using sugarcane-stalk-length-related markers prior to or instead of seedling selection. The use of such sugarcane-stalk-length-related marker(s) makes it possible to significantly reduce the number of excellent lines that need to be cultivated in an actual field. This allows drastic reduction of time-consuming efforts and the cost required to create a novel sugarcane variety.

Examples

[0012] The present invention is hereafter described in greater detail with reference to the following examples, although the technical scope of the present invention is not limited thereto.

1. Production of DNA microarray probes

(1) Materials

The following varieties were used: sugarcane varieties: NiF8, Ni9, US56-15-8, POJ2878, Q165, R570, Co290 and B3439; closely-related sugarcane wild-type varieties: Glagah Kloet, Chunee, Natal Uba, and Robustum 9; and Erianthus varieties: IJ76-349 and JW630.

(2) Restriction enzyme treatment

Genomic DNA was extracted from each of the above sugarcane varieties, closely-related sugarcane wild-type varieties, and Erianthus varieties using DNeasy Plant Mini Kits (Qiagen). Genomic DNAs (750 ng each) were treated with a PstI restriction enzyme (NEB; 25 units) at 37 degrees C for 2 hours. A BstNI restriction enzyme (NEB; 25 units) was added thereto, followed by treatment at 60 degrees C for 2 hours.

(3) Adapter ligation

PstI sequence adapters (5'-CACGATGGATCCAGTGCA-3' (SEQ ID NO: 30) and 5'-CTGGATCCATCGTGCA-3' (SEQ ID NO: 31)) and T4 DNA Ligase (NEB; 800 units) were added to the genomic DNA fragments treated in (2) (120 ng each), and the

obtained mixtures were subjected to treatment at 16 degrees C for a full day. Thus, the adapters were selectively added to genomic DNA fragments having PstI recognition sequences at both ends thereof among the genomic DNA fragments treated in (2).

(4) PCR amplification

A PstI sequence adapter recognition primer (5'-GATGGATCCAGTGCAG-3' (SEQ ID NO: 32)) and Taq polymerase (TAKARA; PrimeSTAR; 1.25 units) were added to the genomic DNA fragment (15 ng) having the adaptors obtained in (3). Then, the genomic DNA fragment was amplified by PCR (treatment at 98 degrees C for 10 seconds, 55 degrees C for 15 seconds, 72 degrees C for 1 minute for 30 cycles, and then at 72 degrees C for 3 minutes, followed by storage at 4 degrees C).

(5) Genome sequence acquisition

The nucleotide sequence of the genomic DNA fragment subjected to PCR amplification in (4) was determined by the Sanger method. In addition, information on a nucleotide sequence sandwiched between PstI recognition sequences was obtained based on the total sorghum genome sequence information contained in the genome database (Gramene: <http://www.gramene.org/>).

(6) Probe design and DNA microarray production

50- to 75-bp probes were designed based on the genome sequence information in (5). Based on the nucleotide sequence information of the designed probes, a DNA microarray having the probes was produced.

2. Acquisition of signal data using a DNA microarray

(1) Materials

Sugarcane varieties/lines (NiF8 and Ni9) and the progeny line (line 191) were used.

(2) Restriction enzyme treatment

Genomic DNAs were extracted from NiF8, Ni9, and the progeny line (line 191) using DNeasy Plant Mini Kits (Qiagen). Genomic DNAs (750 ng each) were treated with a PstI restriction enzyme (NEB; 25 units) at 37 degrees C for 2 hours. Then, a BstNI restriction enzyme (NEB; 25 units) was added thereto, followed by treatment at 60 degrees C for 2 hours.

(3) Adapter ligation

PstI sequence adapters (5'-CACGATGGATCCAGTGCA-3' (SEQ ID NO: 30) and 5'-CTGGATCCATCGTGCA-3' (SEQ ID NO: 31)) and T4 DNA Ligase (NEB; 800 units) were added to the genomic DNA fragments treated in (2) (120 ng each), and the obtained mixtures were treated at 16 degrees C for a full day. Thus, the adaptors were selectively added to a genomic DNA fragment having PstI recognition sequences at both ends thereof among the genomic DNA fragments treated in (2).

(4) PCR amplification

A PstI sequence adapter recognition primer (5'-GATGGATCCAGTGCAG-3' (SEQ ID

NO: 32)) and Taq polymerase (TAKARA; PrimeSTAR; 1.25 units) were added to the genomic DNA fragment (15 ng) having the adapters obtained in (3). Then, the genomic DNA fragment was amplified by PCR (treatment at 98 degrees C for 10 seconds, 55 degrees C for 15 seconds, 72 degrees C for 1 minute for 30 cycles, and then 72 degrees C for 3 minutes, followed by storage at 4 degrees C).

(5) Labeling

The PCR amplification fragment obtained in (4) above was purified with a column (Qiagen). Cy3-labeled 9mers (TriLink; 1 O.D.) was added thereto. The resultant was treated at 98 degrees C for 10 minutes and allowed to stand still on ice for 10 minutes. Then, Klenow (NEB; 100 units) was added thereto, followed by treatment at 37 degrees C for 2 hours. Thereafter, a labeled sample was prepared by ethanol precipitation.

(6) Hybridization/signal detection

The labeled sample obtained in (5) was subjected to hybridization using the DNA microarray prepared in 1 above in accordance with the NimbleGen Array User's Guide. Signals from the label were detected.

3. Identification of QTL for sugarcane stalk length and development of markers

(1) Creation of genetic map datasheet

Genotype data of possible 3004 markers were obtained based on the signal data detected in 2 above of the NiF8 and Ni9 sugarcane varieties and the progeny line (line 191). Based on the obtained genotype data, chromosomal marker position information was obtained by calculation using the gene distance function (Kosambi) and the AntMap genetic map creation software (Iwata H, Ninomiya S (2006) AntMap: constructing genetic linkage maps using an ant colony optimization algorithm, *Breed Sci* 56: 371-378). Further, a genetic map datasheet was created based on the obtained marker position information using Mapmaker/EXP ver. 3.0 (A Whitehead Institute for Biomedical Research Technical Report, Third Edition, January, 1993).

(2) Acquisition of stalk length data

The tested sugarcane varieties (NiF8 and Ni9) and the progeny line (191 lines) were subjected to cultivation with 2 replicates (13 individuals per replicate). In each replicate of cultivation, 5 individuals were subjected to measurement of the height from the plant base to the base of the first leaf (top visible dewlap leaf: +1) on 98 days after planting. The mean values obtained from the results for separate replicates of cultivation were used as stalk-length data. The calculated stalk-length values are shown in fig. 3.

(3) Quantitative trait (quantitative trait loci: QTL) analysis

Based on the genetic map datasheet obtained in (1) above and the stalk-length data obtained in (2) above, QTL analysis was carried out by the composite interval mapping

(CIM) method using the QTL Cartographer gene analysis software (Wang S., C. J. Basten, and Z.-B. Zeng (2010), Windows QTL Cartographer 2.5, Department of Statistics, North Carolina State University, Raleigh, NC). Upon analysis, the LOD threshold was determined to be 2.5. As a result, as shown in figs. 4 to 6, peaks exceeding the LOD threshold were observed in the following ranges for the NiF8 sugarcane variety: the range between markers N826961 and N825180 present in the 18th linkage group; the range between markers N827910 and N811685 present in the 23rd linkage group; and the range between markers N804812 and N826565 present in the 46th linkage group. It was possible to specify the obtained peaks as shown in table 4, suggesting the presence of causative genes (i.e., gene group) each having the function of causing an increase in stalk length at relevant peak positions.

[0013] [Table 4]

Linkage group	Position (cM)	Range (cM)	Adjacent marker	LOD value	Effect (cm)
18	40.1	39.9	N826961-N825180	4.3	3.82
23	28.4	21.1	N827910-N811685	3.1	2.51
46	8.1	15.2	N804812-N826565	3.8	3.39

As shown in figs. 4 to 6, markers located in the vicinity of the relevant peaks are inherited in linkage with causative genes (i.e., gene group) each having the function of causing an increase in stalk length. This shows that the markers can be used as sugarcane-stalk-length-related markers. Specifically, it has been revealed that the 29 types of markers shown in figs. 4 to 6 can be used as sugarcane-stalk-length-related markers. In addition, as examples of signals detected in 2 (6) above, table 5 shows signal levels of 13 types of markers among markers N826961 to N825180 present in the 18th linkage group for NiF8 and Ni9 and their 16 progeny lines (F1_1 to F1_16). In particular, the signal levels of N828509 are shown in fig. 7.

[0014]

[Table 5]

Linkage group	Marker name	Line name								
		NiF8	Ni9	F1_1	F1_2	F1_3	F1_4	F1_5	F1_6	F1_7
18	N826961	3,411	459	448	3,205	381	4,993	837	6,314	546
	N816516	1,317	365	483	1,538	468	1,790	510	3,625	435
	N827098	1,874	565	449	2,780	343	2,136	369	3,426	411
	N829001	1,048	335	379	1,546	472	1,357	403	4,837	361
	N828509	4,120	439	586	3,744	409	3,541	442	3,513	410
	N810652	1,422	389	384	1,740	461	1,373	472	3,328	626
	N812117	2,060	704	640	2,216	394	1,973	953	2,607	614
	N827138	2,921	498	411	3,026	438	2,660	800	2,330	877
	N825829	1,680	479	436	1,366	518	1,492	438	1,832	343
	N813742	3,608	397	793	5,077	374	4,629	855	1,338	369
	N803417	2,226	437	376	3,098	433	3,608	431	2,854	309
	N810475	4,888	646	551	5,038	406	4,463	524	1,631	520
	N825180	5,123	530	417	5,897	445	5,172	379	1,635	416

Linkage group	Marker name	Line name								
		F1_8	F1_9	F1_10	F1_11	F1_12	F1_13	F1_14	F1_15	F1_16
18	N826961	421	592	3,755	426	497	3,958	6,840	9,623	4,188
	N816516	390	555	2,715	483	373	5,037	3,706	3,227	3,376
	N827098	425	507	1,622	610	412	1,517	3,876	3,461	2,314
	N829001	439	396	1,688	363	359	1,410	5,769	6,972	3,498
	N828509	501	582	3,051	490	332	2,956	4,799	4,975	2,744
	N810652	596	412	2,430	643	656	2,122	3,881	5,831	2,994
	N812117	733	650	2,136	439	771	2,194	3,183	2,373	2,176
	N827138	356	425	3,659	660	373	3,669	3,844	1,583	1,041
	N825829	338	888	1,285	590	367	1,115	1,561	2,001	1,649
	N813742	523	752	4,383	496	538	4,924	2,005	2,211	1,241
	N803417	381	344	3,090	348	383	3,256	2,882	4,544	3,016
	N810475	477	857	3,996	453	719	3,396	1,736	2,537	2,057
	N825180	396	654	4,036	670	350	3,796	2,132	3,508	1,748

Signal levels of 13 types of markers were found to be very high for NiF8 and the progeny lines such as F1_2 and F1_4 with relatively long stalk lengths. These results also revealed that 13 types of markers among markers N826961 to N825180 present in the 18th linkage group can be used as sugarcane-stalk-length-related markers.

Similarly, table 6 shows signal levels of 9 types of markers for NiF8 and Ni9, and 16 progeny lines (F1_1 to F1_16) among markers N827910 to N811685 present in the 23rd linkage group. In particular, fig. 8 shows the signal levels of N827910.

[0015]

[Table 6]

Linkage group	Marker name	Line name								
		NiF8	Ni9	F1_1	F1_2	F1_3	F1_4	F1_5	F1_6	F1_7
23	N827910	4,339	579	3,316	4,053	465	3,898	348	423	482
	N815704	4,146	483	3,392	4,225	367	3,660	478	512	355
	N820994	3,258	369	2,103	3,036	579	4,267	386	400	334
	N820544	3,109	434	2,641	3,460	382	3,579	405	340	430
	N819412	8,363	607	4,811	3,033	455	5,263	456	412	447
	N829748	1,259	415	1,774	1,941	347	1,489	764	369	518
	N824769	7,344	793	8,263	8,325	352	5,242	827	433	712
	N826558	3,397	691	3,089	2,494	384	2,596	467	478	528
	N811685	2,195	544	2,386	1,886	376	2,356	332	549	402

Linkage group	Marker name	Line name								
		F1_8	F1_9	F1_10	F1_11	F1_12	F1_13	F1_14	F1_15	F1_16
23	N827910	422	563	4,985	391	4,075	4,445	4,968	437	4,745
	N815704	364	787	3,606	605	3,498	3,089	4,066	349	3,963
	N820994	357	487	4,914	335	4,442	4,498	4,163	552	5,809
	N820544	613	345	4,049	752	4,885	4,539	3,196	468	4,264
	N819412	484	453	3,492	439	4,025	3,991	2,819	410	3,278
	N829748	596	438	1,516	599	1,084	1,614	2,658	453	3,373
	N824769	467	341	5,762	818	7,341	5,939	3,989	372	5,019
	N826558	413	405	2,121	486	1,973	2,351	4,732	384	4,940
	N811685	367	330	2,050	502	1,780	2,282	1,230	400	1,630

Signal levels of 9 types of markers were found to be very high for NiF8 and the progeny lines such as F1_1 and F1_2 with relatively long stalk lengths. These results also revealed that 9 types of the markers among markers N827910 to N811685 present in the 23rd linkage group can be used as sugarcane-stalk-length-related markers. Similarly, table 7 shows signal levels of 7 types of markers for NiF8 and Ni9, and 16 progeny lines (F1_1 to F1_16) among markers N804812 to N826565 present in the 46th linkage group. In particular, fig. 9 shows the signal levels of N804812.

[0016] [Table 7]

Linkage group	Marker name	Line name								
		NiF8	Ni9	F1_1	F1_2	F1_3	F1_4	F1_5	F1_6	F1_7
46	N804812	2,574	484	3,946	3,025	419	3,072	740	607	3,195
	N826007	1,407	418	1,241	1,090	390	1,461	410	424	1,158
	N826674	7,980	542	5,736	4,656	447	5,037	591	433	4,477
	N821523	1,351	290	1,599	1,523	535	1,505	307	585	1,457
	N802816	1,527	595	1,190	1,361	357	1,481	936	385	1,506
	N829577	2,625	593	2,468	1,789	333	1,986	846	328	2,034
	N826565	4,203	745	5,484	5,021	353	3,471	773	498	3,779

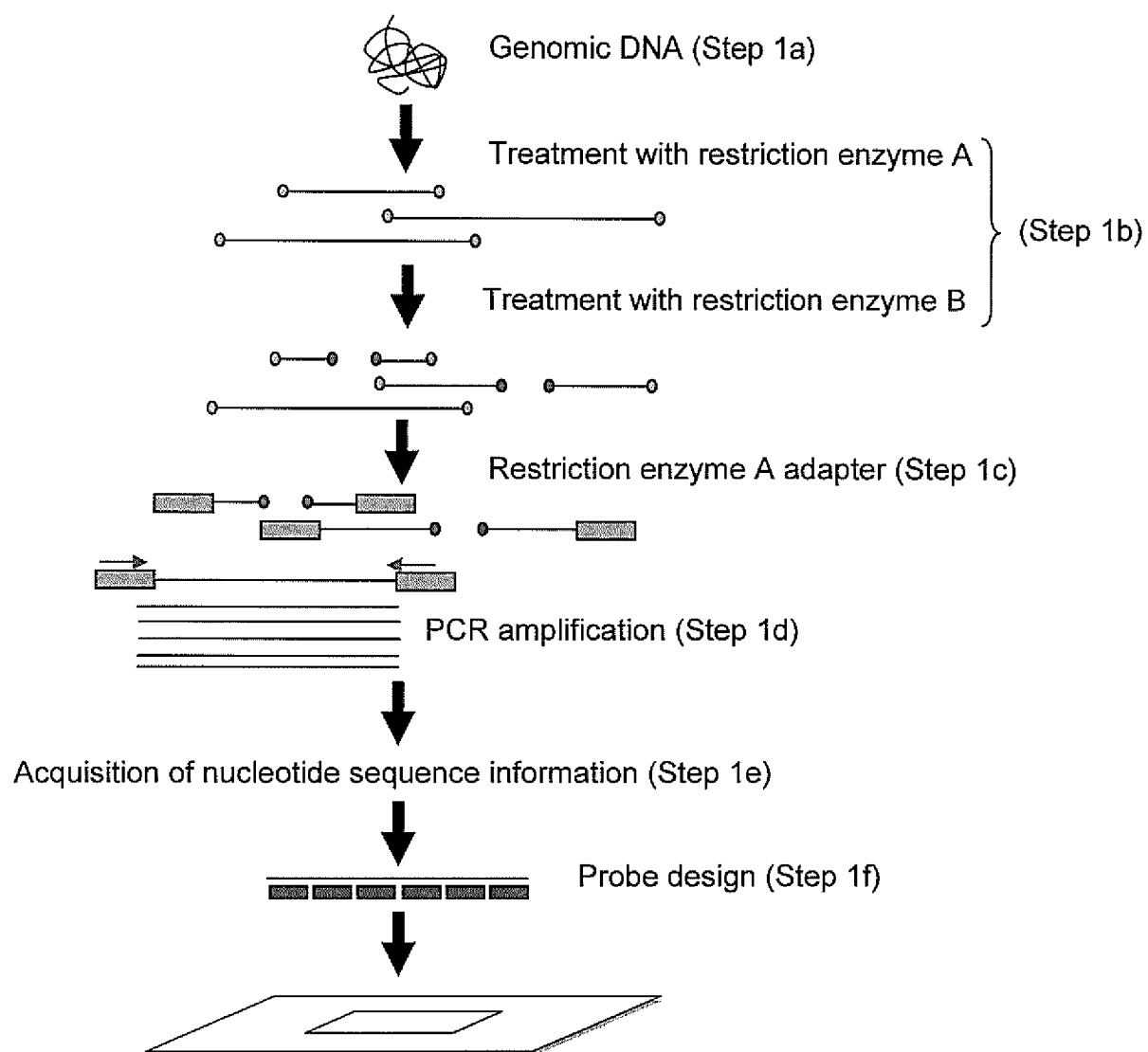
Linkage group	Marker name	Line name								
		F1_8	F1_9	F1_10	F1_11	F1_12	F1_13	F1_14	F1_15	F1_16
46	N804812	3,482	3,537	646	775	653	3,970	548	405	3,611
	N826007	1,114	1,366	492	362	385	1,011	468	381	1,008
	N826674	4,177	2,214	437	496	657	4,836	399	378	1,971
	N821523	1,170	4,469	347	297	326	1,628	722	575	5,021
	N802816	1,677	1,422	495	578	447	1,635	362	459	1,424
	N829577	1,857	1,657	516	566	477	2,511	454	343	1,572
	N826565	3,552	2,311	917	721	954	3,897	660	398	2,639

Signal levels of 7 types of markers were found to be very high for NiF8 and the progeny lines such as F1_1 and F1_2 with relatively long stalk lengths. These results also revealed that 7 types of the markers among markers N804812 to N826565 present in the 46th linkage group can be used as sugarcane-stalk-length-related markers.

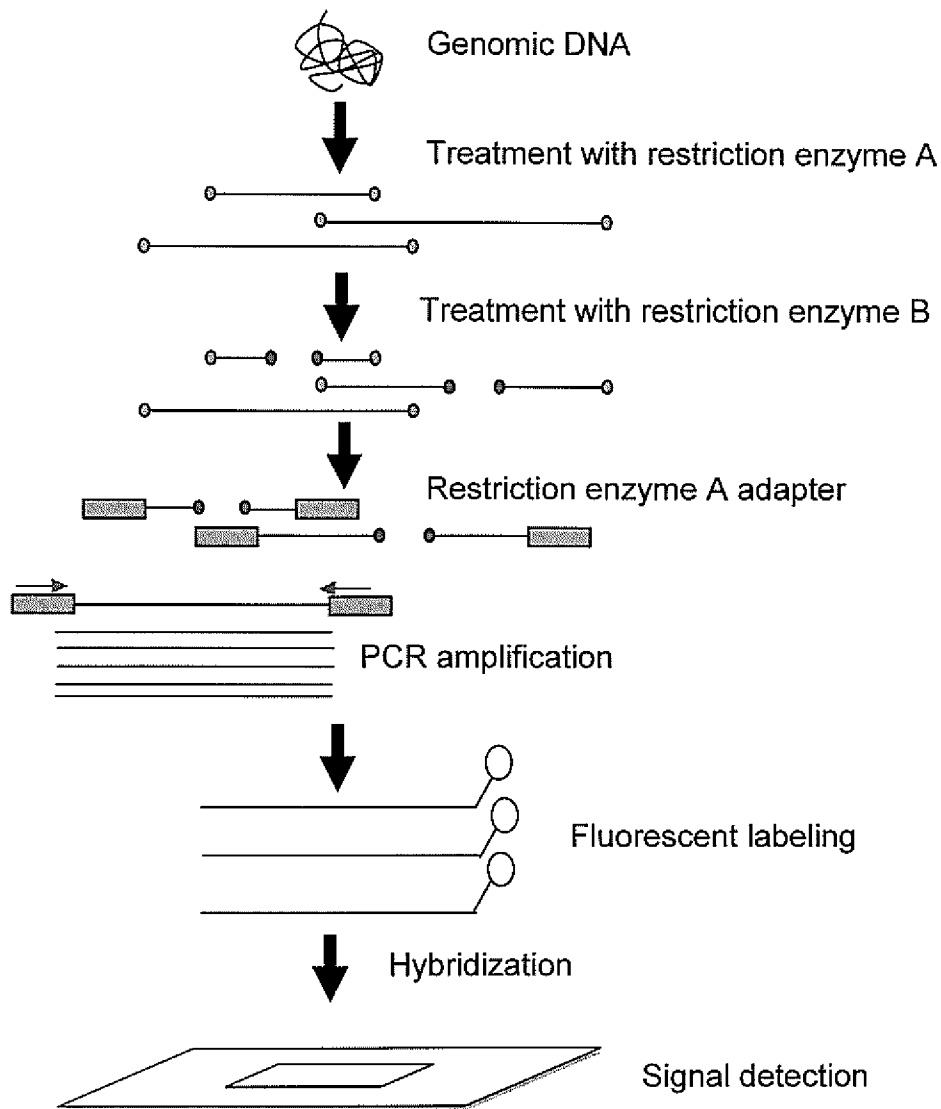
Claims

- [Claim 1] A sugarcane-stalk-length-related marker, which comprises a continuous nucleic acid region existing in a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 1 and the nucleotide sequence shown in SEQ ID NO: 13, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 14 and the nucleotide sequence shown in SEQ ID NO: 22, or a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 23 and the nucleotide sequence shown in SEQ ID NO: 29 of a sugarcane chromosome.
- [Claim 2] The sugarcane-stalk-length-related marker according to claim 1, wherein the continuous nucleic acid region comprises any nucleotide sequence selected from the group consisting of the nucleotide sequences shown in SEQ ID NOS: 1 to 29.
- [Claim 3] The sugarcane-stalk-length-related marker according to claim 1, wherein the continuous nucleic acid region is located at a position in a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 6 and the nucleotide sequence shown in SEQ ID NO: 8, a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 18 and the nucleotide sequence shown in SEQ ID NO: 19, or a region sandwiched between the nucleotide sequence shown in SEQ ID NO: 24 and the nucleotide sequence shown in SEQ ID NO: 28 of a sugarcane chromosome.
- [Claim 4] A method for producing a sugarcane line having an increased stalk length comprising: a step of extracting a chromosome of a progeny plant obtained from parent plants, at least one of which is a sugarcane plant; and a step of determining the presence or absence of the sugarcane-stalk-length-related marker according to any one of claims 1 to 3 in the obtained sugarcane chromosome.
- [Claim 5] The method for producing a sugarcane line according to claim 4, wherein a DNA chip provided with probes each serving as the sugarcane-stalk-length-related marker is used in the determination step.
- [Claim 6] The method for producing a sugarcane line according to claim 4, wherein the progeny plant is in the form of seeds or a young seedling and the chromosome is extracted from the seeds or the young seedling.

[Fig. 1]

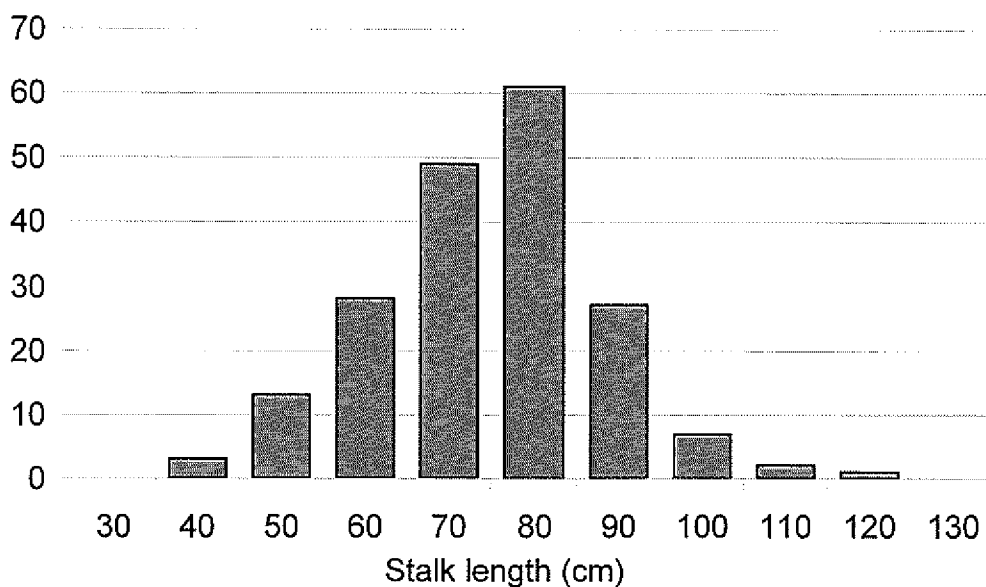


[Fig. 2]

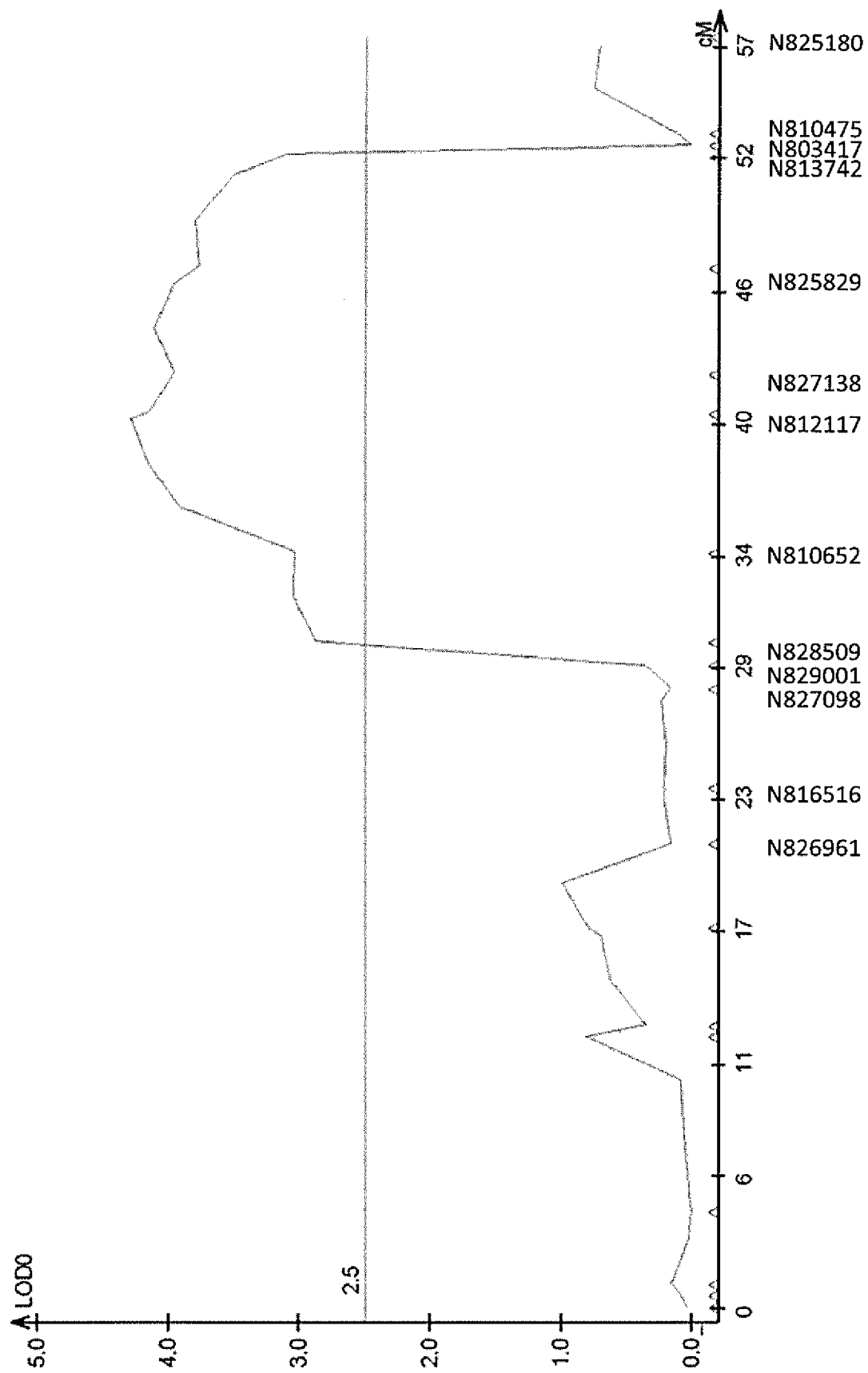


[Fig. 3]

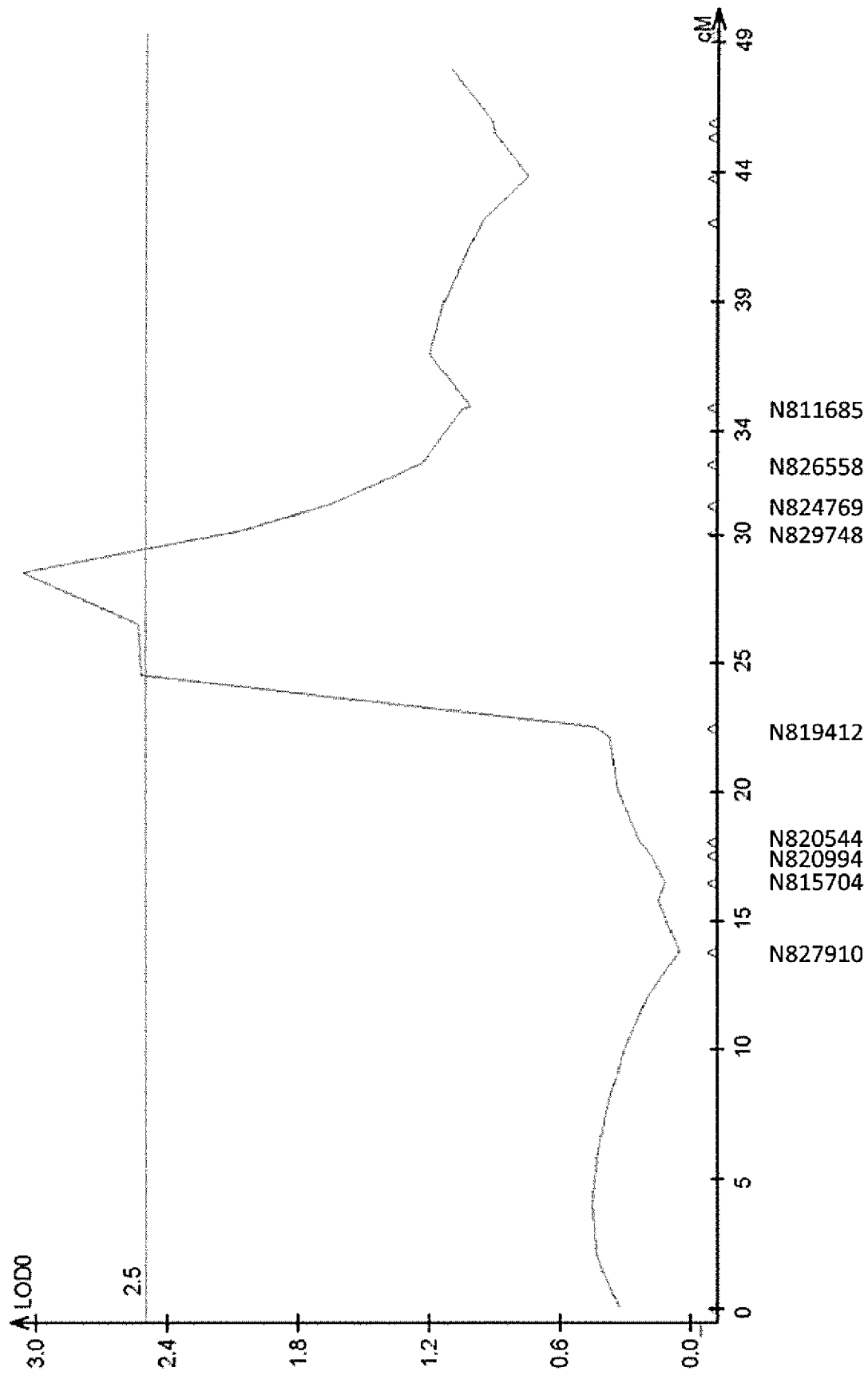
Number of lines



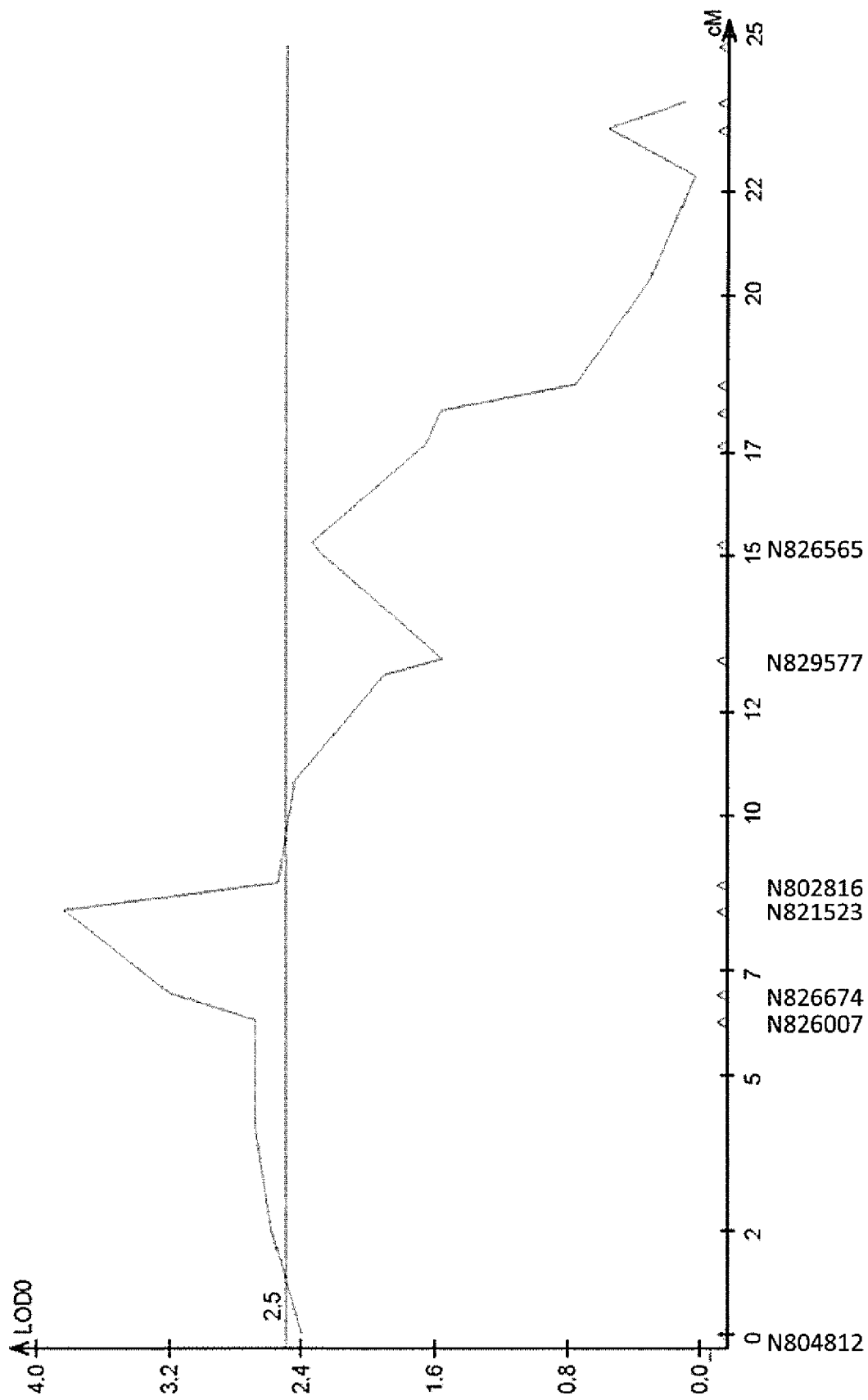
[Fig. 4]



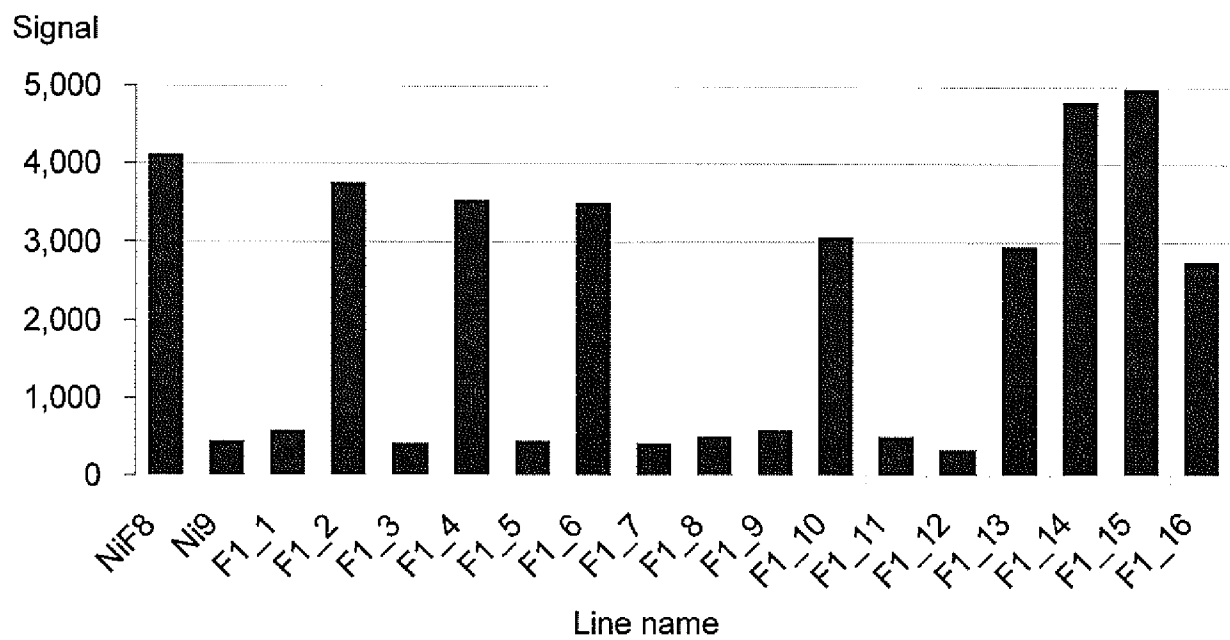
[Fig. 5]



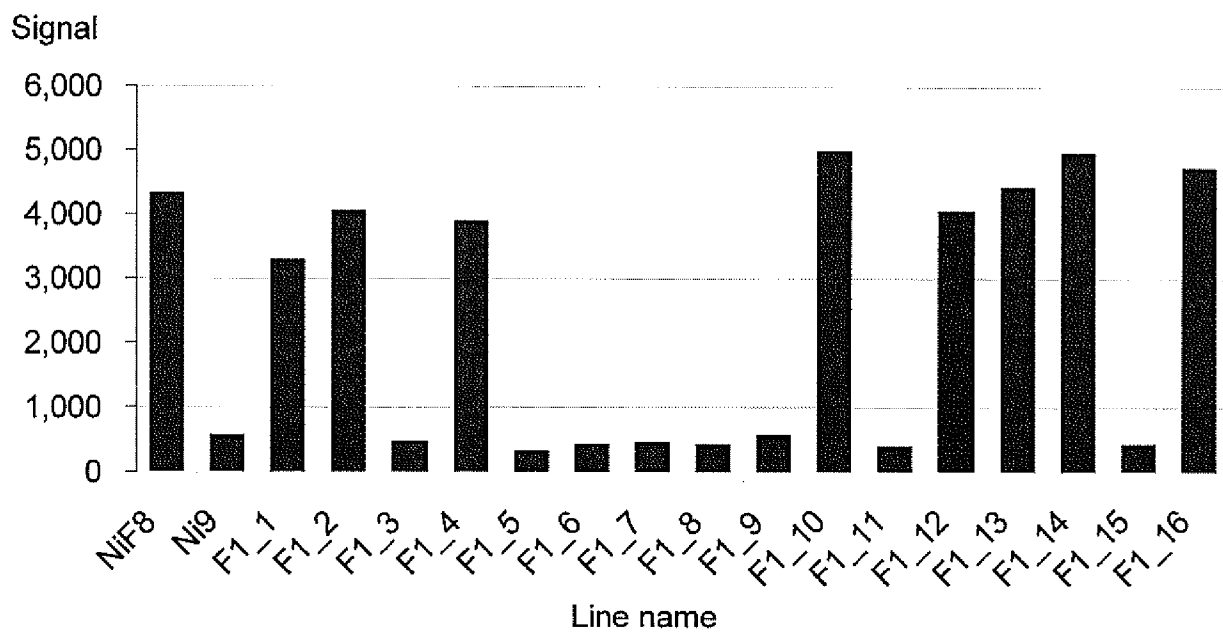
[Fig. 6]



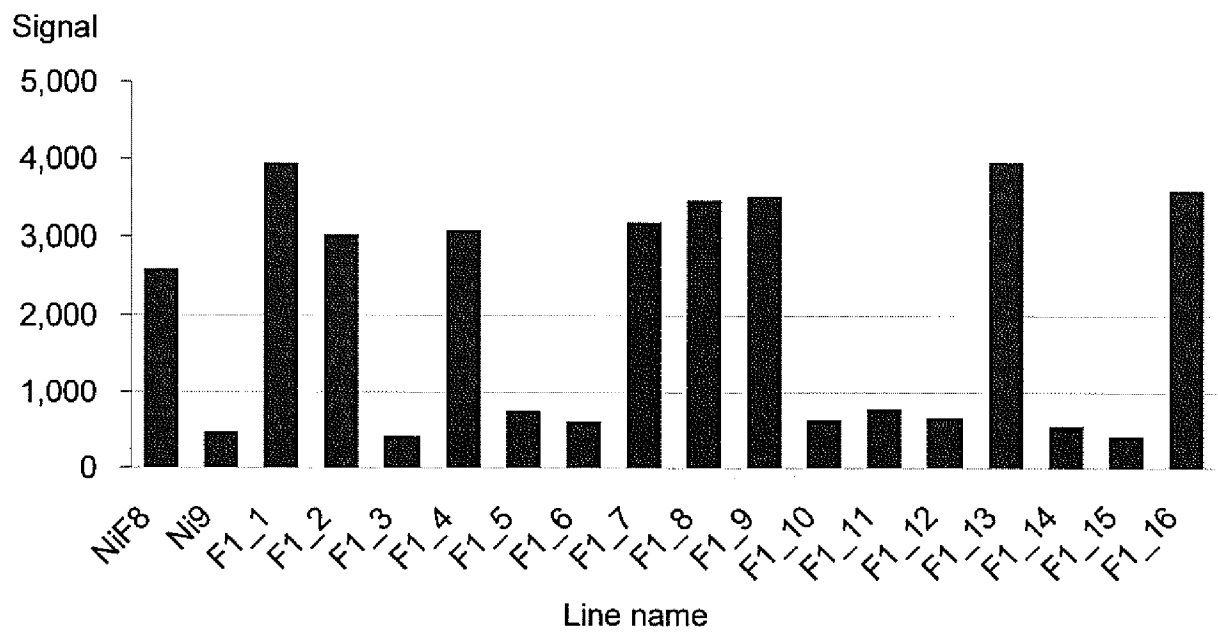
[Fig. 7]



[Fig. 8]



[Fig. 9]



INTERNATIONAL SEARCH REPORT

International application No

PCT/JP2011/004457

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12Q1/68 A01H5/04 A01H1/04
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12Q A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HOARAU J -Y ET AL: "Genetic dissection of a modern sugarcane cultivar (Saccharum spp.). II. Detection of QTLs for yield components.", THEORETICAL AND APPLIED GENETICS, vol. 105, no. 6-7, November 2002 (2002-11) , pages 1027-1037, XP002662392, ISSN: 0040-5752 the whole document	1-6
X	AITKEN K S ET AL: "Genetic control of yield related stalk traits in sugarcane", THEORETICAL AND APPLIED GENETICS, vol. 117, no. 7, November 2008 (2008-11), pages 1191-1203, XP002662393, ISSN: 0040-5752 the whole document	1-6



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

28 October 2011

Date of mailing of the international search report

22/12/2011

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/JP2011/004457

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-6(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-6(partially)

Sugarcane stalk length marker comprising SEQ ID NO:1 and use

2-29. claims: 1-6(partially)

Claimed Inventions 2 to 29: each individual SEQ ID NO: 2 through 29 represents a separate and unlinked alleged invention with its use
