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(54) **Titre : PLANTE RESISTANTE A HELMINTHOSPORIUM TURCIUM**
(54) **Title: HELMINTHOSPORIUM TURCICUM-RESISTANT PLANT**

(57) **Abrégé/Abstract:**
The present invention relates to an improved plant resistant to Helminthosporium turcicum, especially a maize plant, which comprises a polynucleotide having one or more resistance-conferring genes, for example on a truncated chromosome fragment from the accession Pepitilla, and to a cell, a tissue, a part, grain and seed thereof, an isolated polynucleotide comprising one or more genes conferring resistance to Helminthosporium turcicum, a vector, and a transgenic plant cell and a transgenic plant, both containing said polynucleotide. The invention also relates to suitable labels and the use thereof for introducing resistance or the transgene into a plant, and to the identification of improved maize plants comprising a truncated chromosome fragment.

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Abstract

The present invention provides an improved *Helminthosporium turcicum*-resistant plant, in particular a maize plant which comprises a polynucleotide with one or more resistance-conferring genes, for example on a truncated chromosome fragment from the accession Pepitilla, as well as a cell, a tissue, a part, grain and seeds thereof, an isolated polynucleotide which comprises one or more resistance-conferring genes against *Helminthosporium turcicum*, a vector, a transgenic plant cell and a transgenic plant containing this polynucleotide. Furthermore, the invention encompasses suitable markers and their use in introducing resistance or the transgene into a plant, as well as the identification of improved maize plants which comprise a truncated chromosome fragment.

HELMINTHOSPORIUM TURCICUM-RESISTANT PLANT

Field of the invention

The present invention relates to the field of the modification of plants using molecular biological methods and marker technology, along with genetic engineering. It concerns a novel *Helminthosporium turcicum-resistant* plant, in particular a maize plant which comprises a polynucleotide with one or more resistance-conferring genes on a modified chromosome fragment from the accession Pepitilla, as well as a cell, a tissue, a portion, grain and seed thereof, an isolated polynucleotide which comprises one or more resistance-conferring genes against *Helminthosporium turcicum,* a vector, a transgenic plant cell and a transgenic plant containing this polynucleotide. The invention also encompasses suitable molecular markers and their use in introducing the resistance locus or the transgene into a plant, as well as the identification of improved maize plants which comprise a modified chromosome fragment.

Background of the invention

In maize (*Zea mays* L.), there are a large number of fungal pathogens which cause leaf diseases. The fungus which can cause by far the most damage under tropical and also under temperate climatic conditions, such as those in large parts of Europe and North America as well as in Africa and India, is known as *Helminthosporium turcicum* or synonymously as *Exserohilum turcicum* (Pass.) Leonard and Suggs (teleomorph: *Setosphaeria turcica* (Luttrell) Leonard & Suggs). *H. turcicum* is the cause of the leaf spot disease known as "Northern Corn Leaf Blight" (NCLB), which can occur in epidemic proportions during wet years, attacking vulnerable maize varieties and causing a great deal of damage and considerable losses of yield of 30% and more over wide areas (Perkins & Pedersen, 1987; Raymundo & Hooker, 1981a; Ullstrup & Miles, 1957). Since the 1970s, then, natural resistance in genetic material has been sought. Currently, quantitative and qualitative resistances are known. While the oligo- or polygenically inherited quantitative resistance appears incomplete and non-specific as regards race in the phenotype and is influenced by additional and partially dominant genes, qualitative resistance is typically race-specific and can be inherited through individual, mostly dominant genes such as *Ht1*, *Ht2*, *Ht3*, *Htm1* or *Htn1* (Lipps *et al.*, 1997; Welz & Geiger, 2000). Backcrosses in many frequently used inbred maize lines such as W22, A619, B37 or B73 have successfully brought about introgression

of the HT genes, where they exhibit a partial dominance and expression as a function of the respective genetic background (Welz, 1998).

Despite this complex genetic architecture of NCLB resistance in maize, until now principally the use of the *Ht1* gene in maize together with a partial quantitative resistance has been sufficient to control helminthrosporiosis (Welz, 1998). The basis for this is that globally, race 0 of *H. turcicum* dominates as regards use (approximately 55%) (Lipps *et al.*, 1997; Ferguson & Carson, 2007), while other races such as 2N and 23N are only rarely used and even then in a geographically restricted area (Moghaddam & Pataky, 1994; Jordan *et al.*, 1983; Lipps & Hite, 1982; Thakur *et al.*, 1989; Welz, 1998). This race 0 is avirulent having regard to a maize plant with *Ht1*, so that when provided with a suitable quantitative resistance, it exhibits a sufficient general resistance to NCLB. However, many studies have reported an increasing dissemination of the less common races (Jordan *et al.*, 1983; Welz, 1998; Pratt & Gordon, 2006). The reasons for this are linked to the population dynamic of a pathogen which allows changes in pathogen virulence by new mutations on avirulence genes and new combinations of available virulence genes. Finally, this can lead to the occurrence of new, suitable, sometimes more aggressive pathogenic races. In Brazil, for example, the *H. turcicum* population already appears to be substantially more diverse having regard to the race composition than, for example, in North America. Gianasi *et al.* (1996) reported *H. turcicum* races which have already broken through the resistance conferred by the *Ht1* gene. In addition, there is the instability of the resistance genes to certain environmental factors such as temperature and light intensity in some climate zones (Thakur *et al.*, 1989). This development has the consequence that globally, the use of novel *HT* resistance genes or such to which, until now, little attention has been paid for the production of commercial maize plants is growing in importance in order to target a broader and more long-lasting resistance to *H. turcicum* in maize. Initial approaches in this regard were attempted as early as 1998 by Pataky *et al.* The NCLB resistance in *sh2* elite maize was improved by using a combination of *Ht1* and *Htn1*.

A source of monogenic *Htn1* resistance is the Mexican landrace "Pepitilla" (Gevers, 1975). *Htn1* introgression lines exhibit a gene mapping on the long arm of chromosome 8 approximately 10 cM distal from *Ht2* and 0.8 cM distal from the RFLP marker umc117 (bin 8.06) (Simcox & Bennetzen, 1993). In contrast to the usual HT resistance genes, *Htn1* confers resistance by delaying the onset of sporulation, and thus combats the development of lesions. As a result, fewer, smaller lesions as well as reduced sporulation zones are formed (Raymundo *et al.*, 1981b, Simcox & Bennetzen, 1993). Chlorotic-necrotic lesions

such as those which occur with *Ht1*, *Ht2* or *Ht3*-conferred resistance, are not formed (Gevers, 1975). However, the resistance reaction in the heterozygous state of the *Htn1* gene is significantly less effective than in the homozygous state (Raymundo *et al.*, 1981b).

The development of additional specific markers which could further simplify genotype determination would improve the breeding manageability of the *Htn1* gene. Marker assisted selection (MAS) technology thus makes efficient stacking or pyramiding of several resistance genes possible (Min *et al.*, 2012). The introgression lines B37*Htn1* or W22*Htn1* were employed in many studies on mapping the resistance locus and identifying the resistance source (Raymundo *et al.*, 1981a, b; Simcox & Bennetzen, 1993, Bar-Zur *et al.*, 1998; Coates & White, 1998). Available information regarding markers which could be used for selection of the resistance locus for *Htn1* from the accession Pepitilla, however, is still only limited (Simsox & Bennetzen, 1993). The known markers for *Htn1* which are functional for and flank the resistance locus from the accession Pepitilla are still mapped at close to 22.2 cM apart, which in the best case scenario allows selection of a large chromosome fragment. However, there is a frequent risk that within this fragment between the markers, a double genetic recombination occurs which could result in a false positive selection for the *Htn1* resistance locus. In addition, in some cases the probability of unwanted genetic regions being taken into the introgression line rises with the size of the introgressed chromosome fragment and be transmitted over generations of elite lines. Such genetic regions, in particular when they are closely coupled with the *Htn1* locus and lead to unequivocally negative effects on one or more agronomic features, are known as linkage drag. From known studies which investigated and used introgression lines with *Htn1* from Pepitilla, however, such negative effects are unknown. Even the very comprehensive research work by Welz (1998) which, inter alia, was also carried out on B37Htn1, postulated that in view of, for example, yield and ripening, introgression of the *Htn1* locus brought about no significant disadvantages. Thus, no serious efforts have been made in the prior art to deliberately shorten the large chromosome fragment.

In contrast, WO 2011/163590 discloses the genotype PH99N as an alternative source for NCLB resistance on chromosome 8 bin 5 which, however, does not correspond to the accession Pepitilla. Essentially, only resistance as regards *H. turcicum* races 0 and 1 have been identified in backcross populations from PH99N. Even the resistance phenotype was not clearly determined. Nevertheless, the authors concluded that the resistance was due to the *Htn1* gene. But the resistance locus in PH99N was restricted to only a ~224kb long chromosome fragment; a resistant maize plant with the 224 kb fragment and thus the

assumed Htn1 was not disclosed, however. In addition, the genotype PH99N was not made available to the public by deposition.

An alternative approach to making the *Htn1* gene useful is the identification and cloning of the resistance gene and using it in a transgenic strategy.

With the intention of identifying the resistance gene for NCLB, in 2010, Chung *et al.* 2010 published a study for fine mapping the bin 8.06 resistance locus. The chromosome fragment under investigation, however, was not derived from Pepitilla but from the maize hybrid DK888 which exhibits multiple disease resistance. Investigations on *Helminthosporium* race specificity initially made it clear that the resistance locus on DK888, designated $qNLB8.06_{DK888}$, was closely linked or functionally linked with the *Ht2* and *Htn1* genes, since *Helminthosporium* strains 23 and 23N were virulent (Chung *et al.*, 2008). Positive detection of the presence of *Htn1* was not accomplished, however, in the absence of a pure N isolate from *H. turcicum.* In addition, the resistance phenotype with $qNLB8.06_{DK888}$ also did not correspond to the expected phenotype having regard to the appearance of chlorotic lesions and the delay in lesion formation. Further detailed complementation studies in Chung *et al.* (2010) finally provided indications that $qNLB8.06_{DK888}$ was either identical to, allelic, closely linked or functionally linked with *Ht2*, but not with *Htn1*. The resistance locus $qNLB8.06_{DK888}$ could be assigned to a chromosome fragment of 0.46 Mb. Genome annotations of this chromosome fragment hinted at 12 putative open reading frames, of which three could respectively be a tandem protein kinase-like gene (GRMZM2G135202; GRMZM2G164612) or a protein phosphatase-like gene (GRMZM2G119720) and each equally constituted promising candidate genes for the resistance gene *Ht2* (Chung *et al.*, 2010). A functional verification was not described.

Furthermore, WO 2011/163590 A1 also annotated the presumed *Htn1* gene in the resistance source PH99N as a tandem protein kinase-like gene (GRMZM2G451147) and disclosed its genetic sequence, but also did not determine its functionality, for example in a transgenic maize plant.

Summary of the invention

The present invention stems from the prior art described above; the object of the present invention is to provide a maize plant which exhibits resistance to the pathogen *Helminthosporium turcicum* from the donor Pepitilla and wherein the agronomic features of known maize plants can be overlaid with resistance from the donor Pepitilla.

The object is accomplished on the one hand by the provision of a maize plant into the genome of which a chromosome fragment from the donor Pepitilla has been integrated, wherein the chromosome fragment comprises an interval of the donor (hereinafter termed the first interval or interval 1) which exhibits donor alleles in accordance with the haplotype shown in Table 2 and a polynucleotide which confers resistance to *Helminthosporium turcicum* in the maize plant, and wherein the chromosome fragment does not contain a further interval of the donor (hereinafter termed the second interval or interval 2) between a marker in a first marker region (M1) which is flanked by the markers SYN14136 and PZE-108076510 and a marker in a second marker region (M2) which is flanked by the markers SYN24931 and PZE-108077560. These and alternative solutions to the problem, described below, may be based on a breeding programme for integration of the *Htn1* locus from Pepitilla into maize lines. However, genetic engineering approaches may also be selected, by means of which plants in accordance with the present invention may be produced. Examples of genetic engineering strategies are described in more detail below. In order to produce the plants of the present invention, various genotypes from the prior art may be used. In particular, B37HTN1, which comprises the resistance locus for the landrace "Pepitilla", was used as the original line. In addition to Pepitilla itself and B37HTN1 (also known in the prior art as B37HtN), almost any maize genotype may be called upon for integration of the *Htn1* locus in order to produce a maize plant in accordance with the invention into the genome of which, in particular on chromosome 8 bin 5 or 6, an introgression of the *Htn1* resistance locus from Pepitilla has been inserted. In this respect, many examples of genotypes are known in the prior art, for example: W22Htn (e.g. Bar-Zur et al.,1998); H6314*Htn* (e.g. Bar-Zur et al.,1998), B73*HtN* (e.g. Shimoni et al., Journal of Phytopathology 131:4 (1991), 315-321), B68*HtN* and A632*HtN* (e.g. Carson, Plant Disease 79 (1995), 717-720) and A619*HtN* (e.g. Stanković et al, Genetika 39:2 (2007), 227 -240). In a maize plant in accordance with the invention, the chromosome fragment derives from the donor Pepitilla; in a preferred embodiment of the maize plant in accordance with the invention, the chromosome fragment derives from the donor B37HTN1 or from another maize genotype as cited above. As an example, B37HTN1 may be ordered from the Maize Genetics COOP Stock Center using the stock ID 65749.

The chromosome fragment integrated into the genome of the maize plant of the invention derives from the donor Pepitilla which, as is known, comprises the resistance locus HTN1. The introgression of this resistance locus is localized on the long arm of chromosome 8, bin 8.05 – 8.06. The integrated chromosome fragment comprises the first interval of the donor, which comprises a polynucleotide which confers resistance against *Helminthosporium*

turcicum in the maize plant of the invention. In this regard, the polynucleotide comprises one or more resistance-conferring genes of the HTN1 locus from Pepitilla (Table 1) or gene alleles thereof. Under *H. turcicum* infestation conditions, the gene or gene allele may produce a resistance phenotype with features typical of HTN1. Preferably, the polynucleotide comprises one or more resistance-conferring genes of the HTN1 locus, preferably from Pepitilla, selected from RLK1 and EXT1 (see Table 1) or gene alleles thereof which produce a resistance phenotype with the typical features of HTN1 under *H. turcicum* infestation conditions. Particularly preferably, the polynucleotide comprises a nucleotide sequence which codes for a polypeptide in accordance with SEQ ID NO: 2 or SEQ ID NO: 6 or a homologue of a polypeptide in accordance with SEQ ID NO: 2 or SEQ ID NO: 6, which produce a resistance phenotype with the typical features of HTN1 under *H. turcicum* infestation conditions. Examples of these features typical of HTN1 are delayed onset of sporulation, reduced development of lesions, development of smaller lesions, reduced sporulation zones and/or no or only isolated chlorotic-necrotic lesions. Structurally, the polynucleotide is characterized in that it comprises a nucleic acid molecule which (a) comprises a nucleotide sequence in accordance with SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 or 15, (b) comprises a nucleotide sequence with an identity of at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with one of the nucleotide sequences in accordance with SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 or 15, preferably over the entire length of the sequence, (c) which hybridizes with the complementary strand of a nucleic acid molecule in accordance with (a) or (b) under stringent conditions, (d) which codes for a polypeptide with an amino acid sequence in accordance with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16, (e) which codes for a polypeptide with an amino acid sequence which has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with one of the amino acid sequences in accordance with (d), or (f) which comprises a part sequence of a nucleic acid in accordance with (a) to (e). In a preferred embodiment, the polynucleotide is characterized in that it comprises a nucleic acid molecule which (aa) comprises a nucleotide sequence in accordance with SEQ ID NO: 1 or 5, (bb) comprises a nucleotide sequence with an identity of at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with one of the nucleotide sequences in accordance with SEQ ID NO: 1 or 5, preferably over the entire length of the sequence, (cc) which hybridizes with the complementary strand of a nucleic acid molecule in accordance with (aa) or (bb) under stringent conditions, (dd) which codes for a polypeptide with an amino acid sequence in accordance with SEQ ID NO: 2 or 6, (ee) which codes for a polypeptide with an amino acid sequence which has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with one of the

amino acid sequences in accordance with (dd), or (ff) which comprises a part sequence of a nucleic acid in accordance with (aa) to (ee). The expression "part sequence of a nucleic acid molecule" as used in the present invention may be at least 20, 30, 40, 50, 60, 70, 80, 90 or at least 100 successive nucleotides, furthermore at least 150, 200, 250, 300, 350, 400, 450, 500, 600, 700, 800, 900 or 1000 successive nucleotides. The polynucleotide may be in the heterozygous or homozygous state in the genome of the maize plant of the invention; preferably, the polynucleotide is in the homozygous state.

Table 1: Potential resistance-conferring genes of the HTN1 locus from Pepitilla; Gene name (column 1); reference to corresponding SEQ ID Nos in the genomic exon sequence (column 2); reference to corresponding SEQ ID Nos in the predicted amino acid/protein sequence (column 3); annotated homologous gene from the B73 reference genome (column 4).

Gene name	cDNA SEQ ID NO:	Protein sequence SEQ ID NO:	Homologous B73 gene
RLK1	1	2	GRMZM2G451147
RLK4	3	4	GRMZM2G144028
EXT1	5	6	GRMZM2G445338
DUF1	7	8	AC209075.3_FG007
ZNF1	9	10	GRMZM2G175661
CYT1	11	12	GRMZM2G092018
RET1	13	14	GRMZM2G091973
HYD	15	16	GRMZM2G144021

Furthermore, the first interval in the chromosome fragment which exhibits donor alleles in accordance with the haplotype in Table 2 is characterized by the sequence of donor alleles in the haplotype of Table 2, but is not limited to this sequence of donor alleles in accordance with Table 2. This means that the first interval exhibits at least the donor allele which describes the resistance-conferring gene from Table 1, optionally with the donor allele of the marker MA0008. Furthermore, the first interval preferably exhibits at least the donor alleles in accordance with the haplotype of Table 2 from MA0021 to MA0022 (i.e. MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022) or from MA0005 to MA0022 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012 and MA0022) or from MA0005 to MA0013 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022 and MA0013) or from MA0005 to MA0014 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022,

MA0013 and MA0014) or from MA0005 to MA0015 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022, MA0013, MA0014 and MA0015) or from MA0005 to MA0016 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022, MA0013, MA0014, MA0015 and MA0016), particularly preferably from MA0005 to MA0017 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022, MA0013, MA0014, MA0015, MA0016 and MA0017), MA0005 to MA0018 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022, MA0013, MA0014, MA0015, MA0016, MA0017 and MA0018), MA0005 to PZE-108095998 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022, MA0013, MA0014, MA0015, MA0016, MA0017, MA0018 and PZE-108095998), MA0005 to PZE-108096011 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022, MA0013, MA0014, MA0015, MA0016, MA0017, MA0018, PZE-108095998 and PZE-108096011) or MA0005 to MA0019 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022, MA0013, MA0014, MA0015, MA0016, MA0017, MA0018, PZE-108095998, PZE-108096011 and MA0019), more particularly preferably from MA0005 to PZE-108096610 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022, MA0013, MA0014, MA0015, MA0016, MA0017, MA0018, PZE-108095998, PZE-108096011, MA0019 and PZE-108096610), MA0005 to MA0020 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022, MA0013, MA0014, MA0015, MA0016, MA0017, MA0018, PZE-108095998, PZE-108096011, MA0019, PZE-108096610 and MA0020), MA0005 to PZE-108096791 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022, MA0013, MA0014, MA0015, MA0016, MA0017, MA0018, PZE-108095998, PZE-108096011, MA0019, PZE-108096610, MA0020 and PZE-108096791) or MA0005 to MA0006 (i.e. MA0005, MA0021, MA0007, MA0008, MA0009, MA0010, MA0011, MA0012, MA0022, MA0013, MA0014, MA0015, MA0016, MA0017, MA0018, PZE-108095998, PZE-108096011, MA0019, PZE-108096610, MA0020, PZE-108096791 and MA0006). This resistant haplotype unequivocally specifies and identifies the resistance source Pepitilla. In particular, the first interval is localized between the markers MA0004 and PZE-108097482, between the markers MA0004 and MA0022, between the markers MA0005 and PZE-108097482 or between the markers MA0005 and MA0022. Preferably, the first interval describes a segment of the chromosome fragment which can confer the resistance typical of HTN1. As such it is a carrier of the polynucleotide cited above.

Table 2: Resistant haplotype from B37HTN1;

Position in bp on B73 AGPv02	Allele donor B37HTN1	Marker designation
151831049	C	MA0005
151907173	G	MA0021
152045106	T	MA0007
152045141	T	MA0008
152045402	T	MA0009
152045516	C	MA0010
152045912	T	MA0011
152046502	T	MA0012
152046529	A	MA0022
152133057	G	MA0013
152133380	A	MA0014
152144310	A	MA0015
152250992	A	MA0016
152301656	A	MA0017
152304127	A	MA0018
152433358	A	PZE-108095998
152435855	A	PZE-108096011
152630794	C	MA0019
152703579	G	PZE-108096610
152753635	A	MA0020
152887338	G	PZE-108096791
152888374	A	MA0006

Furthermore, every maize plant in accordance with the invention is a HT-resistant maize plant. The HT resistance conferred by integration of the chromosome fragment may be

quantified by determining classification scores in phenotyping experiments in accordance with the scheme in Table 3 and Example 1.A); in this, the resistance level reduces from 1 to 9. HT-resistant maize plants in accordance with the invention exhibit an increased resistance to *H. turcicum* of at least 1 classification score, preferably at least 2 classification scores or at least 3 classification scores and particularly preferably at least 4 classification scores. Preferably, a maize plant in accordance with the invention exhibits resistance to at least one race of *Helminthosporium turcicum* which does not correspond to the known race specificity known in the prior art. In a particularly preferred embodiment, a maize plant in accordance with the invention is resistant to all known races of *Helminthosporium turcicum*, i.e. the conferred resistance is not race-specific and may be particularly advantageous in the formation of a broad resistance to *Helminthosporium turcicum.*

Table 3: Classification score scheme for phenotyping experiments in field trials at various locations with natural and artificial *H. turcicum* inoculation (from the Deutsche Maiskomitee (DMK, German maize committee); AG variety 27.02.02; (DMK J. Rath; RP Freiburg H.J. Imgraben)

Classification score	Phenotype
1	Plants exhibit no symptoms of disease, 0%
2	Beginning of infestation, first small spots (less than 2 cm) visible. Less than 5% of leaf surface affected.
3	Some spots have developed on a leaf stage. Between 5-10% of leaf surface affected.
4	10-20% of leaf surface affected. Clearly visible spots on several leaf stages.
5	20-40% of leaf surface affected. Spots start to coalesce.
6	40-60% of leaf surface affected. Systematic infestation visible on leaves.
7	60-80% of leaf surface affected. Approximately half of leaves destroyed or dried out because of fungal infestation.
8	80-90% of leaf surface affected. More than half of leaves destroyed or dried out because of fungal infestation.
9	90-100% of leaf surface affected. The plants are almost completely dried out.

The description discloses the genetic or molecular structure of the HTN1 locus by providing a haplotype, by mapping prominent markers and also by identifying candidate genes for conferring resistance to the pathogen *Helminthosporium turcicum.*

Surprisingly, the maize plants in accordance with the invention proved to be agronomic in phenotyping experiments carried out in the field and in the greenhouse. This is because, while other converted lines from a breeding programme for integration of the HTN1 locus from Pepitilla as well as from known prior art converted lines such as B37HTN1, in addition to the conferred HT resistance under non-infestation conditions with *H. turcicum* and under comparable environmental conditions (temperature, nutrient supply, location etc) exhibited a significant delay in the male and/or female flowering time compared with the corresponding line without introgression (for example isogenic lines or original lines), in the maize plant of the invention the flowering time corresponded to that of a comparative isogenic maize plant into the genome of which a chromosome fragment from the donor Pepitilla had not been integrated. The "flowering times" correspond when they differ from each other by less than 2 days. The magnitude of the observed delay in this case is strongly dependent on the species of maize or the maize genotype, the prevailing environmental conditions such as the soil condition, humidity, precipitation, temperature etc and/or biotic stress such as pathogen infestation other than with *H. turcicum*. The delay was at least 2 days, at least 3 days, at least 5 days or at least 7 days. This established difference in the flowering time is due to linkage drag as part of the introgression, which is particularly surprising since observations of this type are not known in the prior art. The flowering time is an important agronomic feature. It can directly and substantially influence the yield potential of a maize plant. A delayed flowering time usually results in a reduced yield.

In order to elucidate the genetic cause of this disadvantage and to identify the linkage drag, extensive backcrossing programmes accompanied by genotyping and phenotyping were carried out, for example. The work was supported by intensive development of specific molecular markers on the chromosome fragment carrying the HTN1. The techniques of marker aided selection (MAS) and carrying out focussed backcross programmes (for example "map based cloning") can be found in the prior art (Gupta & Varshney, 2013). The QTL with HTN1 resistance from the donor B37HTN1 or Pepitilla was localized with the aid of the SSR markers bnlg1067, umc1121, MA0002, MA0003, bnlg1782, umc1287, umc1960 and bnlg240 in the descendants on chromosome 8 (bin 8.06) between the markers MA0002 (Table 4) and umc1287 (Table 5) in a region of 23.1 cM (see Figure 1). In maize plants with the delayed flowering time, the locus of the genomic donor sequence segment which is

responsible for the identified linkage drag of the flowering time was successfully determined to be on a further second interval of the donor on the chromosome fragment (Example 3B; Figure 3). In a maize plant in accordance with the invention, a chromosome fragment is integrated into it which does not contain the second interval of the donor. Here, the second interval stems, for example, from a recurrent parent which does not carry the linkage drag for flowering time or from an exogenically introduced homologous DNA fragment which is not a carrier of the linkage drag, on a suitable donor vector for targeted homologous recombination. The second interval is proximal and closely coupled to the resistance locus HTN1 or to the first interval. The second interval is an interval between a marker in a first marker region (M1) which is flanked by the markers SYN14136 and PZE-108076510 and a marker in a second marker region (M2) which is flanked by the markers SYN24931 and PZE-108077560. The flanking markers may be discerned from Table 4. The markers SYN14136, PZE-108076510, SYN24931 and PZE-108077560 are SNP markers for use in the KBioscience-KASP system (www.lgcgenomics.com/genotyping/KASP-genotyping-reagents/KASP-overview/). They clearly define the marker regions M1 and M2 either side of the sequence segment which in the donor B37HTN1 or Pepitilla carry the linkage drag for flowering time. Moreover, as the polymorphic marker, these are also capable of differentiating between Pepitilla donor alleles and, for example, the allele for the recurrent parent. All details regarding the use of these markers as a KASP marker can be obtained from Table 4. Suitable exemplary primer hybridization parameters for the PCR are provided in Example 2. A person skilled in the art is, moreover, also capable of determining other suitable hybridization parameters. Furthermore, it is routine for a person skilled in the art with a knowledge of the described marker regions in addition to the cited markers to develop other markers, in particular polymorphic markers, in M1 and/or M2. Using the markers cited here, namely SYN14136, PZE-108076510, SYN24931 and PZE-108077560 or self-developed markers in M1 and/or M2, the person skilled in the art will readily be able to establish whether in a maize plant into the genome of which a chromosome fragment with HTN1 resistance locus from the donor Pepitilla has been integrated, the second interval of the donor described above is contained therein or not contained therein. The person skilled in the art will also be aware that, for example, during the course of a breeding process or a genetic engineering strategy for targeted recombination, a chromosome interval can be removed from the donor which, for example, comprises genomic sequences which cause linkage drag, by genetic/homologous recombination of the integrated chromosome fragment. In this regard, the interval of the Pepitilla donor can be replaced by the corresponding interval of the recurrent parent genome or by an exogenically introduced homologous DNA fragment.

Markers in general and the markers disclosed here in particular can in particular be used for selection in this regard. As an example, a possible use of markers for the detection of an allele will be given below: detecting an allele may, for example, be carried out by (a) isolating at least one nucleic acid molecule from a genome of a plant or a plant cell/maize plant or maize plant cell, and (b) examining the isolated nucleic acid molecule with at least one marker, as well as optionally (c) sequencing the allele in one and/or more genotypes, (d) detecting one and/or more polymorphisms and/or (e) restriction with a restriction endonuclease which can produce fragments of different sizes at a marker allele.

A preferred embodiment of the maize plant of the invention is a maize plant as described above, wherein the chromosome fragment does not contain the second interval of the donor which is flanked a) by the markers SYN14136 and PZE-108077560, b) by the markers PZE-108076510 and PZE-108077560, c) by the markers SYN14136 and SYN24931 or d) by the markers PZE-108076510 and SYN24931.

In a preferred embodiment, the maize plant of the invention exhibits a deviant male and/or female flowering time compared with the Pepitilla-converted line or Pepitilla-converted plant such as B37HTN1 which contains the interval 2 between a marker in a first marker region (M1) which is flanked by the markers SYN14136 and PZE-108076510, and by a marker in a second marker region (M2) which is flanked by the markers SYN24931 and PZE-108077560, wherein the term “deviant time” means that the converted line or converted plant exhibits a delay of at least 2 days, at least 3 days, at least 5 days or at least 7 days.

A further preferred embodiment of the maize plant of the invention is a maize plant as described above, wherein the chromosome fragment furthermore does not contain an interval of the donor (hereinafter termed the third interval or interval 3) between a marker in the second marker region M2 and a marker in a third marker region M3 which is flanked by the markers PZE-108093423 (Table 4) and PZE-108093748 (Table 4). The markers PZE-108093423 and PZE-108093748 are SNP markers for use in the KBioscience-KASP-System (www.lgcgenomics.com/genotyping/KASP-genotyping-reagents/KASP-overview/). They unequivocally define the marker region M3. As polymorphic markers, they are also suitable for distinguishing between donor alleles and, for example, the allele for the recurrent parent. All details regarding the use of these markers as KASP markers can be obtained from Table 4. Suitable exemplary primer hybridization parameters for PCR are provided in Example 2. A person skilled in the art is also able to determine other suitable hybridization parameters. Furthermore, it is a routine matter for a person skilled in the art

with a knowledge of the described marker region to develop other markers, in particular polymorphic markers, in M3 in addition to the cited markers. Using the markers for M2 as cited above and the markers PZE-108093423 and PZE-108093748 noted herein or self-developed markers in M3, it would be a simple matter for a person skilled in the art to establish whether, in a maize plant into the genome of which a chromosome fragment with a HTN1 resistance locus from the donor Pepitilla has been integrated, contains or does not contain the third interval of the donor as described above.

A further preferred embodiment of the maize plant in accordance with the invention is provided by the maize plant as described above wherein the chromosome fragment does not contain a genetic segment which comprises the second interval and the third interval of the donor and is flanked a) by the markers SYN14136 and PZE-108093423, b) by the markers PZE-108076510 and PZE-108093423, c) by the markers SYN14136 and PZE-108093748 or d) by the markers PZE-108076510 and PZE-108093748.

In a further aspect, further genetic segments may be determined on the chromosome fragment which, under non infestation conditions with *H. turcicum*, could cause a significant negative influence on the yield potential of a maize plant into the genome of which a chromosome fragment with a HTN1 resistance locus from the donor Pepitilla has been integrated. Thus, independently of the delay to the flowering time described above, converted lines as well as known prior art converted lines such as B37HTN1, in addition to the conferred HT resistance, exhibit a substantially reduced yield, in particular a substantially reduced silage yield compared with the corresponding line without introgression (for example isogenic line or original line). This is the case even for lines into the genome of which a genetic segment of the donor consisting of interval 2 (between a marker from M1 and M2) or interval 2 and 3 (between a marker from M1 and M3) is no longer present. Observations of this type would not be expected by the person skilled in the art, since there would be no indication in the prior art of a linkage drag of this type in HTN1 introgression lines. In order to elucidate the genetic cause of this agronomic disadvantage, for example, extended backcrossing programmes accompanied by genotyping and phenotyping were carried out. This work was supported by an intensive development of more accurate and more specific molecular markers on the HTN1-carrying chromosome fragment. In maize plants with the reduced yield (silage yield), the position of the genomic sequence segment which is responsible for the linkage drag of the silage yield was successfully determined on two further intervals of the donor (hereinafter the fourth interval or interval 4 and the fifth interval or interval 5) on the Pepitilla

chromosome fragment (Example 3C; Figure 3). A maize plant in accordance with the invention which comprises a corresponding interval without linkage drag, for example from the recurrent parent, instead of the fourth and/or fifth interval of the donor carrying the linkage drag, exhibits no reduced silage yield, and thus a yield, in particular a silage yield, which is the same as or comparable to a line without introgression (for example isogenic line or original line). Compared with a comparable maize plant with linkage drag for the silage yield, the silage yield of a maize plant in accordance with the invention without fourth and/or fifth intervals of the donors, may be more than 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 15% or 20% higher. The fourth interval is proximally located and closely coupled to the resistance locus HTN1 or the first interval. The fifth interval is distally located and closely coupled with the resistance locus HTN1 or the first interval.

Thus, a particularly preferred embodiment of the maize plant of the invention is a maize plant as described above wherein the chromosome fragment furthermore does not contain i) the fourth interval of the donor between a marker in the third marker region M3 and a marker in a fourth marker region M4 which is flanked by the markers MA0004 and MA0005, or ii) a genetic segment with the fourth interval between a marker in the third marker region M3 and a marker in a seventh marker region M7 which is flanked by the markers MA0005 and MA0021, and/or wherein the chromosome fragment furthermore does not contain i) the fifth interval of the donor between a marker in a fifth marker region M5 which is flanked by the markers MA0006 and PZE-108097482 and a marker in a sixth marker region M6 which is flanked by the markers PZE-108107671 and SYN4196, or ii) a genetic segment with the fifth interval between a marker in an eighth marker region M8 which is flanked by the markers MA0022 and MA0013 and a marker in a sixth marker region M6 which is flanked by the markers PZE-108107671 and SYN4196. The flanking markers may be obtained from Table 4. The markers MA0004, MA0005, MA0006, MA0013, MA0021, MA0022, PZE-108097482, PZE-108107671 and SYN4196 are SNP markers for use in the KBioscience-KASP system (www.lgcgenomics.com/genotyping/KASP-genotyping-reagents/KASP-overview/). They unequivocally define the marker regions M4, M5, M6, M7 and M8 which, together with M3, establish the sequence segments which carry the linkage drag for silage yield in the donor B37HTN1 or Pepitilla. As polymorphic markers, they are also suitable for distinguishing between donor alleles and, for example, the allele for the recurrent parent. All details regarding the use of these markers as KASP markers can be obtained from Table 4. Suitable exemplary primer hybridization parameters for PCR are provided in Example 2. A person skilled in the art is also able to determine other suitable hybridization parameters.

Furthermore, it is a routine matter for a person skilled in the art with a knowledge of the described marker region to develop other markers, in particular polymorphic markers, in M4, in M5, in M6, in M7 and/or in M8. Using the markers MA0004, MA0005, MA0006, MA0013, MA0021, MA0022, PZE-108097482, PZE-108107671 and SYN4196 described here or self-developed markers in M4, in M5, in M6, in M7 and/or M8 together with the markers in M3 described above, it would be a simple matter for a person skilled in the art to establish whether, in a maize plant into the genome of which a chromosome fragment with a HTN1 resistance locus from the donor Pepitilla has been integrated, contains or does not contain the fourth interval of the donor as described above.

A further particularly preferred embodiment of the maize plant of the invention is a maize plant as described above wherein the chromosome fragment i) does not contain a genetic segment which comprises the second interval, the third interval and the fourth interval of the donor and is flanked a) by the markers SYN14136 and MA0004, b) by the markers PZE-108076510 and MA0004, c) by the markers SYN14136 and MA0005 or d) by the markers PZE-108076510 and MA0005, or (ii) does not contain a genetic segment which comprises the second interval and the third interval of the donor and is flanked a) by the markers SYN14136 and PZE-108093423, b) by the markers PZE-108076510 and PZE-108093423, c) by the markers SYN14136 and PZE-108093748 or d) by the markers PZE-108076510 and PZE-108093748, and the fifth interval of the donor, or (iii) does not contain a genetic segment which comprises the second interval, the third interval and the fourth interval of the donor and is flanked a) by the markers SYN14136 and MA0004, b) by the markers PZE-108076510 and MA0004, c) by the markers SYN14136 and MA0005 or d) by the markers PZE-108076510 and MA0005, and the fifth interval of the donor.

A further particularly preferred embodiment of the maize plant in accordance with the invention is a maize plant as described above, wherein the chromosome fragment comprises (i) does not contain a genetic segment which comprises the second interval, the third interval and the fourth interval of the donor and is flanked a) by the markers SYN14136 and MA0021 or b) by the markers PZE-108076510 and MA0021, or (ii) does not contain a genetic segment which comprises the second interval, the third interval and the fourth interval of the donor and is flanked a) by the markers SYN14136 and MA0021 or b) by the markers PZE-108076510 and MA0021, and the fifth interval of the donor, or (iii) does not contain a genetic segment which comprises the second interval, the third interval and the fourth interval of the donor and is flanked a) by the markers SYN14136 and MA0021 or b) by the markers PZE-108076510 and MA0021, and a second genetic segment which

comprises the fifth interval of the donor and is flanked a) by the markers MA0022 and PZE-108107671, b) by the markers MA0022 and SYN4196, c) by the markers MA0013 and PZE-108107671 or by the markers MA0013 and SYN4196, or (iv) does not contain a genetic segment which comprises the second interval and the third interval of the donor and is flanked a) by the markers SYN14136 and PZE-108093423, b) by the markers PZE-108076510 and PZE-108093423, c) by the markers SYN14136 and PZE-108093748 or d) by the markers PZE-108076510 and PZE-108093748, and a second genetic segment which comprises the fifth interval of the donor and is flanked a) by the markers MA0022 and PZE-108107671, b) by the markers MA0022 and SYN4196, c) by the markers MA0013 and PZE-108107671 or by the markers MA0013 and SYN4196, or (v) does not contain a genetic segment which comprises the second interval, the third interval and the fourth interval of the donor and is flanked a) by the markers SYN14136 and MA0021 or b) by the markers PZE-108076510 and MA0021, and a second genetic segment which comprises the fifth interval of the donor and is flanked a) by the markers MA0022 and PZE-108107671, b) by the markers MA0022 and SYN4196, c) by the markers MA0013 and PZE-108107671 or by the markers MA0013 and SYN4196.

The object forming the basis of the present invention is accomplished in an alternative manner by means of a maize plant into the genome of which a chromosome fragment from the donor Pepitilla has been integrated, wherein the chromosome fragment comprises the first interval of the donor which exhibits donor alleles in accordance with the haplotype of Table 2 and comprises the polynucleotide which confers resistance against *Helminthosporium turcicum,* and wherein the chromosome fragment does not contain i) the fourth interval of the donor between a marker in the third marker region which is flanked by the markers PZE-108093423 and PZE-108093748, and a marker in the fourth marker region which is flanked by the markers MA0004 and MA0005, or ii) a genetic segment with the fourth interval between a marker in the third marker region M3 and a marker in the seventh marker region M7 which is flanked by the markers MA0005 and MA0021. The above description, for example, as regards markers the polynucleotide or the phenotyping is also valid in this case and for every other alternative solution to the problem, as well as disclosed embodiments.

A preferred embodiment of this inventive maize plant is a maize plant as described above, wherein the chromosome fragment i) does not contain the fourth interval of the donor which

is flanked a) by the markers PZE-108093423 and MA0004, b) by the markers PZE-108093748 and MA0004, c) by the markers PZE-108093423 and MA0005 or d) by the markers PZE-108093748 and MA0005, or ii) does not contain a genetic segment which comprises the fourth interval of the donor and is flanked a) by the markers PZE-108093423 and MA0021 or b) by the markers PZE-108093748 and MA0021.

A further preferred embodiment of the maize plant in accordance with the invention is a maize plant as hereinbefore described, wherein the chromosome fragment furthermore does not contain the third interval of the donor between a marker in the second marker region M2 and by a marker in the third marker region M3.

A further preferred embodiment of the maize plant in accordance with the invention is a maize plant as described above, wherein the chromosome fragment does not contain a genetic segment which comprises the third interval and the fourth interval of the donor and is flanked a) by the markers SYN24931 and MA0004, b) by the markers PZE-108077560 and MA0004, c) by the markers SYN24931 and MA0005, d) by the markers PZE-108077560 and MA0005, e) by the markers SYN24931 and MA0021 or f) by the markers PZE-108077560 and MA0021.

A further preferred embodiment of the maize plant in accordance with the invention is a maize plant as hereinbefore described, wherein the chromosome fragment i) furthermore does not contain the fifth interval of the donor between a marker in the fifth marker region M5 and a marker in the sixth marker region M6 or ii) does not contain a genetic segment with the fifth interval between a marker in the eighth marker region M8 and a marker in the sixth marker region M6.

A further particularly preferred embodiment of the maize plant in accordance with the invention is a maize plant as hereinbefore described, wherein the chromosome fragment i) does not contain a genetic segment which comprises the third interval and the fourth interval of the donor and is flanked a) by the markers SYN24931 and MA0004, b) by the markers PZE-108077560 and MA0004, c) by the markers SYN24931 and MA0005 or d) by the markers PZE-108077560 and MA0005, and the fifth interval, or ii) does not contain a genetic segment which comprises the third interval and the fourth interval of the donor and is flanked a) by the markers SYN24931 and MA0004, b) by the markers PZE-108077560 and MA0004, c) by the markers SYN24931 and MA0005 or d) by the markers PZE-108077560 and MA0005, and a second genetic segment which comprises the fifth interval

and is flanked a) by the markers MA0022 and SYN4196, b) by the markers MA0022 and PZE-108107671, c) by the markers MA0013 and SYN4196 or by the markers MA0013 and PZE-108107671.

A further particularly preferred embodiment of the maize plant in accordance with the invention is a maize plant as hereinbefore described, wherein the chromosome fragment i) does not contain a genetic segment which comprises the third interval and the fourth interval of the donor and is flanked a) by the markers SYN24931 and MA00021 or b) by the markers PZE-108077560 and MA00021, and the fifth interval, or ii) does not contain a genetic segment which comprises the third interval and the fourth interval of the donor and is flanked a) by the markers SYN24931 and MA00021 or b) by the markers PZE-108077560 and MA00021, and a second genetic segment which comprises the fifth interval and is flanked a) by the markers MA0022 and PZE-108107671, b) by the markers MA0022 and SYN4196, c) by the markers MA0013 and PZE-108107671 or by the markers MA0013 and SYN4196.

Alternatively, the object of the present invention is further accomplished by means of a maize plant, into the genome of which has a chromosome fragment from the donor Pepitilla has been integrated, wherein the chromosome fragment comprises the first interval of the donor which exhibits donor alleles in accordance with the haplotype of Table 2 and which comprises the polynucleotide which confers resistance against *Helminthosporium turcicum* in the maize plant, and wherein the chromosome fragment does not contain i) the fifth interval of the donor between a marker in the fifth marker region which is flanked by the markers MA0006 and PZE-108097482, and a marker in the sixth marker region which is flanked by the markers PZE-108107671 and SYN4196, or ii) a genetic segment with the fifth interval between a marker in the eighth marker region M8 which is flanked by the markers MA0022 and MA0013, and by a marker in the sixth marker region M6 which is flanked by the markers PZE-108107671 and SYN4196.

A further preferred embodiment of the maize plant in accordance with the invention is a maize plant as hereinbefore described, wherein the chromosome fragment furthermore does not contain the third interval of the donor between a marker in the second marker region M2 and a marker in the third marker region M3.

A further particularly preferred embodiment of the maize plant in accordance with the inventions is a maize plant as described above, wherein the chromosome fragment is flanked a) by a marker in the second marker region M2 and by a marker in the sixth marker region M6, b) by a marker in the third marker region M3 and by a marker in the sixth marker region M6, c) by a marker in the fourth marker region M4 and by a marker in the sixth marker region M6, d) by a marker in the seventh marker region M7 and by a marker in the sixth marker region M6, e) by a marker in the marker region M1 and by a marker in the marker region M5, f) by a marker in the second marker region M2 and by a marker in the fifth marker region M5, g) by a marker in the third marker region M3 and by a marker in the fifth marker region M5, h) by a marker in the fourth marker region M4 and by a marker in the fifth marker region M5, i) by a marker in the seventh marker region M7 and by a marker in the fifth marker region M5, j) by a marker in the marker region M1 and by a marker in the marker region M8, k) by a marker in the second marker region M2 and by a marker in the eighth marker region M8, l) by a marker in the third marker region M3 and by a marker in the eighth marker region M8, m) by a marker in the fourth marker region M4 and by a marker in the eighth marker region M8, or n) by a marker in the seventh marker region M7 and by a marker in the eighth marker region M8.

A further particularly preferred embodiment of the maize plant in accordance with the invention is a maize plant as described above, wherein the chromosome fragment is flanked a) by the markers SYN24931 and SYN4196, b) by the markers PZE-108077560 and SYN4196, c) by the markers SYN24931 and PZE-108107671, d) by the markers PZE-108077560 and PZE-108107671, e) by the markers PZE-108093423 and SYN4196, f) by the markers PZE-108093748 and SYN4196, g) by the markers PZE-108093423 and PZE-108107671, h) by the markers PZE-108093748 and PZE-108107671, i) by the markers MA0004 and SYN4196, j) by the markers MA0005 and SYN4196, k) by the markers MA0004 and PZE-108107671, l) by the markers MA0005 and PZE-108107671, m) by the markers MA0021 and SYN4196, n) by the markers MA0021 and PZE-108107671, o) by the markers PZE-108076510 and MA0006, p) by the markers SYN14136 and MA0006, q) by the markers PZE-108076510 and PZE-108097482, r) by the markers SYN14136 and PZE-108097482, s) by the markers SYN24931 and PZE-108097482, t) by the markers PZE-108077560 and PZE-108097482, u) by the markers SYN24931 and MA0006, v) by the markers PZE-108077560 and MA0006, w) by the markers PZE-108093423 and PZE-108097482, x) by the markers PZE-108093748 and PZE-108097482, y) by the markers PZE-108093423 and MA0006, z) by the markers PZE-108093748 and MA0006, aa) by the

markers MA0004 and PZE-108097482, ab) by the markers MA0005 and PZE-108097482, ac) by the markers MA0004 and MA0006, ad) by the markers MA0005 and MA0006, ae) by the markers MA0021 and PZE-108097482, af) by the markers MA0021 and MA0006, ag) by the markers PZE-108076510 and MA0013, ah) by the markers SYN14136 and MA0013, ai) by the markers PZE-108076510 and MA0022, aj) by the markers SYN14136 and MA0022, ak) by the markers SYN24931 and MA0013, al) by the markers PZE-108077560 and MA0013, am) by the markers SYN24931 and MA0022, an) by the markers PZE-108077560 and MA0022, ao) by the markers PZE-108093423 and MA0013, ap) by the markers PZE-108093748 and MA0013, aq) by the markers PZE-108093423 and MA0022, ar) by the markers PZE-108093748 and MA0022, as) by the markers MA0004 and MA0013, at) by the markers MA0005 and MA0013, au) by the markers MA0004 and MA0022, av) by the markers MA0005 and MA0022, aw) by the markers MA0021 and MA0013, ax) by the markers MA0021 and MA0022.

A further particularly preferred embodiment of the maize plant in accordance with the inventions is a maize plant as described above, wherein the chromosome fragment is localized a) between a marker in the second marker region M2 and a marker in the sixth marker region M6, b) between a marker in the third marker region M3 and a marker in the sixth marker region M6, c) between a marker in the fourth marker region M4 and a marker in the sixth marker region M6, d) between a marker in the seventh marker region M7 and a marker in the sixth marker region M6, e) between a marker in the first marker region M1 and a marker in the fifth marker region M5 f) between a marker in the second marker region M2 and a marker in the fifth marker region M5, g) between a marker in the third marker region M3 and a marker in the fifth marker region M5, h) between a marker in the fourth marker region M4 and a marker in the fifth marker region M5, i) between a marker in the seventh marker region M7 and a marker in the fifth marker region M5, j) between a marker in the marker region M1 and a marker in the marker region M8, k) between a marker in the second marker region M2 and a marker in the eighth marker region M8, l) between a marker in the third marker region M3 and a marker in the eighth marker region M8, m) between a marker in the fourth marker region M4 and a marker in the eighth marker region M8, or n) between a marker in the seventh marker region M7 and a marker in the eighth marker region M8.

Table 4: KASP marker primer sequences and assignment to B37HTN1 donor alleles derived from the landrace Pepitilla (allele X and allele Y: describe the biallelic values of the SNPs)

SNP marker	Marker position AGPv02 [bp]	Primer alleles X(5'-3') [SEQ ID NO]	Primer alleles Y(5' -3') [SEQ ID NO]	Common primer (5'-3') [SEQ ID NO]	B37HTN1 donor alleles (SNP)	Marker region
SYN14136	131681497	17	18	19	A	M1
PZE-108076510	131905855	20	21	22	G	M1
SYN24931	132877982	23	24	25	A	M2
PZE-108077560	133189880	26	27	28	A	M2
PZE-108093423	150279048	29	30	31	A	M3
PZE-108093748	150562764	32	33	34	G	M3
PZE-108107671	161543406	35	36	37	C	M6
SYN4196	161766769	38	39	40	C	M6
MA0004	151688652	41	42	43	A	M4
MA0005	151831049	44	45	46	C	M4/M7
MA0021	151907173	241	242	243	G	M7
MA0006	152888310	47	48	49	A	M5
PZE-108097482	153139646	50	51	52	A	M5
MA0002	147720853	53	54	55	A	
MA0003	151346184	56	57	58	C	
MA0007	152045106	59	60	61	T	
MA0008	152045141	62	63	64	T	
MA0009	152045402	65	66	67	T	

MA0010	152045516	68	69	70	C	
MA0011	152045912	71	72	73	T	
MA0012	152046502	74	75	76	A	
MA0022	152046529	244	245	246	A	M8
MA0013	152133057	77	78	79	G	M8
MA0014	152133380	80	81	82	T	
MA0015	152144310	83	84	85	A	
MA0016	152250992	86	87	88	A	
MA0017	152301656	89	90	91	A	
MA0018	152304127	92	93	94	A	
MA0019	152630794	95	96	97	C	
MA0020	152753635	98	99	100	A	
PZE-108095998	152433358	101	102	103	T	
PZE-108096011	152435855	104	105	106	A	
PZE-108096610	152703579	107	108	109	C	
PZE-108096791	152887338	110	111	112	G	

Furthermore, the present invention concerns a seed or grain, a tissue, an organ, a portion and a cell of the maize plants in accordance with the invention described above. In this regard, the seed or the grain is a seed or a grain into the genome of which the chromosome fragment of the embodiment of the invention described above has been integrated.

In a further aspect, the present invention concerns a method for identifying a *H. turcicum*-resistant maize plant into the genome of which a chromosome fragment from the donor Pepitilla has been integrated, comprising the descendants of at least two alleles in the genome of the plant, wherein at least one allele is localized in a genomic segment which is flanked by a

marker in the first marker region M1, the second marker region M2, the third marker region M3, the fourth marker region M4 or the seventh marker region M7, and by the polynucleotide described above which confers resistance to *H. turcicum* in the maize plant, and wherein at least one allele is localized in a genomic segment which is flanked by a marker in the sixth marker region M6, the fifth marker region M5 or the eighth marker region M8. The marker regions and exemplary markers in these marker regions are described above. Preferably, the identified maize plant is a maize plant in accordance with the [illegible] Furthermore, the invention also concerns a maize plant which has been using the identification method which has been mentioned.

In a further aspect, the present invention concerns a method for increasing the yield of a *H. turcicum*-resistant maize plant, into the genome of which a chromosome fragment from the donor Pepitilla has been integrated, wherein the method comprises a step which removes the second interval of the donor, the fourth interval of the donor or the fifth interval of the donor and wherein the chromosome fragment comprises the first interval of the donor described above which comprises donor alleles in accordance with the haplotype of Table 2 and a polynucleotide which confers resistance to *Helminthosporium turcicum* in the maize plant. As an example, removal may be carried out by genetic recombination during a crossing process between two maize plants, wherein a parent maize plant carries the HTN1-resistance locus from Pepitilla. In addition to the use of conventional breeding techniques to produce a genetic recombination which has the result of replacing at least one of the donor intervals with linkage drag identified above with genomic sequences of the recurrent parent which are preferably free from unwanted genes, modern biotechnology offers the person skilled in the art many tools which can enable precise genetic engineering to be carried out. Examples of known tools are meganucleases (Silva et al., 2011), homing endonucleases (Chevalier 2002), zinc finger nucleases, TALE nucleases (WO 2010/079430; WO 2011/072246) or CRISPR (Gaj et al., 2013). These are artificial nuclease fusion proteins which are capable of cleaving double stranded nucleic acid molecules such as plant DNA and thus of producing double strand breaks at desired positions in the genome. By exploiting the cells own mechanisms for repairing induced double strand breaks, a homologous recombination or a "non-homologous end joining" can be carried out, which could lead to the removal of the intervals of the donor carrying linkage drag. Suitable target sequences in the genome for the recognition domain nucleases may be taken, for example, from the sequence information for the SNP markers (Table 4) or in their intervals. However, a person skilled in the art is also able

to identify other sequences, preferably within or between the six marker regions described above, which are suitable as target sequences for the recognition domains of the nucleases.

In this regard we shall now describe two genetic engineering approaches in more detail, with the aid of which the elimination of linkage drag-carrying nucleotide sequences from a plant genome is supported or directly obtained. The following methods as well as the conventional breeding method may be employed for the production of the maize plants in accordance with the invention.

As already stated, current molecular tools are capable of inducing double strand breaks at defined locations in the genome of a plant DNA. In this regard the use of TALE nucleases (TALENs) or zinc finger nucleases (ZFNs) has proved to be particularly advantageous. The TALE or ZF recognition domain enables it to bind specifically to any location in the genome. Knowing the sequence in the target region, the TALE or ZF recognition domains can be tailored so that they exclusively bind to desired locations in the genome. If, for example, the recognition sequence is fused with a non-specific endonuclease such as FokI, a double strand break (DSB) can be induced at defined locations in the genome, enabling targeted genome engineering (Tzfira et al., 2012; Li et al., 2011; Puchta and Hohn 2010). The person skilled in the art will be familiar with handling FokI endonucleases and the provision of suitable TALENs and ZFNs from the prior art.

An induced double strand break may, for example, stimulate a homologous recombination between an endogenic target gene locus (for example one of the above marker regions) and an exogenically introduced homologous DNA fragment which, for example, is not a carrier of linkage drag (for example on a suitable donor vector). This so-called gene replacement or genome editing can be carried out in vitro and does not necessitate any crossing steps between two plants. To this end, the plants to be modified must on the one hand be transiently transformed with nucleic acids coding for the designated TALENs or ZFNs, and on the other hand with the exogenic DNA fragment. The DNA fragment in this regard may originate from a plant of the same species and, for example, corresponds to the chromosomal segment which is to be replaced, but without linkage drag. After completing the induced homologous recombination, cells with a modified genome can be regenerated into plants and then selected as to whether the linkage drag has been successfully removed and the previously transformed DNA elements are once again lost during the regenerative cell division. The markers described above may also be used for this purpose. Methods for the transformation and regeneration are known in the prior art and are also discussed further below.

Furthermore, the present TALENs and ZFNs may also be transgenically introduced during the process of meiosis, where double strand breaks are induced at predetermined locations in the genome and thus the probability for a recombination at these locations in the crossing over step is increased. In this manner, the elimination of linkage drag can be significantly encouraged. A person skilled in the art is aware that after completion of meiosis, linkage drag-free and TALENs or ZFNs-free plants are produced from the haploid cells. In a further aspect, the present invention concerns a method for the production of a maize plant in accordance with the invention, which comprises the following steps: (A) preparing a first maize plant into the genome of which a chromosome fragment from the donor Pepitilla has been integrated, wherein the chromosome fragment comprises a first interval of the donor which exhibits donor alleles in accordance with the haplotype of Table 2 and comprises a polynucleotide which confers resistance against *Helminthosporium turcicum* in the maize plant, and wherein the chromosome fragment contains a second interval of the donor and/or the fourth interval of the donor and/or the fifth interval of the donor, (B) providing a second maize plant, (C) crossing the maize plant from (A) with the maize plant from (B), and (D) selecting a maize plant in accordance with the invention, preferably using at least one of the markers described above. Alternatively, the present invention concerns a method for the production of a maize plant in accordance with the invention which comprises the following steps: (A) transiently transforming a maize plant cell with a first nucleotide sequence which codes for a first protein with endonuclease activity (for example a TALE or ZF endonuclease fusion protein) which is capable of inducing a double strand break of the DNA between the marker regions M2 and M4 in the maize plant cell, and with a second nucleotide sequence which codes for a second protein with endonuclease activity (for example a TALE or ZF endonuclease fusion protein) which is capable of inducing a double strand break of the DNA in the genome of the maize plant cell between marker regions M5 and M6, (B) transiently introducing a donor vector into the first maize plant cell which carries a chromosome fragment from the donor Pepitilla, wherein the chromosome fragment comprises a first interval of the donor which exhibits donor alleles in accordance with the haplotype of Table 2 and comprises a polynucleotide which confers resistance against *Helminthosporium turcicum* in the maize plant, and wherein the chromosome fragment furthermore comprises the chromosomal segments of the donor Pepitilla between the sites of the double strand break from (A) so that a homologous recombination takes place between the genome of the first maize plant cell and the chromosome fragment of the donor vector, (C) regeneration of a maize plant from the maize plant cell, (D) identification of a maize plant in accordance with the invention, preferably using at least one of the markers described above. Particularly preferably, transiently

introduced first and second nucleic acid sequences and donor vectors are then lost. The person skilled in the art will know how to carry this out and detect it.

In a further aspect, the invention encompasses the markers described above as oligonucleotides, in particular primer oligonucleotides. Preferably, the oligonucleotides are isolated oligonucleotides. An oligonucleotide comprises a nucleic acid molecule with a nucleotide sequence selected from one of SEQ ID NOs: 41-49, 53-100 and 229-250. Furthermore, the present invention concerns the use of an oligonucleotide which comprises a nucleic acid molecule with a nucleotide sequence selected from one of the SEQ ID NOs: 17-250, for the identification of a *H. turcicum*-resistant maize plant. Preferably, the resistance derives from the donor Pepitilla and is HTN1.

Furthermore, the problem of the present invention is alternatively solved by means of a transgenic plant, in particular a transgenic maize plant, which comprises a transgenic plant cell as described below. Furthermore, the invention also concerns a portion of this plant in accordance with the invention, wherein a portion may be a cell, a tissue, an organ or a fusion of several cells, tissues or organs. An example of a fusion of several organs is a flower or a seed. In a particular embodiment, the invention concerns a seed from the transgenic plant, wherein the seed comprises the polynucleotide in accordance with the invention as the transgene, as described below. Preferably, a transgenic plant in accordance with the present invention, in particular a plant of the species *Zea mays*, exhibits a higher resistance to *H. turcicum* than a corresponding non-transformed plant (isogenic plant without the transgene). A transgenic HT-resistant plant in accordance with the invention exhibits an increased resistance to *H. turcicum* of at least one classification score, preferably at least 2 classification scores or at least 3 classification scores and particularly preferably at least 4 classification scores (see classification score scheme in Table 3).

Furthermore, the invention provides a method for the production of a transgenic plant which comprises a step for introducing the polynucleotide of the invention or the vector of the present invention described below into a plant cell, and optionally a step for selection of a transgenic plant cell. Furthermore, a method of this type for the production of a transgenic plant is characterized by a subsequent step which includes the regeneration of the transgenic plant from the transgenic plant cell produced in the first step. Methods for regeneration are known to the person skilled in the art from the prior art.

In an additional aspect, the present invention discloses the polynucleotide which contains one or more resistance-conferring genes of the HTN1 locus from Pepitilla (table 1) or selected from RLK1 and EXT1 (see Table 1) or gene alleles thereof. Genes or gene alleles may bring about a resistance phenotype with the features typical of HTN1 under infestation conditions with *H. turcicum*. Structurally, the polynucleotide is characterized in that it comprises a nucleic acid molecule that (a) comprises a nucleotide sequence in accordance with SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 or 15, (b) comprises a nucleotide sequence with an identity of at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with one of the nucleotide sequences in accordance with SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 and 15, preferably over the entire length of the sequence, (c) which hybridizes with the complementary strand of a nucleic acid molecule in accordance with (a) or (b) under stringent conditions, (d) which codes for a polypeptide with an amino acid sequence in accordance with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16, or (e) which codes for a polypeptide with an amino acid sequence which has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with one of the amino acid sequences in accordance with (d). In a preferred embodiment, the polynucleotide is characterized in that it comprises a nucleic acid molecule which (aa) comprises a nucleotide sequence in accordance with SEQ ID NO: 1 or 5, (bb) comprises a nucleotide sequence with an identity of at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% with one of the nucleotide sequences in accordance with SEQ ID NO: 1 or 5, preferably over the entire length of the sequence, (cc) which hybridizes with the complementary strand of a nucleic acid molecule in accordance with (aa) or (bb) under stringent conditions, (dd) which codes for a polypeptide with an amino acid sequence in accordance with SEQ ID NO: 2 or 6, or (ee) which codes for a polypeptide with an amino acid sequence which has at least 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with one of the amino acid sequences in accordance with (dd). Preferably, the polynucleotide can be isolated and/or purified from its natural genetic environment or is present essentially in the pure or homogeneous form. Preferably, the polynucleotide is DNA, and particularly preferably cDNA, i.e. the polynucleotide comprises the cDNA from one or more resistance-conferring genes (Table 1). However, it may also be present as RNA. The person skilled in the art will know how to deduce the genomic DNA sequence from the sequence information disclosed herein. A polynucleotide in accordance with the invention codes for at least one polypeptide which is capable of conferring a resistance against the pathogen *Helminthosporium turcicum* in a plant in which the polypeptide is expressed. Preferably, the polypeptide which is coded by the polynucleotide of the invention or portions thereof,

preferably confers resistance to the pathogen *Helminthosporium turcicum*, in particular in a plant of the genus *Zea* or in a plant of the species *Zea mays*.

Furthermore, the present invention also concerns a polypeptide which is capable of conferring resistance to *H. turcicum* in a plant in which the polypeptide is expressed and which is encoded by the polynucleotide of the invention or a portion thereof. Preferably, the polypeptide comprises an amino acid sequence in accordance with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16 or, particularly preferably, an amino acid sequence in accordance with SEQ ID NO: 2 or 6. The polypeptide may be an isolated polypeptide.

In a further aspect, the present invention concerns a vector which comprises the polynucleotide in accordance with the invention. The vector may be a plasmid, a cosmid, a phage or an expression vector, a transformation vector, shuttle vector or cloning vector, it may be double or single stranded, linear or circular, or it may be a prokaryotic or eukaryotic host, either by integration into its genome or transforming extrachromosomally. Preferably, the polynucleotide of the invention is operatively linked in an expression vector with one or more regulatory sequences which allow transcription and optionally expression in a prokaryotic or eukaryotic host cell. As an example, the polynucleotide may be under the control of suitable promoters or a terminator. Suitable promoters may be promoters which are constitutively induced (example: 35S promoter from the "cauliflower mosaic virus" (Odell et al. 1985); particularly suitable promoters are those promoters which are pathogen-inducible (example: PR1 promoter from parsley (Rushton et al., 1996)). Particularly suitable pathogen-inducible promoters are synthetic or chimeric promoters which do not occur in nature, are composed of several elements and contain a minimum promoter as well as, upstream of the minimum promoter, at least one cis-regulatory element which act as the binding site for special transcription factors. Chimeric promoters are custom-designed and are induced by various factors or re-primed. Examples of such promoters can be found in WO 2000/29592 and WO 2007/147395. An example of a suitable terminator is the nos-terminator (Depicker et al., 1982).

In addition to the vectors described above, the present invention also provides a method which comprises introducing a vector as described into a host cell. The vector may, for example, be introduced by conjugation, mobilization, biolistic transformation, agrobacterium-conferred transformation, transfection, transduction, vacuum infiltration or electroporation. Methods of this type as well as methods for the preparation of the vectors described are familiar to the person skilled in the art (Sambrook et al. 2001).

In a further aspect, the present invention concerns a host cell which comprises the polynucleotide of the invention or a vector of the present invention. In the context of the invention, a host cell may be a prokaryotic cell (for example bacterial) or eukaryotic cell (for example a plant cell or a yeast cell). Preferably, the enzyme is an agrobacterium such as *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes*, or a plant cell which comprises the polynucleotide of the invention or the vector of the present invention. The person skilled in the art will be familiar with the many methods such as conjugation or electroporation for introducing the polynucleotide of the invention or the vector of the present invention into an agrobacterium, and also methods such as various transformation methods (biolistic transformation, agrobacterium-conferred transformation) with which the polynucleotide of the invention or the vector of the present invention can be introduced into a plant cell (Sambrook et al. 2001).

In a further aspect, the present invention concerns a transgenic plant cell which comprises the polynucleotide in accordance with the invention as a transgene or the vector of the present invention. A transgenic plant cell of this type is, for example, a plant cell which is transformed with the polynucleotide in accordance with the invention or with the vector of the present invention, preferably in a stable manner. In a preferred embodiment of the transgenic plant cell, the polynucleotide is operatively linked with one or more regulatory sequences which allow transcription and optionally expression in the plant cell. The total construct of the polynucleotide in accordance with the invention and the regulatory sequence(s) may then constitute the transgene. Examples of regulatory sequences of this type are a promoter or a terminator. The person skilled in the art will be familiar with many functional promoters and terminators which can be used in plants. Preferably, a transgenic plant cell in accordance with the present invention, in particular a cell of a plant of the species *Zea mays*, exhibits a higher resistance to *H. turcicum* than a corresponding non-transformed plant cell (the (isogenic) plant cell without the transgene). Transgenic HT-resistant plant cells of the invention exhibit an increased resistance to *H. turcicum* by at least one classification score, preferably at least 2 classification scores or at least 3 classification scores and particularly preferably at least 4 classification scores (see the classification scheme in Table 3). Furthermore, the present invention also concerns a method for the production of a transgenic plant cell of the present invention, which comprises a step for introducing the polynucleotide in accordance with the invention or the vector of the present invention into a plant cell. As an example, the introduction may be carried out by transformation, preferably by stable transformation. Suitable techniques for introduction such as biolistic transformation, agrobacterium-conferred

transformation or electroporation are known to the person skilled in the (Sambrook et al. 2001).

In a further aspect, the present invention also concerns a method for conferring or increasing a resistance to *H. turcicum* in a plant, preferably a plant of the species *Zea mays*, which comprises a step for transformation of a plant cell with a polynucleotide in accordance with the invention or with the vector of the present invention. Preferably, this method results in enhanced resistance to *H. turcicum* by at least 1 classification score, preferably at least 2 classification scores or at least 3 classification scores and particularly preferably at least 4 classification scores (see the classification scheme in Table 3).

In an additional aspect, the present invention also encompasses a method for modification of the resistance phenotype of a plant, in particular a maize plant, to the pathogen *Helminthosporium turcicum*, which comprises a step for mutation of the resistance-conferring gene of the HTN1 locus from Pepitilla or a gene allele comprised therein. Preferably, the resistance-conferring gene of the HTN1 locus from Pepitilla codes for a polypeptide in accordance with SEQ ID NO: 2 or a homologue of a polypeptide in accordance with SEQ ID NO: 2 which produces a resistance phenotype with the features typical of HTN1 under infestation conditions with *H. turcicum*. The resistance-conferring gene of the HTN1 locus from Pepitilla or a gene allele thereof can be transgenic or endogenic in the genome of the plant. Modification of the resistance phenotype can mean a change in the pathogen race specificity and/or a change in the resistance level, measured as the classification score based on the phenotypical characteristics such as the affected leaf surface (see Table 3) or measured as an AUDPC value (see Example 1.C). Preferably, the resistance level after modification of the resistance phenotype is between the resistance level of a plant which expresses the non-mutated resistance-conferring gene of the HTN1 locus from Pepitilla and the resistance level of an isogenic plant which does not express the resistance-conferring gene of the HTN1 locus from Pepitilla; however, it may also be above the resistance level of a plant which expresses the non-mutated resistance-conferring gene of the HTN1 locus from Pepitilla. Particularly preferably, the resistance level is between the resistance level of a plant which expresses the polypeptide in accordance with SEQ ID NO: 2 and the resistance level of an isogenic plant which does not express the polypeptide in accordance with SEQ ID NO: 2; it may also, however, be above the resistance level of a plant which expresses the polypeptide in accordance with SEQ ID NO: 2. The expression “mutate” as used herein may be a change carried out by man in the genetic sequence (mutation). Examples in this regard are that plants, plant cells or plant portions receiving a high dose of chemical, radiological or other mutating

agents and then selecting for mutants. Alternatively, the mutation may also be carried out with, for example, the help of TILLING nucleases, TALE nucleases, zinc finger nucleases or a CRISPR/Cas system, or by fusion, insertion, deletion or exchange in the DNA sequence or the amino acid sequence. The person skilled in the art may receive sufficient technical instruction from the prior art regarding carrying out the mutation steps. Preferably, mutation of the resistance-conferring gene of the HTN1 locus from Pepitilla results in at least one amino acid exchange, at least two amino acid exchanges, at least three amino acid exchanges, or at least five or more amino acid exchanges. In the case of a plurality of amino acid exchanges, they may be carried out on different gene alleles for the resistance-conferring gene of the HTN1 locus from Pepitilla, i.e. the mutation may be heterozygous or it may also be homozygous.

In a preferred embodiment of the method for the modification of the resistance phenotype of a plant, mutation of the resistance-conferring gene of the HTN1 locus from Pepitilla results in a point mutation in the nucleotide sequence in accordance with SEQ ID NO: 1 at position 1365 with base exchange of a G for an A or at position 1490 with base exchange of a G for an A. Furthermore, this embodiment also concerns a mutation which leads to an amino acid exchange in the amino acid sequence in accordance with SEQ ID NO: 2 at position 455 from M (methionine) to I (isoleucine) or at position 497 from G (glycine) to E (glutamic acid). In a further preferred embodiment of the method, mutation of the resistance-conferring gene of the HTN1 locus from Pepitilla results in a point mutation, which results in an amino acid exchange in the nucleotide sequence in accordance with SEQ ID NO: 1 between position 1365 and position 1490, or the embodiment concerns the mutation which leads to an amino acid exchange in the amino acid sequence in accordance with SEQ ID NO: 2 between position 455 and position 497.

In a further aspect, the invention concerns a method for producing a plant, in particular a maize plant, having a modified resistance phenotype as regards the pathogen *Helminthosporium turcicum*, which comprises a step for mutation of the resistance-conferring gene of the HTN1 locus from Pepitilla or a gene allele thereof in at least one cell of the plant or in at least one cell from which the plant is regenerated. Furthermore, the method can thus comprise a step for regeneration of at least one plant from at least one mutated cell and selection of the regenerated plants on the basis of the modified resistance phenotype as regards the pathogen *Helminthosporium turcicum*. Preferably, the resistance-conferring gene of the HTN1 locus from Pepitilla codes for a polypeptide in accordance with SEQ ID NO: 2 or a homologue of a polypeptide in accordance with SEQ ID NO: 2, which

produces a resistance phenotype with the features typical of HTN1 under infestation conditions with *H. turcicum*. The resistance-conferring gene of the HTN1 locus from Pepitilla or a gene allele thereof may be present in the plant transgenically or endogenically. Modification of the resistance phenotype can mean a change in the pathogen race specificity and/or a change in the resistance level, measured as the classification score based on the phenotypical characteristics such as the affected leaf surface (see Table 3) or measured as an AUDPC value (see Example 1.C). Preferably, the resistance level of the modified resistance phenotype lies between the resistance level of a plant which expresses the non-mutated resistance conferred gene of the HTN1 locus from Pepitilla and the resistance level of an isogenic plant which does not express the resistance conferred gene of the HTN1 locus from Pepitilla; however, it may be above the resistance level of a plant which expresses the non-mutated resistance conferred gene of the HTN1 locus from Pepitilla. Particularly preferably, the resistance level is between the resistance level of a plant which expresses the polypeptide in accordance with SEQ ID NO: 2 and the resistance level of an isogenic plant which does not express the polypeptide in accordance with SEQ ID NO: 2; however, it can also be above the resistance level of a plant which expresses the polypeptide in accordance with SEQ ID NO: 2.

The expression "mutation" herein may be understood to be a change in the genetic sequence (mutation) carried out by man. Examples in this regard are plants, plant cells or plant parts receiving a high dose of chemical, radiological or other mutagens and then being selected for mutants. Alternatively, mutation may also be carried out, for example, with the aid of TILLING nucleases, TALE nucleases, zinc finger nucleases or a CRISPR/Cas system or by fusion, insertion, deletion or exchanges in the DNA sequence or the amino acid sequence. The person skilled in the art may receive sufficient technical instruction from the prior art regarding carrying out the mutation steps. Preferably, mutation of the resistance-conferring gene of the HTN1 locus from Pepitilla results in at least one amino acid exchange, at least two amino acid exchanges, at least three amino acid exchanges, at least five or in more amino acid exchanges. In the case of a plurality of amino acid exchanges, these may also be present on different gene alleles of the resistance-conferring gene of the HTN1 locus from Pepitilla, i.e. the mutation may be heterozygous or even homozygous.

In a preferred embodiment of a method for the production of a plant having a modified resistance phenotype as regards the pathogen *Helminthosporium turcicum*, mutation of the resistance-conferring gene of the HTN1 locus from Pepitilla results in a point mutation in the nucleotide sequence in accordance with SEQ ID NO: 1 at position 1365 with base exchange of a G for an A or at position 1490 with base exchange of a G for an A.

Furthermore, this embodiment also concerns a mutation which leads to an amino acid exchange in the amino acid sequence in accordance with SEQ ID NO: 2 at position 455 from M (methionine) to I (isoleucine) or at position 497 from G (glycine) to E (glutamic acid). In a further preferred embodiment of the method, mutation of the resistance-conferring gene of the HTN1 locus from Pepitilla results in a point mutation, which results in an amino acid exchange in the nucleotide sequence in accordance with SEQ ID NO: 1 between position 1365 and position 1490, or the embodiment concerns the mutation which leads to an amino acid exchange in the amino acid sequence in accordance with SEQ ID NO: 2 between position 455 and position 497.

The invention also concerns plants or parts thereof which may be produced by a method for the production of a plant with a modified resistance phenotype as regards the pathogen *Helminthosporium turcicum*.

Further, the invention encompasses a plant or a part thereof which comprises a mutation in the resistance-conferring gene of the HTN1 locus from Pepitilla or a gene allele thereof. Preferably, the mutation results in a modified resistance phenotype as described above. Preferably, the resistance-conferring gene of the HTN1 locus from Pepitilla codes for a polypeptide in accordance with SEQ ID NO: 2 or a homologue of a polypeptide in accordance with SEQ ID NO: 2, which produces a resistance phenotype with the features typical of HTN1 under infestation conditions with *H. turcicum*. The resistance-conferring gene of the HTN1 locus from Pepitilla or a gene allele thereof may be present in the plant transgenically or endogenically. In a preferred embodiment, of the plant or the part thereof, the mutation is a point mutation in the nucleotide sequence in accordance with SEQ ID NO: 1 at position 1365 with base exchange of a G for an A or at position 1490 with base exchange of a G for an A. Furthermore, this embodiment also concerns a mutation which leads to an amino acid exchange in the amino acid sequence in accordance with SEQ ID NO: 2 at position 455 from M (methionine) to I (isoleucine) or at position 497 from G (glycine) to E (glutamic acid). In a further preferred embodiment of the plant or the part thereof, the mutation of the resistance-conferring gene of the HTN1 locus from Pepitilla is a point mutation which results in an amino acid exchange in the nucleotide sequence in accordance with SEQ ID NO: 1 between the position 1365 and the position 1490, or the embodiment concerns a mutation which leads to an amino acid exchange in the amino acid sequence in accordance with SEQ ID NO: 2 between position 455 and position 497.

Some of the terms used in this application will now be explained in more detail:

The term “allele” refers to one or two or more nucleotide sequences at a specific locus in the genome. A first allele is on a chromosome, a second on a second chromosome at the same position. If the two alleles are different, they are heterozygous, and if they are the same, they are homozygous. Various alleles of a gene (gene alleles) differ in at least one SNP. Depending on the context of the description, an allele also means a single SNP which, for example, allows for a distinction between the donor of HTN1 (Pepitilla) and recurrent parent.

The expression “chromosome fragment” means a specific chromosomal DNA segment of a specific chromosome which comprises at least one gene. An integrated chromosome fragment derives from a donor source. In the context of the invention, the sequential succession of the genes within an integrated chromosome fragment corresponds to that sequence as it is present in the original chromosome fragment of the donor source. In this manner, the integrated chromosome fragment may be present over the whole length unchanged compared with the corresponding chromosome fragment in the donor source. A chromosome fragment or a part thereof may constitute a specific “haplotype”, wherein the chromosome fragment may comprise specific SNPs through which the haplotype can also be unequivocally specified and identified.

The terms “distal” and “proximal” describe the position of a chromosomal interval or a genetic segment in relation to a specific reference point (for example a specific polynucleotide, another chromosomal interval or a gene) on a whole chromosome; “distal” means that the interval or the segment is localized on the side of the reference point distant from the chromosome centromere, and “proximal” means that the interval or the segment is localized on the side of the reference point close to the chromosome centromere.

“close coupled” or “closely linked” means two loci, two intervals, two genetic segments or two markers (marker loci) which are less than 15 cM, less than 12 cM, less than 10 cM, less than 8 cM, less than 7 cM, less than 6 cM, less than 5 cM, less than 4 cM, less than 3 cM, less than 2 cM, less than 1 cM, less than 0.5 cM, less than 0.2 cM, less than 0.1 cM distant from each other, established using the IBM2 neighbors 4 genetic map which is publicly available on the Maize GDB website.

The term “yield” as used in the context of the present invention refers to the productivity per unit area of a specific plant product with commercial value. As an example, the yield of maize is usually measured in metric tonnes of seed or grain per hectare (ha) and season or in metric tonnes of dry biomass per hectare (ha) and season. Unless otherwise specifically

stated or specified, the yield may mean the absolute fresh or dry matter, the relative fresh or dry matter, the silage yield (also known as the silo maize yield or total dry matter yield) or the grain yield. The yield is influenced by genetic and environmental factors and in principle is a combination of many agronomic properties which are built up of features based on genetic elements of a plant and contribute to the final yield during the season. Examples of these individual agronomic properties are seed emergence, vegetative vitality, stress tolerance, disease resistance or tolerance, herbicide resistance, branching tendency, flowering time, seed clusters, seed density, stability and storeability, threshing capability (uniform ripening), etc.

The expression "genetic segment with" a more precisely specified interval should be understood to mean a genetic segment which encloses or comprises the more precisely specified interval, i.e. is not limited to the more precisely specified interval. As an example, a "genetic segment with the fifth interval between a marker in the eighth marker region M8 which is flanked by the markers MA0022 and MA0013, and a marker in the sixth marker region M6 which is flanked by the markers PZE-108107671 and SYN4196" means that the genetic segment comprises the fifth interval and the genetic segment are localized between a marker in the eighth marker region M8 which is flanked by the markers MA0022 and MA0013 and a marker in the sixth marker region M6 which is flanked by the markers PZE-108107671 and SYN4196.

The term "hybridize" or "hybridization" should be understood to mean a procedure in which a single stranded nucleic acid molecule agglomerates with a nucleic acid strand which is as complementary as possible, i.e. base-pairs with it. Examples of standard methods for hybridization have been described in 2001 by Sambrook et al. Preferably, this should be understood to mean that at least 60%, more preferably at least 65%, 70%, 75%, 80% or 85%, particularly preferably 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% of the bases of the nucleic acid molecule undergo base pairing with the nucleic acid strand which is as complementary as possible. The possibility of such agglomeration depends on the stringency of the hybridization conditions. The term "stringency" refers to the hybridization conditions. High stringency is when base pairing is more difficult, low stringency is when base pairing is easier. The stringency of the hybridization conditions depends, for example, on the salt concentration or ionic strength and the temperature. In general, the stringency can be increased by raising the temperature and/or by reducing the salt content. The term "stringent hybridization conditions" should be understood to mean those conditions under which a hybridization takes place primarily only between homologous

nucleic acid molecules. The term "hybridization conditions" in this respect refers not only to the actual conditions prevailing during actual agglomeration of the nucleic acids, but also to the conditions prevailing during the subsequent washing steps. Examples of stringent hybridization conditions are conditions under which primarily only those nucleic acid molecules that have at least 70%, preferably at least 75%, at least 80%, at least 85%, at least 90% or at least 95% sequence identity undergo hybridization. Stringent hybridization conditions are, for example: 4 x SSC at 65°C and subsequent multiple washes in 0.1 x SSC at 65°C for approximately 1 hour. The term "stringent hybridization conditions" as used herein may also mean: hybridization at 68°C in 0.25 M sodium phosphate, pH 7.2, 7 % SDS, 1 mM EDTA and 1 % BSA for 16 hours and subsequently washing twice with 2 x SSC and 0.1 % SDS at 68°C. Preferably, hybridization takes place under stringent conditions.

The term "interval" or "chromosomal interval" means a continuous linear segment on a genomic DNA which is present in an individual chromosome in a plant or on a chromosome fragment and which is usually defined through two markers which represent the end points of the interval on the distal and proximal side. In this regard, the markers which define the ends of the interval may themselves also be a part of the interval. Furthermore, two different intervals might overlap. In the description, an interval is specified by the statement "between marker A and marker B". An end marker of an interval may also be localized in a defined marker region to one side of the interval. A marker region is then defined by providing two flanking markers and constitutes a chromosomal segment on which more markers might be located, in addition to the flanking markers. Flanking markers determine the end points of a marker region and are themselves still a part of the marker region. If both end markers of an interval are markers in different marker regions on both sides of an interval, the description specifies an interval by stating "between a marker in a marker region X which is flanked by the markers C and D and a marker in a marker region Y which is flanked by markers E and F". A marker region may extend over up to 500 000 base pairs (bp), and can preferably be between 100 000 and 400 000 bp in size, or can particularly preferably be between 140 000 and 315 000 bp in size.

The term "introgression" as used in connection with the present invention means the transfer of at least one desired gene allele on a genetic locus of a genetic background into another. As an example, an introgression of a desired gene allele at a specific locus may be transferred to a descendant by sexual crossing between two parents of the same species. Alternatively, for example, the transfer of a gene allele may also occur by recombination between two donor genomes in a fused protoplast, wherein at least one donor protoplast carries the desired gene

allele in its genome. In each case the descendants, which then comprise the desired gene allele, can then be backcrossed again with a line which comprises a preferred genetic background and can be selected for the desired gene allele. The result is fixing of the desired gene allele in a selected genetic background.

The term “isolated nucleic acid molecule” or “isolate polynucleotide” should be understood to mean a nucleic acid molecule or polynucleotide removed from its natural or original environment. The term also encompasses a synthetically produced nucleic acid molecule. An “isolated polypeptide” should be understood to mean a polypeptide which has been removed from its natural or original environment. The term also encompasses a synthetically produced polypeptide.

The term “pathogen infection” should be understood to mean the earliest time at which a pathogen interacts with a plant host tissue. Examples in fungi such as ascomycetes or oomycetes are the growth of hyphae or the formation of specific infection structures such as penetration hyphae and the appressorium. In detail, an infection with *Helminthosporium turcicum* may be investigated using various stain techniques (for example trypan blue) (Chung et al., *BMC Plant Biology* 10 (2010), 103; Walsh et al. (2008), Poster presentation P192, 50th Maize Genetics Conference in Washington D.C.).

“Donor Pepitilla”, “accession Pepitilla” or “Pepitilla” means, in addition to the landrace Pepitilla itself, other maize genotypes into the genome of which, in particular on chromosome 8 bin 5 or 6, an introgression of the *HTN1* resistance locus, preferably from Pepitilla, has been inserted. Examples of these are W22Htn (e.g. Bar-Zur et al. 1998), H6314*Htn* (e.g. Bar-Zur et al. 1998), B73*HtN* (e.g. Shimoni et al., Journal of Phytopathology 131:4 (1991), 315-321), B68*HtN* and A632*HtN* (e.g. Carson, Plant Disease 79 (1995), 717-720) and A619*HtN* (e.g. Stanković et al, Genetika 39:2 (2007), 227 -240). Furthermore, Pepitilla includes any source of resistance which confers the resistance phenotype with the features typical of HTN1 after introgression into a vulnerable maize line/maize plant. Examples of these HTN1-specific features are delayed onset of sporulation, reduced development of lesions, development of smaller lesions, reduced sporulation zones and/or no or only isolated chlorotic-necrotic lesions.

A "Locus" is a position on a chromosome where one or more genes are found which cause an agronomic feature or influence one. In particular, “locus” as used here means the HTN1-resistance locus which confers resistance against the pathogen *Helminthosporium turcicum* or at least against a race of *Helminthosporium turcicum*.

A "maize plant" should be understood to mean a plant from the species *Zea mays* as well as its subspecies such as, for example, *Zea mays* ssp. *mays*, *Zea mays* ssp. *mexicana* or *Zea mays* ssp. *parviglumis*.

A "marker" is a nucleotide sequence which is used as a reference or orientation point. A marker for recognizing a recombination event should be suitable for monitoring differences or polymorphisms in a plant population. For markers, these differences are on a DNA level and, for example, are polynucleotide sequence differences such as, for example, SSRs (*simple sequence repeats*), RFLPs (*restriction fragment length polymorphisms*), FLPs (fragment length polymorphisms) or SNPs (single nucleotide polymorphisms). The markers may be derived from genomic or expressed nucleic acids such as spliced RNA, cDNA or ESTs and may be based on nucleic acids which are used as probes or primer pairs and as such are suitable for amplifying a sequence fragment using PCR-based methods. Markers which concern genetic polymorphisms between parts of a population can be detected using established methods from the prior art (An Introduction to Genetic Analysis. 7th Edition, Griffiths, Miller, Suzuki et al., 2000). These include, for example: DNA sequencing, PCR-based, sequence-specific amplification, assaying of RFLPs, assaying of polynucleotide polymorphisms using allele-specific hybridization (ASH), detection of SSRs, SNPs or AFLPs. Methods for detecting ESTs (*expressed sequence tags*) and RAPD (*randomly amplified* polymorphic DNA) are also known. Depending on the context, the term "marker" in the description may also mean a specific chromosome position in the genome of a species where a specific marker (for example SNP) can be found. A marker position of this type can be used in order to monitor the presence of a coupled locus, for example a coupled locus which contributes to the expression of a specific phenotypical feature (e.g. HTN1 or linkage drag). As an example, the marker locus may also be used to observe the segregation of alleles at a locus (QTL or individual gene) which are genetically or physically closely coupled with the marker position.

"Operatively linked" means linked in a common nucleic acid molecule in a manner such that the linked elements are positioned and orientated with respect to each other such that transcription of the nucleic acid molecule can take place. A DNA which is operatively linked with a promoter is under the transcriptional control of this promoter.

Examples of plant "organs" are leaves, plant stems, stems, roots, vegetative buds, meristems, embryos, anthers, ovulae or fruit. Plant "parts" means a fusion of several organs, for example a flower or a seed or a part of an organ, for example a cross segment from the stem. Examples of plant "tissues" are callus tissue, soft tissue, meristem tissue, leaf tissue, bud

tissue, root tissue, plant tumour tissue or reproductive tissue. The term “cells” should be understood to mean isolated plant cells with a cell wall or aggregates thereof or protoplasts, for example.

In the context of the invention, unless stated otherwise, a “plant” may be any species of dicotyledon, monocotyledon or gymnosperm plant. Preferably, the plants are monocotyledon plants and are of interest in agriculture or horticulture or for the production of bioenergy (bioethanol, biogas, etc). Examples are *Gossypium* sp., *Zea mays*, *Brachypodium distachyon, Triticum* sp., *Hordeum vulgare*, *Oryza sativa*, *Sorghum* sp., *Musa* sp., *Saccharum officinarum*, *Secale cereale*, *Avena* sp., turf grass and forage grass. A preferred plant in accordance with the invention is a plant from the genus *Zea,* in particular the species *Zea mays,* or *Sorghum*.

In connection with the present invention, the term “regulatory sequence” means a nucleotide sequence which influences the specificity and/or strength of expression, for example insofar as the regulatory sequence confers a specific tissue specificity. A regulatory sequence of this type may be localized upstream of the transcription initiation point of a minimum promoter, but also downstream thereof, for example in a transcribed but not translated leader sequence or within an intron.

The expression “resistance” or “resistant” as regards a pathogen should be understood to mean the ability of a plant or plant cell to resist the damaging effects of the pathogen and extends from a delay in the development of disease to complete suppression of the development of the disease. In connection with the present invention, a plant/plant cell is resistant or a plant/plant cell has a resistance to the pathogen *Helminthosporium turcicum (H. turcicum* or *HT)*, i.e. to the leaf disease Northern Corn Leaf Blight (NCLB). The resistance is conferred by one or more proteins which are coded by a gene or by genes (resistance-conferring genes) from the accession Pepitilla. The resistance may be complete or partial and may be specific or non-specific to the pathogen race. In the event of a pathogen race-specific resistance, the virulent races of *Helminthosporium turcicum* may, for example, include N, 1N, 2N, 23N or 123N; the avirulent races may, for example, include 0, 1, 2, 3, 12, 23 or 123. A conferred resistance may be a newly inherited resistance or an increase in a partial resistance which is already extant.

A “transgenic plant” is a plant into the genome of which at least one polynucleotide, preferably a heterologous polynucleotide, has been integrated. Preferably, the polynucleotide has been integrated in a stable manner, which means that the integrated polynucleotide

remains stable in the plant, is expressed and can also be stably inherited to descendants. The stable introduction of a polynucleotide into the genome of a plant also includes integration into the genome of a plant of the previous parental generation, whereby the polynucleotide can be further inherited in a stable manner. The term "heterologous" means that the introduced polynucleotide originates, for example, from a cell or an organism with another genetic background of the same species or from another species, or is homologous with the prokaryotic or eukaryotic host cell, but then is localized in a different genetic environment and thus is different from any possible corresponding naturally occurring polynucleotide. A heterologous polynucleotide can be present in addition to a corresponding endogenous gene.

Embodiments and variations of the present invention will now be described with reference to the accompanying figures and sequences in which:

Figure 1: Calculated QTL region of 23.11 cM on chromosome 8 using 8 markers in 528 F2 individuals of the RP1 x RP1HTN1 cross. The black bars (HtN) show the *confidence* interval. Positions of the markers are in cM.

Figure 2: Silage yield test on 5 locations in Germany and in two duplications, with the recurrent parent RP3 and the A version of the donor fragment from B37HTN1 (RP3HTNA) and the K version of the donor fragment from B37HTN1 (RP3HTNK). The bars show significant differences using the t-test, with $p = 0.05$.

Figure 3: Description of the marker regions M1 to M6 which define the chromosomal intervals (Int. 1 to Int. 5) which exhibit the resistance-conferring polynucleotide in the introgression lines and carry linkage drag in the chromosome fragment originating from the donor. Chromosomal segments of the donor (Pepitilla" are shown as dotted areas, those of the recurrent parent (without linkage drag) are shown as areas with diagonal stripes. Interval 1 (Int. 1) covers the resistance locus HTN1, interval 2 (Int. 2) covers sequence regions which are responsible in the donor for the linkage drag of the flowering time, intervals 4 and 5 (Int. 4 and Int. 5) cover sequence regions which are responsible for linkage drag of the silage yield in the donor.

Figure 4: BAC contig on its RP4HTN1 BAC bank with corresponding sequence scaffold and gene annotations. Candidate genes are shown in squared boxes. The black arrows represent further annotated genes which are not candidate genes for HTN resistance.

1. Phenotyping experiments

A) Carrying out field trials to determine the HT resistance under natural and artificial inoculation/infection conditions and the flowering time:

At a location, at least 20 individuals per maize genotype to be investigated were planted out in a row. Inoculation was carried out naturally or artificially. Natural inoculation/infection was carried out using naturally occurring spores of *H. turcicum*. Artificial inoculation/infection was carried out using infected and ground leaf material which was administered to the plants to be tested. The latter type of inoculation allowed a comparable *H. turcicum* infestation to be simulated in different test years and at different locations independently of the prevailing natural infestation conditions there. A vulnerable parent and a parent with HTN1 introgression were cultivated from the donor B37HTN1 as control genotypes, depending on the test cross population. The classification score of the HT resistance feature was noted at least three times during the vegetative period. Only the classification score scheme shown in Table 3 was used.

The donor B37HTN1 as the source of HT resistance was crossed into various genetic backgrounds from elite lines with various levels of vulnerability to *H. turcicum* and near-isogenic lines were developed which were different from the vulnerable original lines essentially only by the introgression from B37HTN1. In phenotyping experiments, after artificial inoculation as described above, lines were selected which exhibited an improvement in the HT resistance by at least 2 to 3 classification scores, preferably 3 to 4 classification scores by introducing the resistance-conferring introgression from B37HTN1. The present invention will be described below in more detail by way of example using the two selected recurrent parents RP1 and RP3. The results for the phenotyping experiments described are summarized in Table 5. The recurrent parent RP1 without introgression exhibited average classification scores of 7 to 9, which were improved by 3 to 4 classification scores by the introgression from B37HTN1. The recurrent parent RP3 exhibited classification scores between 4 and 6 without introgression and an improvement of 2 to 3 classification scores by the introgression. The recurrent parent RP4 exhibited a classification score of 6 without introgression and an improvement of 2-3 classification scores by the introgression.

Table 5: Phenotyping data for HT resistance from genotypes RP1, RP3, and RP4 with and without resistance conferred introgression from B37HTN1 (classification scores were determined in accordance with the scheme in Table 3).

Genotype	Average classification scores (n=20) without introgression from B37HTN1	Improvement in HT resistance with introgression from B37HTN1
RP1	7 to 9	3 to 4
RP3	4 to 6	2 to 3
RP4	6	2 to 3

In addition to the HT resistance, for each genotype the time of female and male flowering was determined as "days after sowing". The time for female flowering was determined by silk emergence; of male flowering by the appearance of panicles. The results are shown in more detail in Example 3.B).

B) Carrying out field trials to determine grain and silage yields:

In addition to the above data regarding HT resistance and flowering time, yield data for RP3 containing different lengths of resistance-conferring introgression fragments from B37HTN1 or Pepitilla and for a comparative elite line were determined. The lines RP3, RP3HTNA and RP3HTNK were dusted with a tester (flint maize, interpool single cross) of the complementary gene pool (flint maize) in order to produce seed stock for test hybrids. These test hybrids were each grown in duplicate in a field trial at five representative locations for maize crops in Germany. The test hybrids are well suited to these growing regions having regard to ripening. The field trial was carried out in two duplications in 4-row parcels 6 m in length and with a 0.75 m row separation. The density was 9 plants per m^2 in the first and 11 plants per m^2 in the second duplication. At the time of the silo maize harvest only the two central rows of each parcel were harvested in order to minimize competition effects. The weight per parcel and the water content were determined for the harvested material in order to calculate the silo maize yield (also known as the silage yield or the total dry matter yield) and the dry matter content (total dry matter content).

C) Carrying out greenhouse trials in order to determine the HT resistance:

20 individuals per genotype were grown in pots. The controls were genotypes of a vulnerable parent and a near-isogenic parent (NIL) with resistance-conferring introgression from B37HTN1, depending on the cross. 14 days after sowing, an artificial infection was carried out (see above). After a further 2 to 3 weeks, the first symptoms of disease developed. From the time of the appearance of the first symptoms, every other day the classification scores of the HT resistance feature as well as the number of plants with symptoms were determined. From this, the AUDPC (*area under disease progress curve*) was determined. The infestation frequency (as the %/time × period) was used to classify the plants under investigation; here, an AUDPC from 0 – 100 was resistant, 101 – 450 was heterozygous, and > 450 was vulnerable.

2. Marker development for the HTN1-Target-Region

In addition to the classification score tests, the target region around the HTN1 resistance locus on chromosome 8 (bin 8.06) in many genotypes was examined in more detail and mapped finely using novel and/or optimized molecular markers. The molecular markers used herein were developed on the basis of single nucleotide polymorphisms (SNP) or already publically available *simple sequence repeat* markers (SSR):

The DNA from the genotypes for use as markers was either isolated using the NucleoSpin 96 Plant II method following the manufacturer's instructions (MACHEREY-NAGEL GmbH & Co. KG, Germany) or using the Klear Gene DNA Extraction 384 method (LGC Genomics GmbH, Germany).

The primer sequences for the SSR markers were already known from the public database from the National Center for Biotechnology Information (NCBI) at http://www.ncbi.nlm.nih.gov/unists; the primer sequences for the markers bnlg1782, umc1960, bnlg240, umc1121, bnlg1067 and umc1287, which were used to examine the target region, are summarized in Table 6, together with the modifications made.

Table 6: Primer sequences for the SSR marker (NED: 2'-chloro-5'-fluoro-7',8'-fused phenyl-1.4-dichloro-6-carboxyfluorescein; FAM: 6-carboxyfluorescein; M13: core sequence for phage M13)

Marker	Forward primer sequence (5'-3') [SEQ ID NO]	Modification	Reverse primer sequence (5'-3') [SEQ ID NO]	Modification	Additional primer + modification
bnlg1782	113	NED	114	none	
umc1960	115	NED	116	none	
bnlg240	117	FAM	118	none	
umc1121	119	FAM	120	none	
bnlg1067	121	FAM	122	none	
umc1287	123	none	124	none	M13 + FAM

The volume of the PCR reaction mixture of bnlg1782, umc1960, bnlg240, umc1121 and bnlg1067 was 10 µl and consisted of a single concentration of the 4x buffer B (Solis BioDyne, Estonia), 0.5 pmol of the forward primer, 0.5 pmol of the reverse primer, 10-30 ng of DNA, 0.25 units of HotFirepol TAQ-Polymerase (Solis BioDyne, Estonia). The volume of the reaction mixture of umc1287 was 10 µl and consisted of a single concentration of the 4x bufferB (Solis BioDyne, Estonia), 0.5 pmol of the forward primer, 2,5 pmol of the reverse primer, 0.3 pmol of the additional primer M13, 10-30 ng of DNA, 0.25 units of HotFirepol TAQ-Polymerase (Solis BioDyne, Estonia).

The PCR reaction was carried out with an initial denaturing period of 900 seconds at 94°C, an amplification cycle of 25-40 cycles with 15 seconds at 94°C, 30 seconds at 50-55°C and 120 seconds at 72°C, and a final step of 300 seconds at 72°C. Next, the PCR reaction was incubated for 2 h at 65°C. The PCR products were separated on an ABI3730xl (Life Technologies™, USA) following the manufacturer's instructions for the separation of 50-400 bp fragments.

The SNP markers were developed and used either (a) from publically available resources, (b) from a comparative amplicon sequencing or (c) from a sequence comparison of BAC sequences from RP4HTN1 (see Molecular Analysis segment) and B73 reference genome AGPv02 (www.maizesequence.org).

(a) SNPs were transformed into KASP markers (LGC Genomics GmbH, Germany) from the publically available SNP resource of the Maize Community 50K-Illumina-Chip (Ganal et al., 2011). To this end, novel primers were developed which ensured the amplification of the decisive alleles in the KASP marker assay (see Table 4). The whole operation was carried out using Kraken™ Software (LGC Genomics GmbH, Germany). For a KASP marker assay, 5-20 ng DNA, 0.02 µl of an oligo assay mixture (12 µM primer allele 1 (forward); 12 µM of primer allele 2 (forward); 30 µM of reverse primer) and 1.5 µl of a 1xKASPar Reagent Kit for 1536 plates was used. A standard PCR setup consisted of 94°C for 15 min, 10 cycles at 94°C for 20 seconds, 61-55°C touchdown for 1 minute, 26 cycles at 94°C for 20 seconds and 55°C for 1 minute. The evaluation of the alleles per genotype was carried out using Kraken™ software (LGC Genomics GmbH, Germany).

(b) The comparative amplicon sequencing was carried out using Sanger sequencing. The genotypes in the comparative sequences each comprised the donor B37HTN1 as well as B37, RP1, RP1HTN1, RP3, RP3HTN1 (versions A, B, K), RP4, RP4HTN1. The DNA was isolated from ground grains using the CTAB method (Maniatis et al., 1982). The primer sequences for the amplicon sequencing are listed in Table 4. A standard PCR protocol for amplification of the corresponding regions consisted of denaturing at 94°C for 5 minutes, 35 cycles each at 94°C for 1 minute, 60°C for 1 minute and 72°C for 2 minutes and a subsequent step at 72°C for 10 minutes. The PCR products were sequenced with the Sanger method (Sanger & Coulson, 1975). The sequence evaluation was carried out using DNAStar Lasergene software (DNASTAR Inc., USA). The detected polymorphisms were transformed into KASP markers as described in (a).

(c) The BAC sequence contigs were projected against the B73 reference genome AGPv02 using Blast algorithms(http://blast.ncbi.nlm.nih.gov/Blast.cgi) in order to detect single nucleotide polymorphisms (SNP). The polymorphisms were detected using Lasergene software (DNASTAR Inc., USA) and are shown in Table 4 along with the flanking sequences. Primers were developed for the flanking sequences of an SNP and the identified SNPs were transformed into KASP markers as described in (a).

3. Localization of the HTN1 resistance locus on chromosome 8 using the SSR marker

A) Localization of the HTN1 resistance locus:

The HTN1 resistance locus from the B37HTN1 donors were crossed into elite lines as described in Example 1.A) and localized on chromosome 8 (bin 8.06) with the aid of the SSR and SNP markers from Example 2 (see Figure 1). NILs from the crosses RP1 x RP1HTN1 and RP3 x RP3HTN1 were phenotyped at two locations over several years with two duplications under natural infection conditions using the classification score scheme of Table 3. The NILs showed, on average, a resistance response which was improved by 4 classification scores compared with the original line. The development of local lesions on the leaves was shifted by approximately 2 weeks compared with the vulnerable genotype. QTL mapping was carried out with 528 F2 individuals (RP1 x RP1HTN1 cross) using the 8 markers (Tables 4 and 6 are from the QTL mapping markers of Figure 1). The QTL region which covered the HTN1 resistance locus was localized on chromosome 8 between the markers MA0002 and umc1287, in a 23.1 cM region.

B) Crossing the B37HTN1 donor fragment into an elite maize line and identification and elimination of linkage drag for delayed flowering time:

The donor B37HTN1 was crossed with KWS.elite, an elite maize line from KWS SAAT AG (Germany) and then backcrossed over five generations with KWS.elite. In each backcross generation, molecular markers were used in order to select plants which were heterozygous for the HTN target region. Next, a selected plant from the fifth backcross generation was self-fertilized and homozygous plants for the HTN target region were identified with molecular markers.

These lines were tested in field trials at several locations. In this regard, for the genotypes B37HTN1, KWS.elite and KWS.elite.B37HTN1, the phenotypical data of HT resistance and the flowering times were determined as described in Example 1. The genotypes with HTN1 introgression exhibited the expected HT resistance with classification scores of 1 to 3, while the original line KWS.elite exhibited classification scores of 5-7. Unexpectedly, in addition, compared with the KWS.elite, the KWS.elite.B37HTN1exhibited a flowering time both for the female and for the male flowers which was shifted by at least 2 days. These shifted flowering times constitute a negative agronomic feature for maize based on linkage drag which has not yet been

described in this form following introgression of HT resistance from B37HTN1. Marker analyses found the localization of the linkage drag which is responsible for the delayed flowering time to be in a region between two marker regions on the introgression from B37HTN1, between M1 and M2. In this regard, the genotypes B37HTN1, KWS.elite and KWS.elite.B37HTN1 were, for example, analysed with the KASP markers SYN14136, PZE-108076510, SYN24931 and PZE-108077560 (see Figure 3 and Table 4). SYN14136 and PZE-108076510 were used for the specific detection of the marker region M1, SYN24931 and PZE-108077560 for the specific detection of the region M2. According to this, the marker region M1 lies 5' from the locus of the linkage drag and the marker region M2 is 3' thereto. The marker analysis showed that B37HTN1 and KWS.elite.B37HTN1, both with a flowering delayed by two days, exhibited common alleles for the regions M1 and M2 as well as the interval between these regions, while KWS.elite has a normal flowering time and has other alleles for the regions M1 and M2 and the interval between them.

The donor B37HTN1 was crossed with RP3 and then backcrossed over three generations with RP3. Molecular markers were used in each backcross generation. Initially, plants which were heterozygous for the HTN1 target region were selected and then these plants were investigated with markers which were distributed uniformly over the genome in order to select against the donor genome. Next, a selected plant from the third backcross generation was self-fertilized and homozygous plants for the HTN1 target region were identified with molecular markers.

Furthermore, the donor B37HTN1 was also crossed with the recurrent parent RP3 and RP4 and a RP3HTNA and RP4HTNA line produced over several backcrossing steps. The phenotyping on HT resistance showed an improvement in the classification scores of 5 to 7 for the original line RP3 to 1 to 3 for RP3HTNA and an improvement in the classification scores from 6 for the original line RP4 to 2 to 3 for RP4HTNA. The phenotyping for flowering time exhibited comparable flowering times for RP3 and RP3HTNA and RP4 and RP4HTNA. Using the KASP markers SYN14136, PZE-108076510, SYN24931 and PZE-108077560 showed that RP3 and RP3HTNA carry common alleles for the regions M1 and M2. These did not correspond to the donor B37HTN1. As a result, then, the flowering time-delaying chromosomal segment of the introgression from B37HTN1 lies on a chromosomal interval between the marker regions M1 and M2. With the line RP3HTNA, then, this linkage drag was successfully

removed. The KASP markers used, SYN14136, PZE-108076510, SYN24931 and PZE-108077560, proved to be effective tools for "assisted selection".

Phenotyping of RP3 and RP3HTNA also comprised recording the grain and silage yield. While the grain yield in the genotypes was not significantly different, the silage yield feature in RP3HTNA exhibited an unequivocal, statistically significant reduction of at least 14 decitonnes per hectare (dt/ha) over RP3, or a reduction of more than 5%.

With the aid of the designed KASP markers SYN14136, PZE-108076510, SYN24931 and PZE-108077560, a RP1HTN1 line could be selected from the cross of B37HTN1 and the recurrent parent RP1 which did not exhibit any more flowering time-delaying linkage drag, but rather a silage yield reduction, as was observed for RP3HTNA. For the purposes of more accurate molecular characterization, RP1HTN1 was developed further and a F2 population was set up with 724 individuals from the cross RP1 x RP1HTN1. Next, the F3 generation was self-fertilized and selected F4 plants were genotyped and phenotyped. Genotyping was carried out using markers from Table 6 in the detected QTL region of 23.1 cM. Phenotyping was carried out at several locations in two duplications (see Example 1). Recombinant plants for the QTL region were selected and correlated with the phenotype data. The selection comprised plants which covered different regions of the target region as well as heterozygous plants, with the aim of obtaining new recombinant plants. Each year, two backcrosses with RP1 were carried out and individual plants were selected, and thus new recombinants were produced. New recombinants were phenotyped in field and greenhouse tests (see under 1.) and genotyped for the development of novel molecular markers in accordance with 2.

The use of these novel markers on the RP3HTNA genotype allowed a marker region M3 to be identified which limited the introgression in the 5' region and can be described with the flanking markers PZE-108093423 and PZE-108093748. In this regard, PZE-108093423 should exhibit the alleles of the recurrent parent RP3 and PZE-108093748 should exhibit the alleles of the donor B37HTN1. In the 3' region, the introgression of RP3HTNA by the markers PZE-108107671 and SYN4196 in a further marker region M6 is described (see Figure 3). In this regard, PZE-108107671 carries the alleles of the donor B37HTN1 and SYN4196 carries the alleles of the recurrent parent RP3. The introgression from RP3HTNA (hereinafter termed version

A) corresponds, between the marker regions M3 and M6, to the donor B37HTN1, but outside this region it corresponds to the recurrent parent or another line which does not carry the alleles in the region of the donor B37HTN1 between M1 and M2. This version A was introduced into various other genetic backgrounds and fresh yield tests, resistance phenotyping and flowering time determinations were undertaken. The results were comparable with those described for RP3HTNA. Thus, the flowering time was not shifted compared with the corresponding line without introgression and the line exhibited an improved resistance to *Helminthosporium turcicum* compared with the original line, or at least the reduction of the silage yield.

C) Identification and elimination of linkage drag for reduced silage yield:

The donor used was the RP3HTNA line. This was crossed with RP3 and self-fertilized over six further generations. In each self-fertilization generation, molecular markers were used in the target region in order to reduce the donor fragment. Since all regions of the genome outside the target region had already been selected in the RP3HTNA line on the RP3 genome, only the region around the HTN target region was investigated with markers. In this regard, homozygous plants were identified for a reduced HTN target region. At the same time, intensive marker development was carried out in the target region. In addition to many others, a RP3HTNK line was identified which described the B37HTN1 donor fragment from a marker region M4 flanked by the markers MA0004 and MA0005, wherein MA0004 describes the alleles of the recurrent parent RP3 and MA0005 describes the alleles of the donor B37HTN1 in RP3HTNK, up to a marker region M5, flanked by the markers MA0006 and PZE-108097482, wherein MA0006 describes the alleles of the donor B37HTN1 and PZE-108097482 describes the alleles of the recurrent parent RP3. In RP3HTNK, the introgression from RP3HTNK (hereinafter termed version K) causes an improved HTN1 resistance of 3 to 4 classification scores compared with RP3, the same flowering time as its original line RP3 (no delay in flowering) and no further significant reduction in the silage yield (see Figure 2). In addition, with the aid of the described markers, linkage drag-free lines could be produced from the original line RP1 by crossing, which lines exhibited version K of the introgression.

D) Resistance-conferring haplotype from B37HTN1 or from Pepitilla

Version K possesses a haplotype from B37HTN1 or from Pepitilla which carries the donor alleles described in Table 4 at the physical positions with respect to B73 AGPv02 in bp. As an example, the haplotype at marker MA0008 will be described: using the marker MA0008 and specifying the alleles for B37HTN1, RP3, RP3HTNA, RP3HTNK, then the allele "T" is for B37HTN1, RP3HTNA, RP3HTNK and the allele "C" is for RP3. For this locus, this marker also distinguishes the assumed HTN1 resistance source PH99N (WO 2011/163590), which also contains an allele "C" at this position, from the resistance source used here.

4. Molecular analysis of the fine-mapped region

Furthermore, the chromosome fragment which had been inserted and truncated by introgression was investigated on a molecular level. The resistance locus *Htn1* from the accession Pepitilla was thus reduced to a distinct target region, a chromosome interval of 700 kb, and sequenced in the genotype RP4HTN1. As will be described in more detail below, BAC clones from RP4HTN1 were isolated, sequenced and assembled into a sequence scaffold. The sequence scaffold was annotated and the annotated genes in this interval were set against EST/cDNA sequence information. Differential expression studies were then carried out from a multiplicity of annotated genes to identify the candidate genes (see Table 1).

A) BAC bank and pool construction, BAC bank screening, BAC sequencing

A BAC bank was produced from the genotype RP4HTN1. This was followed by constructing the BAC bank and the 3D matrix pool from leaf material as well as by screening the 3D matrix pool. The primers for screening the 3D matrix pool were based on the B73 AGPv01 sequence from 149957158 bp to 152977351 bp on chromosome 8 (www.maizesequence.org) and the primer program 3 (http://simgene.com/primer3; Rozen & Skaletsky, 2000). The parameters for the primer selection were a mean GC content of 50%, primer length of 20-25 bp, melting temperature between 70-90°C and amplicon length between 70-80 bp. Using the primer pairs in Table 7, the 3D pools were screened using RT-PCR. The values of the two parameters, namely melting temperature and CP value, are given for the BAC clone. 26 BAC clones could be identified for the selected region. All BAC clones were

isolated from the BAC bank and used as *E.coli* culture for DNA isolation and sequencing. Sequencing was carried out with a standard GS-FLX titanium kit (454 Life Sciences, USA). The sequence information obtained for the BAC clones 144N24, 119F13, 219G11, 86N21, 16B06, 84L18, 128D02, 25M23, 96H10, 19J24, 136A01, 75H06, 135F07 is summarized in Table 8.

Table 7: Primer pairs for detection of BAC clones from the RP4HTN BAC bank

BAC clone ID	Primer pair 1 Primer pair 2	Sequence, primer pair 1 (5'-3')	Melting temp, °C (50% of amplicon is single stranded) in genotype RP4HTN1	CP value (cycle when the exponential phase of the PCR begins)	Amplicon size (bp)
58A14	579ZMPM0_2F; 579ZMPM0_2R	125; 126	77.4	28.5	74
	579ZMPM0_4F; 579ZMPM0_4R	127; 128	80.96	26.52	77
144N24	579ZMPM0_5F; 579ZMPM0_5R	129; 130	79.09	27.09	76
	579ZMPM0_17F; 579ZMPM0_17R	131; 132	83.06	25.53	78
219G11	579ZMPM0_16F; 579ZMPM0_16R	133; 134	84.7	25.96	78
	579ZMPM0_25F; 579ZMPM0_25R	135; 136	78.95	26.09	80
119F13	579ZMPM0_22F; 579ZMPM0_22R	137; 138	80.89	25.98	73
	579ZMPM0_34F; 579ZMPM0_34R	139; 140	80.1	24.43	76
86N21	579ZMPM0_35F; 579ZMPM0_35R	141; 142	80.9	25.27	70
	579ZMPM0_38F; 579ZMPM0_38R	143; 144	83.86	26.01	71
16B6	579ZMPM0_37F; 579ZMPM0_37R	145; 146	79.22	25.71	80
	579ZMPM0_41F; 579ZMPM0_41R	147; 148	75.93	26.6	74
84L18	579ZMPM0_41F; 579ZMPM0_41R	149; 150	75.93	26.6	74
	579ZMPM0_46F; 579ZMPM0_46R	151; 152	80.54	25.68	78
128D2	579ZMPM0_180F; 579ZMPM0_180R2	153, 154	84.41	25.99	77
	579ZMPM0_48F; 579ZMPM0_48R	155; 156	83.96	25.33	77

25M23	579ZMPM0_48F; 579ZMPM0_48R	157; 158	83.96	25.33	77
	579ZMPM0_56F; 579ZMPM0_56R	159; 160	77	29.12	79
19J24	579ZMPM0_51F; 579ZMPM0_51R	161; 162	87.76	27.75	77
	579ZMPM0_199F; 579ZMPM0_199R	163; 164	82.49	26.56	79
96H10	579ZMPM0_63F; 579ZMPM0_63R	165; 166	85.78	26.08	63
	579ZMPM0_208F; 579ZMPM0_208R	167; 168	79.87	26.84	79
136A1	579ZMPM0_206F; 579ZMPM0_206R	169; 170	89.81	32.09	70
	579ZMPM0_86F; 579ZMPM0_86R	171; 172	81.81	30.07	71
135F7	579ZMPM0_79F; 579ZMPM0_79R	173; 174	75.82	25.43	72
	579ZMPM0_278F; 579ZMPM0_278R	175; 176	78.13	22.69	78
75H6	579ZMPM0_209F; 579ZMPM0_209R	177; 178	75.41	24.93	77
	579ZMPM0_86F; 579ZMPM0_86R	179; 180	81.81	30.07	71
117O2	579ZMPM0_87F; 579ZMPM0_87R	181; 182	81.89	27.7	76
	579ZMPM0_91F; 579ZMPM0_91R	183; 184	80.13	26.93	75
173H23	579ZMPM0_216F; 579ZMPM0_216R	185; 186	82.3	25.76	80
	579ZMPM0_95F; 579ZMPM0_95R	187; 188	79.5	24.97	73
118N19	579ZMPM0_99F; 579ZMPM0_99R	189; 190	76.84	24.69	80
	579ZMPM0_244F; 579ZMPM0_244R	191; 192	80.07	25.38	80
42L23	579ZMPM0_241F; 579ZMPM0_241R	193; 194	81.16	25.79	79
	579ZMPM0_109F; 579ZMPM0_109R	195; 196	77.89	25.28	74
112N13	579ZMPM0_109F; 579ZMPM0_109R	197; 198	77.89	25.28	74
	579ZMPM0_247F; 579ZMPM0_247R	199; 200	80.76	24.82	71
97K23	579ZMPM0_112F; 579ZMPM0_112R	201; 202	79.22	25.2	77
	579ZMPM0_125F; 579ZMPM0_125R	203; 204	83.44	28.17	74
18J17	579ZMPM0_253F; 579ZMPM0_253R	205; 206	77.5	32.34	71
	579ZMPM0_125F;	207;	83.44	28.17	74

	579ZMPM0_125R	208			
5M22	579ZMPM0_128F; 579ZMPM0_128R	209; 210	77.99	24.05	77
	579ZMPM0_136F; 579ZMPM0_136R	211; 212	78.65	26.46	78
146I6	579ZMPM0_131F; 579ZMPM0_131R	213; 214	76.58	26.54	78
	579ZMPM0_137F; 579ZMPM0_137R	215; 216	83.7	25.42	73
147O15	579ZMPM0_138F; 579ZMPM0_138R	217; 218	79.38	24.8	79
	579ZMPM0_147F; 579ZMPM0_147R	219; 220	79.63	26.77	80
88K17	579ZMPM0_145F; 579ZMPM0_145R	221; 222	81.51	27.61	76
	579ZMPM0_262F; 579ZMPM0_262R	223; 224	75.7	25.82	80
180G22	579ZMPM0_161F; 579ZMPM0_161R	225; 226	80.21	25.16	73
	579ZMPM0_265F; 579ZMPM0_265R	227; 228	79.3	24.7	79

Table 8: Sequence content of the 13 analysed BAC clones

BAC	# Reads	# Reads without *E.coli*	Sequence quantity in bp	Sequence quantity in bp without *E.coli*
144N24	10967	10849	3646226	3591222
119F13	17987	17847	6033910	5957456
219G11	32904	32484	10553629	10381924
86N21	39606	39106	12991596	12750077
16B06	36198	35849	12523123	12357036
84L18	50537	34162	15991645	10776458
128D02	15998	15847	5138442	5064677
25M23	22551	22416	7864493	7786402
96H10	7723	7614	2569604	2525488
19J24	21953	21775	7327364	7234315
136A01	31998	31724	10298869	10158900
75H06	24345	24121	8021727	7920125
135F07	29702	29484	9721708	9604010

B) BAC sequence assembly, annotation and candidate gene selection:

Production of a sequence scaffold: the BAC clones 144N24, 119F13, 219G11, 86N21, 16B06, 84L18, 128D02, 25M23, 19J24, 96H10, 136A01, 75H06, 137F07 were sequenced using the 454 technique (Margulies et al., 2005).

Automatic assembly of the raw sequences of the BAC clones was carried out with the "Newbler" software (454 runAssembly software, software release 2.3). The pro BAC sequence contigs produced in this manner were arranged correctly by manual analysis, in which the following techniques were applied:

1. Sequences of overlapping BACs could be roughly divided into overlapping and non-overlapping zones.
2. Sequence contigs from various overlapping BACs were compared in the overlapping zones. Approximately 20% of the sequence contigs could be arranged in this manner and gaps between them could be closed (for example when a contig of one BAC covered or connected to two contigs of the other BACs).
3. All sequence contigs were manually annotated. In this regard, initially only repetitive elements (transposons and retrotransposons, abbreviated to "TEs") were annotated. Since sequence gaps occur primarily in TEs, the TE annotation can help to correctly arrange sequence contigs. This means that when one end of a TE is on one sequence contig and the other end is on another, the two contigs can be ordered appropriately. In such cases, a sequence of 100 Ns is respectively inserted in order to fill the gaps between the sequence contigs. In addition, the information from TEs which are nested (i.e. TEs which have been inserted into other TEs) was used in order to arrange sequence contigs.
4. In some zones, neither information from overlapping BACs nor TE annotations could be used (this was the case, for example, in zones which were only covered by one BAC). Here, the sequence contigs were arbitrarily arranged and the gaps between them filled with sequences of 200 Ns.
5. Many of the TEs in the maize genome are "long terminal repeat" (LTR) retrotransposons which are flanked by long (1-2 kb) LTR sequences. These LTRs may be up to 100% identical. In some cases, then, raw sequences of the two LTRs were assembled into a consensus sequence (i.e. a copy of the LTR is not present in the assembly). In these cases, the sequence gaps were filled with the number of Ns which would correspond to the length of the second LTR.
6. Genes were manually annotated. To this end, the coding sequences (CDS) for the published B73 maize genome was used as the reference (http://www.maizegdb.org/gene_model.php). The CDS were aligned with the RP4HTN sequence using DotPlot (http://www.dotplot.org/) and so the positions of exons and

introns were determined. Candidate genes were on the one hand determined by describing their function (if publically known). On the other hand, the CDS of the resistant RP4HTN was compared with the vulnerable B73 AGPv02. If differences occurred, the respective gene was placed in the list of candidates. The prepared sequence had a length of 1'328'253 bp. The list of candidate genes is given in Table 1.

5. Molecular analysis of the candidate genes:

Expression analysis: the expression of the various candidate genes was tested on 21 day old (following sowing), uninfected plants (infection day = 0 dpi) and also at 36 days old with plants which had been infected and also which had not been infected with *H. turcicum* (15 days after infection = 15 dpi inf/ni).

RNA from the second leaf was extracted from the tested maize plants, reverse transcribed into cDNA and the expression was measured using qPCR. In each case the second leaf was harvested, frozen and the RNA was extracted, quantified and tested for quality and purity using the SV Total RNA Isolation System Kit (Z3100; Promega, Dübendorf, Switzerland), exactly as described (Brunner et al., 2011; Risk et al., 2012).
1 µg of RNA was reverse transcribed using the iScript RT Supermix (170-8841; Bio-Rad, Cressier, Switzerland) in a reaction volume of 20 µl, following the manufacturer's instructions. In order to exclude the possibility of contamination by genomic DNA (RT minus), at the same time, a reaction without adding the reverse transcriptase was incubated for each sample.
Quantitative Real Time PCR (RT-qPCR) was carried out in technical triplicate or duplicate in a reaction volume of 10 µl and with the addition of 4 µl of 1:10 diluted (10mM Tris HCL pH8, 0.1 mM EDTA) cDNA, 5 ul of SsoFast EvaGreen® Supermix (172-5201; Bio-Rad, Switzerland) and a primer concentration of 400 nM on the C1000 Touch Cycler (Bio-Rad, Switzerland). For amplification, the following program was used: 95° C for 30 seconds, followed by 40 cycles at 95°C for 3 seconds, then 60-63°C (see Table 2) for 5 seconds. To analyse the melting curve (exclusion of primer dimers), the PCR product was heated in 0.5°C steps from 65°C to 95°C. Amplification curves and melting curves were checked in the CFX Manager V 3.0 (Bio-Rad, Switzerland) program and the Cq values (quantification cycle) were exported into the qbasePLUS V 2.3 (Biogazelle, Zwijnaarde, Belgium) program to determine the relative expressions.

The primers for the candidate genes were determined with the aid of primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast/), in order, as far as possible, to exclude non-specific amplification on transcripts which were already known. In order to evaluate suitable amplicons, the PCR products were separated using agarose gel electrophoresis and their sizes were examined using isolated bands. Furthermore, amplicons from RP1HtN and also NILHtN as set out in Table 1were sequenced. In order to normalize the expression data, 1-3 reference genes (LUG, MEP, FPGS) were used (Manoli et al., 2012).
All of the candidate genes were expressed in the vulnerable genotype RP1 and in the resistant genotype RP1HTN. A differential expression between RP1 and RP1HTN could be demonstrated for RLK1. RLK1 in the vulnerable plants is expressed up to 4 times more strongly than in the resistant plants.

Table 9: Primer pairs for candidate genes, with their amplicon length in bp and the appropriate annealing temperature.

Gene name	Primer name	SEQ ID NO:	Primer sequences (F = Forward sequence; R = Reverse sequence)	Length (in bp) in RP1	Length (in bp) in RP1HTN	Annealing temperature
ZNF1	GH034 GH033	229 230	F: TGGTTGGTGTCGAAGCTGAG R: ATTTATCCCGGCCTTTGCAT	130	130	60°C
HYD	GH039 GH040	231 232	F: GATCTACAGGGAAGCCCACTGA R: TTTTTCCTTGAGGCAGTTATATGCT	74	74	60°C
RLK4	GH220 GH221	233 234	F: TTGTGCAGCGGAGGGAA R: CCAGGGCACCAGCAAGAAT	91	85	63°C
EXT1	GH168 GH170	235 236	F: CGACTACAAGACGCGTACC R: GGTGTCGATGGTGAGGTTC	103	103	60°C
RLK1	GH138 GH139	237 238	F: TATTGTTGGTGCTGTTGCCG R: GGACTCAATCCTTGTCCCTG	121	121	60°C

RET1	GH055 GH056	239 240	F: CGCTCGTTTGCCAGATAGC C R: CACGGTGTGTGCCAGTTTGT	165	165	60°C

TILLING population screening and detection of mutants: For the candidate genes (Table 1), screening of a TILLING population of 10000 plants which carries the introgression from Pepitilla on chromosome 8 in the region from 151688552 - 153139596 bp compared to the B73 reference AGPv02 (www.maizesequence.org) (RP3HTN1) and which exhibits a resistance to *Helminthosporium turcicum* was carried out. The mutations could be either silent nucleotide exchanges, amino acid exchanges or stop codons and acted to detect the function of the candidate genes.

Transformation: Candidate genes could, for example, be introduced into the vulnerable genotype A188 by means of *Agrobacterium tumefanciens*-conferred transformation. This genotype is characterized by AUDPC values of 702 in the GWH-Test (n=18 plants), so that a transformation with the resistance gene produces a clear resistance response.

6. Determination of race specificity: proof that HTN1 also confers race-non-specific resistance

Screening of the genotypes with the HtN gene was carried out at many locations in all of the infestation regions of Europe. Until now, this resistance has not been broken, so that we started with the assumption that until now they were not race-specific until a race N was found. Race 1 predominates in Europe, but in some individual regions, races 2 or 3 or a combination thereof could be detected (Hanekamp et al., 2013).

7. Phenotype test on other recombination plants

New recombination plants were tested for the QTL region and correlated with the phenotype data. The selection comprised plants which covered different regions of the target region. Recombinant plants could be identified which exhibited an introgression of the donor B37HTN1 between the markers MA0005 and MA0021 – marker region M7 and the markers

MA0013 and MA0022 – marker region M8, in the genetic background of RP1. Figure 4 shows that this region only comprises the three genes RLK4, EXT1 and RLK1. These recombination plants, which comprise the region M7 – M8, exhibit the resistance phenotype both in the field with artificial inoculation and also in the greenhouse test.

8. Identification of the resistance-conferring candidate gene

In order to identify the resistance-conferring gene, screening of the TILLING population of 10000 plants which exhibited the introgression from Pepitilla on chromosome 8 in the region from 151688552 - 153139596 bp compared with the B73 reference AGPv02 (http://www.genome.arizona.edu) (RP3HTN1) and a resistance to *Helminthosporium turcicum* was carried out.

Amplicons were developed for genes RLK4 and RLK1 (Table 10) and after amplification of the individual plant DNA of the TILLING population, these were sequenced by means of Sanger sequencing.

Table 10: Primer sequences for amplicons

Gene name	Primer name	SEQ ID NO:	Primer sequences (F = Forward sequence; R = Reverse sequence)	Length of amplicons (in bp)	Annealing temperature (°C)
RLK4	MA04916-6f MA04916-6r	247 248	F: TGTTTCAG GAATCACG CAACTGGA R: GCACCAC GCCATGAC CAACATC	399	60
RLK1	TG10013-10.f TG10013-11.r	249 250	F: CTTCCTAC AGAAGAAC GAGAGT R:	804	60

60

			TTCCTCAC GAGCTCTG TGGTC		

The amplicon sequences were evaluated using DNASTAR Lasergene software and base mutations were identified. Table 11 lists a selection of the mutations found.

Table 11: Identified mutations for the genes RLK4 and RLK1

Gene name	Mutation name	Position of the mutation in cDNA of RP3HTN1 (bp)	Base exchange	Position of mutation in protein sequence of RP3HTN1 (bp)	Amino acid exchange effect
RLK4	RLK4d	977 in SEQ ID NO: 3	G > A	326 in SEQ ID NO: 4	G > D
	RLK4f	1169 in SEQ ID NO: 3	C > T	390 in SEQ ID NO: 4	T > I
RLK1	RLK1b	1365 in SEQ ID NO: 1	G > A	455 in SEQ ID NO: 2	M > I
	RLK1d	1490 in SEQ ID NO: 1	G > A	497 in SEQ ID NO: 2	G > E

The identified mutants were self-fertilized in the greenhouse and seed stock was harvested from the homozygous plants with the wild type allele and mutation allele per mutation event for a phenotype test.

15 homozygous individual plants with a wild type allele and mutation allele for the mutants RLK1b, RLK1d, RLK4d and RLK4f and the controls RP1 and RP1HTN1 were inoculated with *H. turcicum* as described above, in a greenhouse. In the period from 11 to 25 days following inoculation, the infestation was determined every day. The AUDPC values for all of the test plants are summarized in Table 12. Changing the amino acid in the resistant parent of the RP3HTN1 TILLING population was expected to make the homozygous mutants vulnerable.

Table 12: AUDPC values for homozygous plants with wild type allele and mutation allele of the genes RLK1 and RLK4. In the phenotype column, 0 – 100 means resistant, 101 – 450 means heterozygous, and > 450 means vulnerable.

Mutant name	Alleles	AUDPC	Phenotype
RLK4d	Hom. Mutant	33.3	resistant
	Hom. Wild type	0.0	resistant
RLK4f	Hom. Mutant	46.7	resistant
	Hom. Wild type	96.7	resistant
RLK1b	Hom. Mutant	346.7	heterozygous
	Hom. Wild type	46.4	resistant
RLK1d	Hom. Mutant	406.7	heterozygous
	Hom. Wild type	83.3	resistant
RP1		1030.0	vulnerable
RP1HTN1		0.0	resistant

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Patent Claims

1. A maize plant into the genome of which a chromosome fragment from the donor Pepitilla has been integrated, wherein the chromosome fragment comprises an interval of the donor which exhibits donor alleles in accordance with the haplotype of Table 2 and exhibits a polynucleotide which confers resistance to *Helminthosporium turcicum* in the maize plant, and wherein the chromosome fragment

a) does not contain an interval of the donor between a marker in a first marker region which is flanked by the markers SYN14136 and PZE-108076510 and a marker in a second marker region which is flanked by the markers SYN24931 and PZE-108077560, and/or

b) does not contain an interval of the donor between a marker in a third marker region which is flanked by the markers PZE-108093423 and PZE-108093748 and a marker in a fourth marker region which is flanked by the markers MA0004 and MA0005, and/or

c) does not contain an interval of the donor between a marker in a fifth marker region which is flanked by the markers MA0006 and PZE-108097482 and a marker in a sixth marker region which is flanked by the markers PZE-108107671 and SYN4196.

2. The maize plant as claimed in claim 1, wherein the chromosome fragment furthermore does not contain an interval of the donor between a marker in the second marker region and a marker in the third marker region.

3. The maize plant as claimed in claim 1, wherein the flowering time for the maize plant and/or the silage yield of the maize plant corresponds to that of a comparative maize plant into the genome of which the chromosome fragment from the donor Pepitilla has not been integrated.

4. The maize plant as claimed in one of claims 1 to 3, wherein the polynucleotide comprises a nucleic acid molecule:

(a) which comprises a nucleotide sequence in accordance with SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 or 15,

(b) which comprises a nucleotide sequence with an identity of at least 80% with one of the nucleotide sequences in accordance with SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 or 15, preferably over the entire length of the sequence,

(c) which hybridizes with the complementary strand of a nucleic acid molecule in accordance with (a) or (b) under stringent conditions,

(d) which codes for a polypeptide with an amino acid sequence in accordance with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16,

(e) which codes for a polypeptide with an amino acid sequence which has at least 80% identity with one of the amino acid sequences in accordance with (d), or

(f) which comprises a part sequence of a nucleic acid in accordance with (a) to (e).

5. Cells, tissues or parts of the maize plant as claimed in one of claims 1 to 4.

6. Grain or seeds from the maize plant as claimed in one of claims 1 to 4.

7. A method for identifying a *Helminthosporium turcicum*-resistant maize plant, the genome of which has a chromosome fragment from the donor Pepitilla integrated therein, comprising detecting at least two alleles in the genome of the plant, wherein at least one allele is localized in a genomic segment which is flanked by a marker in the first marker region, the second marker region, the third marker region or the fourth marker region and by the polynucleotide which confers resistance against *Helminthosporium turcicum* in the maize plant, and wherein at least one allele is localized in a genomic segment which is flanked by the polynucleotide and by a marker in the sixth marker region or the fifth marker region.

8. A method for increasing the yield of a *H. turcicum*-resistant maize plant, the genome of which has a chromosome fragment from the donor Pepitilla integrated therein, wherein the chromosome fragment comprises an interval of the donor which exhibits donor alleles in accordance with the haplotype of Table 2 and comprises a polynucleotide which confers resistance against *Helminthosporium turcicum* in the maize plant, comprising the steps of removing

a) an interval of the donor between a marker in a first marker region which is flanked by the markers SYN14136 and PZE-108076510 and a marker in a second marker region which is flanked by the markers SYN24931 and PZE-108077560, and/or

b) an interval of the donor between a marker in a third marker region which is flanked by the markers PZE-108093423 and PZE-108093748 and a marker in a fourth marker region which is flanked by the markers MA0004 and MA0005, and/or

c) an interval of the donor between a marker in a fifth marker region which is flanked by the markers MA0006 and PZE-108097482 and a marker in a sixth marker region which is flanked by the markers PZE-108107671 and SYN4196.

9. An oligonucleotide which comprises a nucleic acid molecule with a nucleotide sequence selected from one of the SEQ ID NOs: 41-49, 53-100 or 229-250.

10. Use of an oligonucleotide which comprises a nucleic acid molecule with a nucleotide sequence selected from one of the SEQ ID NOs: 17-250, for the identification of a *Helminthosporium turcicum*-resistant maize plant.

11. A polynucleotide which comprises a nucleic acid molecule which,

(a) comprises a nucleotide sequence in accordance with SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 or 15,

(b) comprising a nucleotide sequence with an identity of at least 80% with one of the nucleotide sequences in accordance with SEQ ID NO: 1, 3, 5, 7, 9, 11, 13 or 15, preferably over the entire length of the sequence,

(c) which hybridizes with the complementary strand of a nucleic acid molecule in accordance with (a) or (b) under stringent conditions,

(d) which codes for a polypeptide with an amino acid sequence in accordance with SEQ ID NO: 2, 4, 6, 8, 10, 12, 14 or 16, or

(e) which codes for a polypeptide with an amino acid sequence which has at least 80% identity with one of the amino acid sequences in accordance with (d).

12. A vector or mobile genetic element comprising a polynucleotide as claimed in claim 11.

13. A transgenic plant cell comprising a polynucleotide as claimed in claim 11 as a transgene or a vector or mobile genetic element as claimed in claim 12.

14. A transgenic plant comprising a polynucleotide as claimed in claim 11 as a transgene or a vector or mobile genetic element as claimed in claim 12, or a plant cell as claimed in claim 13.

15. Grain or seeds from the plant as claimed in claim 14, wherein the grain or the seed comprises a polynucleotide as claimed in claim 11 as a transgene.

Figure 1

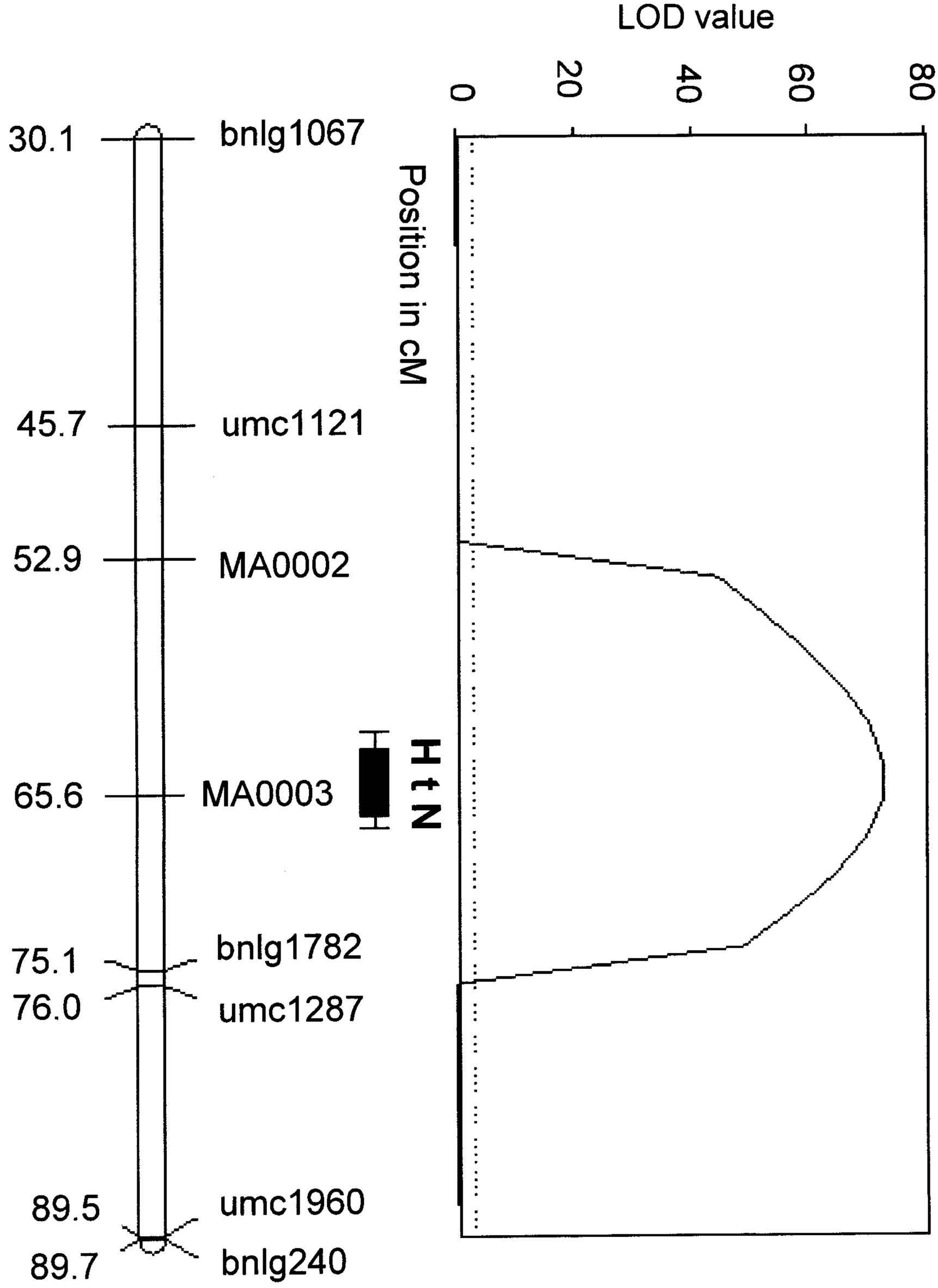
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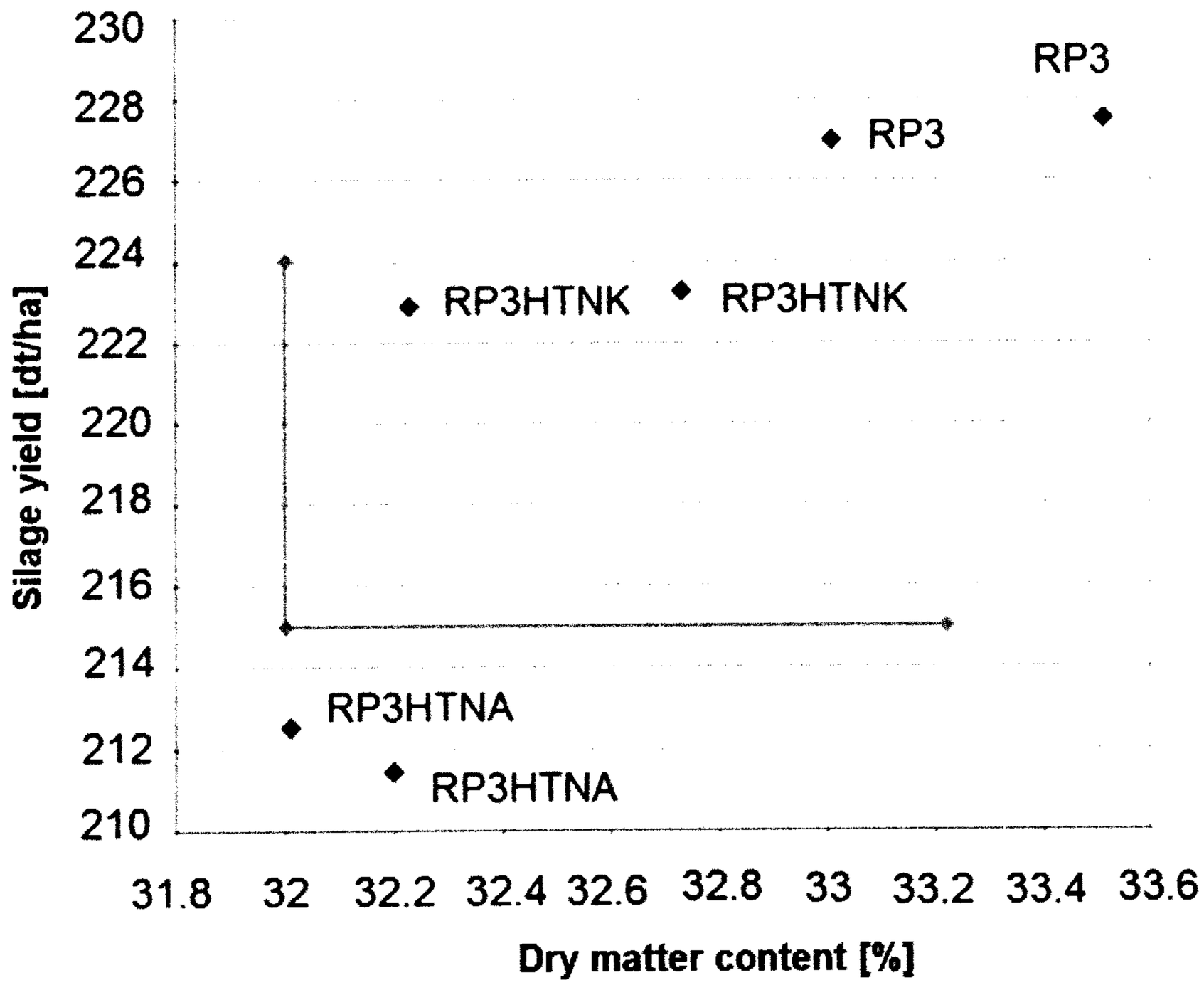
Figure 2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RP3HTNA
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Figure 3

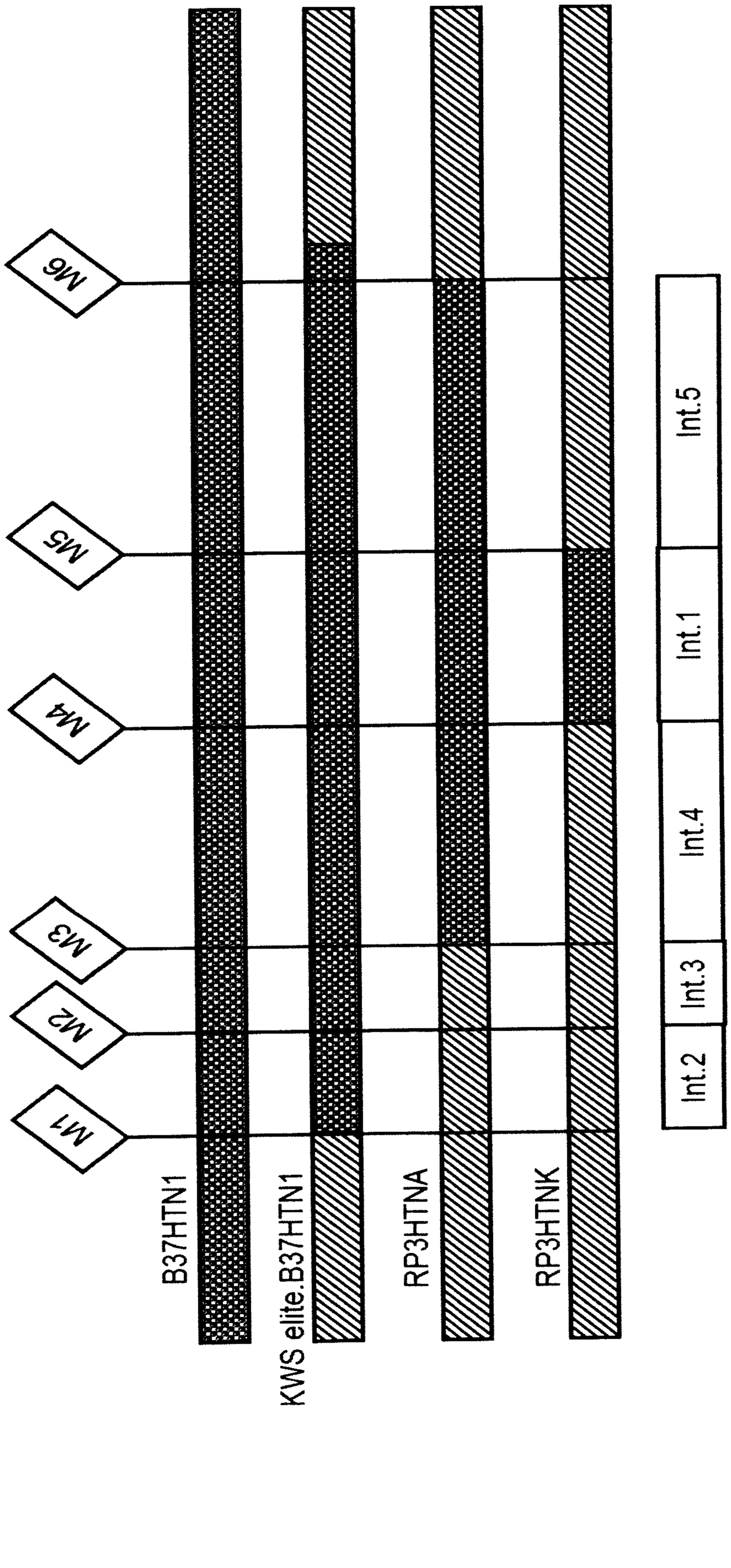
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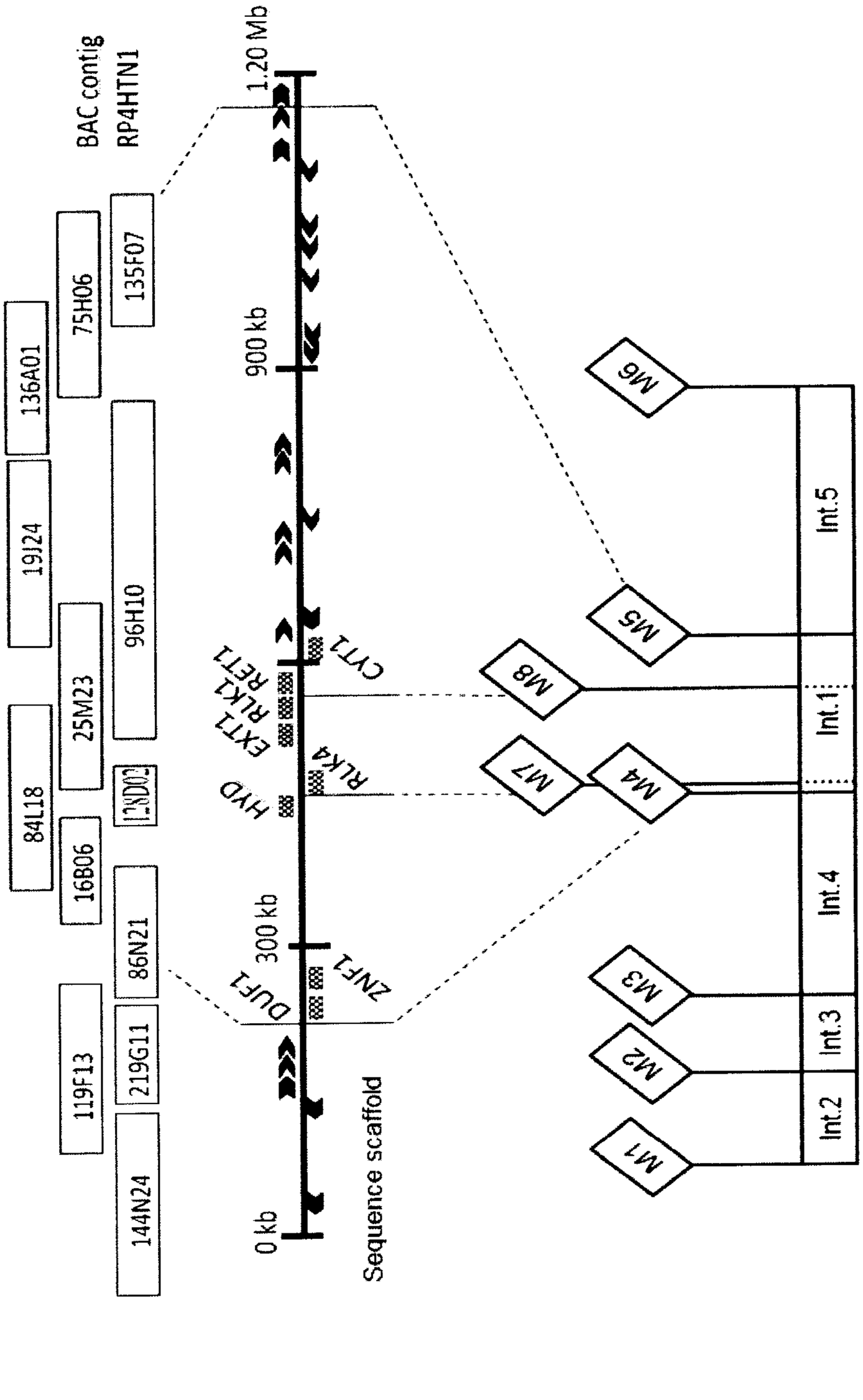
Figure 4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EXT1
RLK1
RET1
RLK4
HYD
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ZNF1
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