



(43) International Publication Date
28 April 2016 (28.04.2016)

(10) International Publication Number
WO 2016/064597 A1

(51) International Patent Classification:

A01H 5/10 (2006.01) *A01H 1/04* (2006.01)
C07K 14/415 (2006.01) *C12Q 1/68* (2006.01)
G01N 33/53 (2006.01) *G01N 33/00* (2006.01)

(21) International Application Number:

PCT/US2015/054858

(22) International Filing Date:

9 October 2015 (09.10.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/062,520 10 October 2014 (10.10.2014) US

(71) Applicant: **DOW AGROSCIENCES LLC** [US/US];
9330 Zionsville Road, Indianapolis, IN 46268 (US).

(72) Inventors: **CORAM, Tristan E.**; 9330 Zionsville Road,
Indianapolis, IN 46268 (US). **JAYNE, Susan M.**; 9330
Zionsville Road, Indianapolis, IN 46268 (US). **ALABED,
Diaa**; 13310 Golden Gate Drive East, Carmel, IN 46074
(US). **FOULK, Stephen**; 9330 Zionsville Road, Indiana-
polis, IN 46268 (US). **AGGARWAL, Rajat**; 9330 Zions-
ville Road, Indianapolis, IN 46268 (US). **DANIELS,
Natae**; 9330 Zionsville Road, Indianapolis, IN 46268
(US).

(74) Agent: **KRAUS, Eric**; Dow AgroSciences LLC, 9330
Zionsville Road, Indianapolis, Indiana 46268 (US).

(81) Designated States (*unless otherwise indicated, for every
kind of national protection available*): AE, AG, AL, AM,

AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every
kind of regional protection available*): ARIPO (BW, GH,
GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,
TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- *as to applicant's entitlement to apply for and be granted a
patent (Rule 4.17(ii))*
- *as to the applicant's entitlement to claim the priority of the
earlier application (Rule 4.17(iii))*

Published:

- *with international search report (Art. 21(3))*
- *with sequence listing part of description (Rule 5.2(a))*

(54) Title: GENETIC LOCI ASSOCIATED WITH CULTURE AND TRANSFORMATION IN MAIZE

(57) Abstract: This invention relates to methods for identifying maize plants having increased culturability and/or transformability. The methods use molecular markers to identify and to select plants with increased culturability and/or transformability or to identify and deselect plants with decreased culturability and/or transformability. Maize plants generated by the methods of the invention are also a feature of the invention.



WO 2016/064597 A1

GENETIC LOCI ASSOCIATED WITH CULTURE AND TRANSFORMATION IN MAIZE

This application claims a priority based on provisional application 62/062,520 which was filed in the U.S. Patent and Trademark Office on October 10, 2014, the entire disclosure of which is hereby incorporated by reference.

FIELD OF THE INVENTION

[0001] The present invention relates to methods useful in improving culturability and transformability in maize plants.

BACKGROUND OF THE INVENTION

[0002] Maize transformation has historically been practiced using genotypes that are amenable to tissue culture and gene delivery techniques. However, from a product development stand point, creation of transgenic events using these genotypes is undesirable because they are often agronomically poor. In many instances, however, plants with superior agronomic traits, such as elite lines, tend to exhibit poor culturing and transformability characteristics. Thus, there is an opportunity to develop elite lines that can be cultured and transformed at efficiencies suitable for routine use in maize transformation. Advantages of having such a line include faster and more precise event and trait evaluation, enhanced trait, yield, and regulatory trials, and faster product development.

[0003] A methodology for developing elite transformable inbred lines involves introgression of culturability and transformability traits from donor material into the desired elite line. Having genetic markers for these traits would be valuable for aiding this introgression. The culturability and transformability traits in the Hi-II and A188 germplasm have been well studied, and several molecular markers linked to these traits have been identified (Armstrong *et al.* 1992; Lowe and Chomet 2004; Lowe *et al.* 2006; Zhao *et al.* 2008). Hi-II, a novel line with improved culturability and transformability, was developed from an initial cross between A188 and B73 (Armstrong *et al.* 1991), wherein A188 was the donor of the culturability and transformability traits.

[0004] The present invention addresses the need for more culturable and transformable elite lines and provides improved methods to facilitate the development of new, agronomically superior corn lines with enhanced culturability and transformability.

SUMMARY OF THE INVENTION

[0005] In one embodiment, methods of identifying a maize plant that displays increased culturability and transformability, comprising detecting in germplasm of the maize plant at least one allele of a marker locus *are provided*. The marker locus is located *within* a *chromosomal* interval comprising and flanked by idp8516 and magi87535 (Bin 1.07); and at least one allele is associated with increased culturability and transformability. The marker locus can be selected from any of the following marker loci PZA01216.1, DAS-PZ-7146, DAS-PZ-12685, and magi17761, as well as any other marker that is linked to these markers. The marker locus can be found on chromosome 1, within the interval comprising and flanked by PZA01216.1 and magi17761, and comprises at least one allele that is associated with increased culturability and transformability.

[0006] In other embodiments of the invention, the marker locus is located within a chromosomal interval comprising and flanked by npi386a and gpm174b (Bin 4.04); and at least one allele is associated with increased culturability and transformability. The marker locus can be Mo17-100177, as well as any other marker that is linked to this marker, and comprises at least one allele that is associated with increased culturability and transformability.

[0007] In other embodiments of the invention, the marker locus is located within a chromosomal interval comprising and flanked by agr37b and nfa104 (Bin 4.05); and at least one allele is associated with increased culturability and transformability. The marker locus can be selected from the following marker loci DAS-PZ-5617, DAS-PZ-2343, PZA03203-2, Mo17-100291, PZA03409, and DAS-PZ-19188, as well as any other marker that is linked to these markers. The marker locus can be found on chromosome 4, within the interval comprising and flanked by DAS-PZ-5617 and DAS-PZ-19188, and comprises at least one allele that is associated with increased culturability and transformability.

[0008] In other embodiments of the invention, the marker locus is located within a chromosomal interval comprising and flanked by umc156a and pco061578 (Bin 4.06); and at least one allele is associated with increased culturability and transformability. The marker locus can be DAS-PZ-2043, as well as any other marker that is linked to this marker, and comprises at least one allele that is associated with increased culturability and transformability.

[0009] In other embodiments of the invention, the marker locus is located within a chromosomal interval comprising and flanked by php20608a and idp6638 (Bin 4.10); and at least one allele is associated with increased culturability and transformability. The marker locus can be DAS-PZ-20570, as well as any other marker that is linked to this marker, and comprises at least one allele that is associated with increased culturability and transformability.

[0010] In other embodiments of the invention, the marker locus is located within a chromosomal interval comprising and flanked by bn14.36 and umc1482 (Bin 5.04); and at least one allele is associated with increased culturability and transformability. The marker locus can be PZA02965, as well as any other marker that is linked to this marker, and comprises at least one allele that is associated with increased culturability and transformability.

[0011] In other embodiments of the invention, the marker locus is located within a chromosomal interval comprising and flanked by umc126a and idp8312 (Bin 5.06); and at least one allele is associated with increased culturability and transformability. The marker locus can be selected from the following marker loci Mo17-14519 and DAS-PZ-12236, as well as any other marker that is linked to these markers. The marker locus can be found on chromosome 5, within the interval comprising and flanked by Mo17-14519 and DAS-PZ-12236, and comprises at least one allele that is associated with increased culturability and transformability.

[0012] In other embodiments of the invention, the marker locus is located within a chromosomal interval comprising and flanked by bn19.11a and gpm609a (Bin 8.02); and at least one allele is associated with increased culturability and transformability. The marker locus can be magi52178, as well as any other marker that is linked to this marker, and

comprises at least one allele that is associated with increased culturability and transformability.

[0013] In other embodiments of the invention, the marker locus is located within a chromosomal interval comprising and flanked by wx1 and bnlgl209 (Bin 9.03); and at least one allele is associated with increased culturability and transformability. The marker locus can be DAS-PZ-366, as well as any other marker that is linked to this marker, and comprises at least one allele that is associated with increased culturability and transformability.

[0014] In some embodiments, the maize plant belongs to the Stiff Stalk heterotic group. Maize plants identified by this method are also of interest.

[0015] In another embodiment, methods for identifying maize plants with increased culturability and transformability by detecting a haplotype in the germplasm of the maize plant are provided. The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 1 within the interval comprising and flanked by idp8516 and magi87535 (Bin 1.07). The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 1 and are selected from the group consisting of PZA01216.1, DAS-PZ-7146, DAS-PZ-12685, and magi17761, as well as any other marker that is linked to these markers. The haplotype is associated with increased culturability and transformability.

[0016] In another embodiment, methods for identifying maize plants with increased culturability and transformability by detecting a haplotype in the germplasm of the maize plant are provided. The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 4 within the interval comprising and flanked by npi386a and gpm174b (Bin 4.04). The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 4 and are selected from the group consisting of Mo17-100177, as well as any other marker that is linked to this marker. The haplotype is associated with increased culturability and transformability.

[0017] In another embodiment, methods for identifying maize plants with increased culturability and transformability by detecting a haplotype in the germplasm of the maize plant are provided. The haplotype comprises alleles at one or more marker loci, wherein the

one or more marker loci are found on chromosome 4 within the interval comprising and flanked by agr37b and nfa104 (Bin 4.05). The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 4 and are selected from the group consisting of DAS-PZ-5617, DAS-PZ-2343, PZA03203-2, Mo17-100291, PZA03409, and DAS-PZ-19188, as well as any other marker that is linked to these markers. The haplotype is associated with increased culturability and transformability.

[0018] In another embodiment, methods for identifying maize plants with increased culturability and transformability by detecting a haplotype in the germplasm of the maize plant are provided. The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 4 within the interval comprising and flanked by umc156a and pco061578 (Bin 4.06). The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 4 and are selected from DAS-PZ-2043, as well as any other marker that is linked to this marker. The haplotype is associated with increased culturability and transformability.

[0019] In another embodiment, methods for identifying maize plants with increased culturability and transformability by detecting a haplotype in the germplasm of the maize plant are provided. The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 4 within the interval comprising and flanked by php20608a and idp6638 (Bin 4.10). The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 4 and are selected from DAS-PZ-20570, as well as any other marker that is linked to this marker. The haplotype is associated with increased culturability and transformability.

[0020] In another embodiment, methods for identifying maize plants with increased culturability and transformability by detecting a haplotype in the germplasm of the maize plant are provided. The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 5 within the interval comprising and flanked by bn14.36 and umc1482 (Bin 5.04). The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 5 and are selected from PZA02965, as well as any other marker that is linked to this marker. The haplotype is associated with increased culturability and transformability.

[0021] In another embodiment, methods for identifying maize plants with increased culturability and transformability by detecting a haplotype in the germplasm of the maize plant are provided. The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 5 within the interval comprising and flanked by umc126a and idp8312 (Bin 5.06). The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 5 and are selected from Mo17-14519 and DAS-PZ-12236, as well as any other marker that is linked to these markers. The haplotype is associated with increased culturability and transformability.

[0022] In another embodiment, methods for identifying maize plants with increased culturability and transformability by detecting a haplotype in the germplasm of the maize plant are provided. The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 8 within the interval comprising and flanked by bn19.11a and gpm609a (Bin 8.02). The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 8 and are selected from magi52178, as well as any other marker that is linked to this marker. The haplotype is associated with increased culturability and transformability.

[0023] In another embodiment, methods for identifying maize plants with increased culturability and transformability by detecting a haplotype in the germplasm of the maize plant are provided. The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 9 within the interval comprising and flanked by wx1 and bnlgl209 (Bin 9.03). The haplotype comprises alleles at one or more marker loci, wherein the one or more marker loci are found on chromosome 9 and are selected from DAS-PZ-366, as well as any other marker that is linked to this marker. The haplotype is associated with increased culturability and transformability.

[0024] In a further embodiment, methods of selecting plants with increased culturability and transformability are provided. In one aspect, a first maize plant is obtained that has at least one allele of a marker locus wherein the allele is associated with increased culturability and transformability. The marker locus can be found on chromosome 1, within the interval comprising and flanked by idp8516 and magi87535 (Bin 1.07). The first maize plant can be crossed to a second maize plant, and the progeny resulting from the cross can be evaluated for the allele of the first maize plant. Progeny plants that possess the allele from

the first maize plant can be selected as having increased culturability and transformability. Maize plants selected by this method are also of interest.

[0025] In a further embodiment, methods of selecting plants with increased culturability and transformability are provided. In one aspect, a first maize plant is obtained that has at least one allele of a marker locus wherein the allele is associated with increased culturability and transformability. The marker locus can be found on chromosome 4, within the interval comprising and flanked by *npi386a* and *gpm174b* (Bin 4.04). The first maize plant can be crossed to a second maize plant, and the progeny resulting from the cross can be evaluated for the allele of the first maize plant. Progeny plants that possess the allele from the first maize plant can be selected as having increased culturability and transformability. Maize plants selected by this method are also of interest.

[0026] In a further embodiment, methods of selecting plants with increased culturability and transformability are provided. In one aspect, a first maize plant is obtained that has at least one allele of a marker locus wherein the allele is associated with increased culturability and transformability. The marker locus can be found on chromosome 4, within the interval comprising and flanked by *agrr37b* and *nfa104* (Bin 4.05). The first maize plant can be crossed to a second maize plant, and the progeny resulting from the cross can be evaluated for the allele of the first maize plant. Progeny plants that possess the allele from the first maize plant can be selected as having increased culturability and transformability. Maize plants selected by this method are also of interest.

[0027] In a further embodiment, methods of selecting plants with increased culturability and transformability are provided. In one aspect, a first maize plant is obtained that has at least one allele of a marker locus wherein the allele is associated with increased culturability and transformability. The marker locus can be found on chromosome 4, within the interval comprising and flanked by *umc156a* and *pco061578* (Bin 4.06). The first maize plant can be crossed to a second maize plant, and the progeny resulting from the cross can be evaluated for the allele of the first maize plant. Progeny plants that possess the allele from the first maize plant can be selected as having increased culturability and transformability. Maize plants selected by this method are also of interest.

[0028] In a further embodiment, methods of selecting plants with increased culturability and transformability are provided. In one aspect, a first maize plant is obtained

that has at least one allele of a marker locus wherein the allele is associated with increased culturability and transformability. The marker locus can be found on chromosome 4, within the interval comprising and flanked by php20608a and idp6638 (Bin 4.10). The first maize plant can be crossed to a second maize plant, and the progeny resulting from the cross can be evaluated for the allele of the first maize plant. Progeny plants that possess the allele from the first maize plant can be selected as having increased culturability and transformability. Maize plants selected by this method are also of interest.

[0029] In a further embodiment, methods of selecting plants with increased culturability and transformability are provided. In one aspect, a first maize plant is obtained that has at least one allele of a marker locus wherein the allele is associated with increased culturability and transformability. The marker locus can be found on chromosome 5, within the interval comprising and flanked by bnl4.36 and umc1482 (Bin 5.04). The first maize plant can be crossed to a second maize plant, and the progeny resulting from the cross can be evaluated for the allele of the first maize plant. Progeny plants that possess the allele from the first maize plant can be selected as having increased culturability and transformability. Maize plants selected by this method are also of interest.

[0030] In a further embodiment, methods of selecting plants with increased culturability and transformability are provided. In one aspect, a first maize plant is obtained that has at least one allele of a marker locus wherein the allele is associated with increased culturability and transformability. The marker locus can be found on chromosome 5, within the interval comprising and flanked by umc126a and idp8312 (Bin 5.06). The first maize plant can be crossed to a second maize plant, and the progeny resulting from the cross can be evaluated for the allele of the first maize plant. Progeny plants that possess the allele from the first maize plant can be selected as having increased culturability and transformability. Maize plants selected by this method are also of interest.

[0031] In a further embodiment, methods of selecting plants with increased culturability and transformability are provided. In one aspect, a first maize plant is obtained that has at least one allele of a marker locus wherein the allele is associated with increased culturability and transformability. The marker locus can be found on chromosome 8, within the interval comprising and flanked by bnl9.11a and gpm609a (Bin 8.02). The first maize plant can be crossed to a second maize plant, and the progeny resulting from the cross can be

evaluated for the allele of the first maize plant. Progeny plants that possess the allele from the first maize plant can be selected as having increased culturability and transformability. Maize plants selected by this method are also of interest.

[0032] In a further embodiment, methods of selecting plants with increased culturability and transformability are provided. In one aspect, a first maize plant is obtained that has at least one allele of a marker locus wherein the allele is associated with increased culturability and transformability. The marker locus can be found on chromosome 9, within the interval comprising and flanked by wx1 and bnlgl209 (Bin 9.03). The first maize plant can be crossed to a second maize plant, and the progeny resulting from the cross can be evaluated for the allele of the first maize plant. Progeny plants that possess the allele from the first maize plant can be selected as having increased culturability and transformability. Maize plants selected by this method are also of interest.

BRIEF DESCRIPTION OF FIGURES AND SEQUENCE LISTINGS

[0033] The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application. The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in Nucleic Acids Research 13:3021-3030 (1985) and in the Biochemical Journal 219 (No. 2): 345-373 (1984) which are herein incorporated by reference in their entirety. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

[0034] SEQ ID NO: 1 contains the DAS-PZ-7146 SNP and flanking sequence.

[0035] SEQ ID NO: 2 contains the DAS-PZ-12685 SNP and flanking sequence.

[0036] SEQ ID NO: 3 contains the Mo17-100177 SNP and flanking sequence.

[0037] SEQ ID NO: 4 contains the DAS-PZ-5617 SNP and flanking sequence.

[0038] SEQ ID NO: 5 contains the DAS-PZ-2343 SNP and flanking sequence.

[0039] SEQ ID NO: 6 contains the Mo17-100291 SNP and flanking sequence.

[0040] SEQ ID NO: 7 contains the DAS-PZ-19188 SNP and flanking sequence.

[0041] SEQ ID NO: 8 contains the DAS-PZ-2043 SNP and flanking sequence.

[0042] SEQ ID NO: 9 contains the DAS-PZ-20570 SNP and flanking sequence.

[0043] SEQ ID NO: 10 contains the Mo17-14519 SNP and flanking sequence.

[0044] SEQ ID NO: 11 contains the DAS-PZ-12236 SNP and flanking sequence.

[0045] SEQ ID NO: 12 contains the DAS-PZ-366 SNP and flanking sequence.

[0046] SEQ ID NO: 13 is a forward PCR primer for the amplification of PZA01216.1.

[0047] SEQ ID NO: 14 is a forward PCR primer for the amplification of PZA01216.1.

[0048] SEQ ID NO: 15 is a reverse PCR primer for the amplification of PZA01216.1.

[0049] SEQ ID NO: 16 is a forward PCR primer for the amplification of SEQ ID NO: 1.

[0050] SEQ ID NO: 17 is a forward PCR primer for the amplification of SEQ ID NO: 1.

[0051] SEQ ID NO: 18 is a reverse PCR primer for the amplification of SEQ ID NO: 1.

[0052] SEQ ID NO: 19 is a forward PCR primer for the amplification of SEQ ID NO: 2.

[0053] SEQ ID NO: 20 is a forward PCR primer for the amplification of SEQ ID NO: 2.

[0054] SEQ ID NO: 21 is a reverse PCR primer for the amplification of SEQ ID NO: 2.

[0055] SEQ ID NO: 22 is a forward PCR primer for the amplification of magi17761.

[0056] SEQ ID NO: 23 is a forward PCR primer for the amplification of magi17761.

[0057] SEQ ID NO: 24 is a reverse PCR primer for the amplification of magi17761.

[0058] SEQ ID NO: 25 is a forward PCR primer for the amplification of SEQ ID NO: 3.

[0059] SEQ ID NO: 26 is a forward PCR primer for the amplification of SEQ ID NO: 3.

[0060] SEQ ID NO: 27 is a reverse PCR primer for the amplification of SEQ ID NO: 3.

[0061] SEQ ID NO: 28 is a forward PCR primer for the amplification of SEQ ID NO: 4.

4. [0062] SEQ ID NO: 29 is a forward PCR primer for the amplification of SEQ ID NO:
4. [0063] SEQ ID NO: 30 is a reverse PCR primer for the amplification of SEQ ID NO:
5. [0064] SEQ ID NO: 31 is a forward PCR primer for the amplification of SEQ ID NO:
5. [0065] SEQ ID NO: 32 is a forward PCR primer for the amplification of SEQ ID NO:
5. [0066] SEQ ID NO: 33 is a reverse PCR primer for the amplification of SEQ ID NO:
2. [0067] SEQ ID NO: 34 is a forward PCR primer for the amplification of PZA03203-
2. [0068] SEQ ID NO: 35 is a forward PCR primer for the amplification of PZA03203-
- [0069] SEQ ID NO: 36 is a reverse PCR primer for the amplification of PZA03203-2.
- [0070] SEQ ID NO: 37 is a forward PCR primer for the amplification of SEQ ID NO:
6. [0071] SEQ ID NO: 38 is a forward PCR primer for the amplification of SEQ ID NO:
6. [0072] SEQ ID NO: 39 is a reverse PCR primer for the amplification of SEQ ID NO:
6. [0073] SEQ ID NO: 40 is a forward PCR primer for the amplification of PZA03409.
- [0074] SEQ ID NO: 41 is a forward PCR primer for the amplification of PZA03409.
- [0075] SEQ ID NO: 42 is a reverse PCR primer for the amplification of PZA03409.
- [0076] SEQ ID NO: 43 is a forward PCR primer for the amplification of SEQ ID NO:
7. [0077] SEQ ID NO: 44 is a forward PCR primer for the amplification of SEQ ID NO:
7. [0078] SEQ ID NO: 45 is a reverse PCR primer for the amplification of SEQ ID NO:
- 7.

8. [0079] SEQ ID NO: 46 is a forward PCR primer for the amplification of SEQ ID NO:
8. [0080] SEQ ID NO: 47 is a forward PCR primer for the amplification of SEQ ID NO:
8. [0081] SEQ ID NO: 48 is a reverse PCR primer for the amplification of SEQ ID NO:
9. [0082] SEQ ID NO: 49 is a forward PCR primer for the amplification of SEQ ID NO:
9. [0083] SEQ ID NO: 50 is a forward PCR primer for the amplification of SEQ ID NO:
9. [0084] SEQ ID NO: 51 is a reverse PCR primer for the amplification of SEQ ID NO:
- [0085] SEQ ID NO: 52 is a forward PCR primer for the amplification of PZA02965.
- [0086] SEQ ID NO: 53 is a forward PCR primer for the amplification of PZA02965.
- [0087] SEQ ID NO: 54 is a reverse PCR primer for the amplification of PZA02965.
10. [0088] SEQ ID NO: 55 is a forward PCR primer for the amplification of SEQ ID NO:
10. [0089] SEQ ID NO: 56 is a forward PCR primer for the amplification of SEQ ID NO:
10. [0090] SEQ ID NO: 57 is a reverse PCR primer for the amplification of SEQ ID NO:
11. [0091] SEQ ID NO: 58 is a forward PCR primer for the amplification of SEQ ID NO:
11. [0092] SEQ ID NO: 59 is a forward PCR primer for the amplification of SEQ ID NO:
11. [0093] SEQ ID NO: 60 is a reverse PCR primer for the amplification of SEQ ID NO:
- [0094] SEQ ID NO: 61 is a forward PCR primer for the amplification of magi52178.
- [0095] SEQ ID NO: 62 is a forward PCR primer for the amplification of magi52178.
- [0096] SEQ ID NO: 63 is a reverse PCR primer for the amplification of magi52178.

[0097] SEQ ID NO: 64 is a forward PCR primer for the amplification of SEQ ID NO:
12.

[0098] SEQ ID NO: 65 is a forward PCR primer for the amplification of SEQ ID NO:
12.

[0099] SEQ ID NO: 66 is a reverse PCR primer for the amplification of SEQ ID NO:
12.

DETAILED DESCRIPTION OF THE INVENTION

[00100] The present invention provides methods for identifying and selecting maize plants with increased culturability and transformability. The following definitions are provided as an aid to understand the invention.

[00101] The term "allele" refers to one of two or more different nucleotide sequences that occur at a specific locus.

[00102] An "amplicon" is amplified nucleic acid, e.g., a nucleic acid that is produced by amplifying a template nucleic acid by any available amplification method (e.g., PCR, LCR, transcription, or the like).

[00103] The term "amplifying" in the context of nucleic acid amplification is any process whereby additional copies of a selected nucleic acid (or a transcribed form thereof) are produced. Typical amplification methods include various polymerase based replication methods, including the polymerase chain reaction (PCR), ligase mediated methods such as the ligase chain reaction (LCR) and RNA polymerase based amplification (e.g., by transcription) methods.

[00104] The term "assemble" applies to BACs and their propensities for coming together to form contiguous stretches of DNA. A BAC "assembles" to a contig based on sequence alignment, if the BAC is sequenced, or via the alignment of its BAC fingerprint to the fingerprints of other BACs. The assemblies can be found using the Maize Genome Browser, which is publicly available on the internet.

[00105] An allele is "associated with" a trait when it is linked to it and when the presence of the allele is an indicator that the desired trait or trait form will occur in a plant comprising the allele.

[00106] A "BAC", or bacterial artificial chromosome, is a cloning vector derived from the naturally occurring F factor of *Escherichia coli*. BACs can accept large inserts of DNA sequence. In maize, a number of BACs, or bacterial artificial chromosomes, each containing a large insert of maize genomic DNA, have been assembled into contigs (overlapping contiguous genetic fragments, or "contiguous DNA").

[00107] "Backcrossing" refers to the process whereby hybrid progeny are repeatedly crossed back to one of the parents. In a backcrossing scheme, the "donor" parent refers to the parental plant with the desired gene or locus to be introgressed. The "recipient" parent (used one or more times) or "recurrent" parent (used two or more times) refers to the parental plant into which the gene or locus is being introgressed. For example, see Ragot, M. et al. (1995) Marker-assisted backcrossing: a practical example, in *Techniques et Utilisations des Marqueurs Moléculaires Les Colloques*, Vol. 72, pp. 45-56, and Openshaw et al., (1994) Marker-assisted Selection in Backcross Breeding, *Analysis of Molecular Marker Data*, pp. 41-43. The initial cross gives rise to the F1 generation: the term "BC1" then refers to the second use of the recurrent parent, "BC2" refers to the third use of the recurrent parent, and so on.

[00108] "Bins" refer to chromosomal segments. Genetic maps are divided into 100 segments, called bins, of approximately 20 centiMorgans between two fixed Core Markers (Gardiner et al. 1993 *Genetics* 134:917-930). The segments are designated with the chromosome number followed by a two-digit decimal (e.g., 1.00, 1.01, 1.02, etc). A bin is the interval that includes all loci from the leftmost or top Core Marker to the next Core Marker. Placement of a locus to a bin is dependent on the precision of mapping data, and increases in certainty as markers increase in number or populations increase. Whenever the placement is statistically uncertain, 'Bin1' refers to the beginning of a range, and 'Bin2' refers to the end of the range.

[00109] A centimorgan ("cM") is a unit of measure of recombination frequency. One cM is equal to a 1% chance that a marker at one genetic locus will be separated from a marker at a second locus due to crossing over in a single generation.

[00110] "Chromosomal interval" designates a contiguous linear span of genomic DNA that resides in a plant on a single chromosome. The genetic elements or genes located on a single chromosomal interval are physically linked. The size of a chromosomal

interval is not particularly limited. In some aspects, the genetic elements located within a single chromosomal interval are genetically linked, typically with a genetic recombination distance of, for example, less than or equal to 20 cM, or alternatively, less than or equal to 10 cM. That is, two genetic elements within a single chromosomal interval undergo recombination at a frequency of less than or equal to 20% or 10%. The term "chromosomal interval" designates any and all intervals defined by any of the markers set forth in this invention. Chromosomal intervals that correlate with increased culturability and transformability are provided.

[00111] The term "complement" refers to a nucleotide sequence that is complementary to a given nucleotide sequence, i.e., the sequences are related by the base-pairing rules.

[00112] The term "contiguous DNA" refers to overlapping contiguous genetic fragments.

[00113] The term "Core Bin Marker (CBM)" refers to fixed core markers that define the boundaries of chromosomal bins.

[00114] The term "crossed" or "cross" means the fusion of gametes via pollination to produce progeny (e.g., cells, seeds or plants). The term encompasses both sexual crosses (the pollination of one plant by another) and selfing (self-pollination, e.g., when the pollen and ovule are from the same plant). The term "crossing" refers to the act of fusing gametes via pollination to produce progeny.

[00115] A "favorable allele" is the allele at a particular locus that confers, or contributes to, a desirable phenotype, e.g., increased culturability and transformability, or alternatively, is an allele that allows the identification of plants with decreased culturability and transformability that can be removed from a breeding program or planting ("counterselection"). A favorable allele of a marker is a marker allele that segregates with the favorable phenotype, or alternatively, segregates with the unfavorable plant phenotype, therefore providing the benefit of identifying plants.

[00116] "Fragment" is intended to mean a portion of a nucleotide sequence. Fragments can be used as hybridization probes or PCR primers using methods disclosed herein.

[00117] A "genetic map" is a description of genetic linkage relationships among loci on one or more chromosomes (or chromosomes) within a given species, generally depicted in a diagrammatic or tabular form. For each genetic map, distances between loci are measured by the recombination frequencies between them, and recombinations between loci can be detected using a variety of molecular genetic markers (also called molecular markers). A genetic map is a product of the mapping population, types of markers used, and the polymorphic potential of each marker between different populations. The order and genetic distances between loci can differ from one genetic map to another. However, information can be correlated from one map to another using a general framework of common markers. One of ordinary skill in the art can use the framework of common markers to identify the positions of markers and other loci of interest on each individual genetic map.

[00118] The term "Genetic Marker" shall refer to any type of nucleic acid based marker, including but not limited to, Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeat (SSR) Random Amplified Polymorphic DNA (RAPD), Cleaved Amplified Polymorphic Sequences (CAPS) (Rafalski and Tingey, 1993, Trends in Genetics 9:275-280), Amplified Fragment Length Polymorphism (AFLP) (Vos et al, 1995, Nucleic Acids Res. 23:4407-4414), Single Nucleotide Polymorphism (SNP) (Brookes, 1999, Gene 234:177-186), Sequence Characterized Amplified Region (SCAR) (Pecan and Micheltore, 1993, Theor. Appl. Genet, 85:985-993), Sequence Tagged Site (STS) (Onozaki et al. 2004, Euphytica 138:255-262), Single Stranded Conformation Polymorphism (SSCP) (Orita et al., 1989, Proc Natl Acad Sci USA 86:2766-2770), Inter-Simple Sequence Repeat (ISR) (Blair et al. 1999, Theor. Appl. Genet. 98:780-792), Inter-Retrotransposon Amplified Polymorphism (IRAP), Retrotransposon-Microsatellite Amplified Polymorphism (REMAP) (Kalendar et al., 1999, Theor. Appl. Genet 98:704-711), an RNA cleavage product (such as a Lynx tag), and the like.

[00119] "Genetic recombination frequency" is the frequency of a crossing over event (recombination) between two genetic loci. Recombination frequency can be observed by following the segregation of markers and/or traits following meiosis.

[00120] "Genome" refers to the total DNA, or the entire set of genes, carried by a chromosome or chromosome set.

[00121] The term "genotype" is the genetic constitution of an individual (or group of individuals) at one or more genetic loci, as contrasted with the observable trait (the phenotype). Genotype is defined by the allele(s) of one or more known loci that the individual has inherited from its parents. The term genotype can be used to refer to an individual's genetic constitution at a single locus, at multiple loci, or, more generally, the term genotype can be used to refer to an individual's genetic make-up for all the genes in its genome.

[00122] "Germplasm" refers to genetic material of or from an individual (e.g., a plant), a group of individuals (e.g., a plant line, variety or family), or a clone derived from a line, variety, species, or culture. The germplasm can be part of an organism or cell, or can be separate from the organism or cell. In general, germplasm provides genetic material with a specific molecular makeup that provides a physical foundation for some or all of the hereditary qualities of an organism or cell culture. As used herein, germplasm includes cells, seed or tissues from which new plants may be grown, or plant parts, such as leaves, stems, pollen, or cells that can be cultured into a whole plant.

[00123] A "haplotype" is the genotype of an individual at a plurality of genetic loci, i.e. a combination of alleles. Typically, the genetic loci described by a haplotype are physically and genetically linked, i.e., on the same chromosome segment. The term "haplotype" can refer to sequence, polymorphisms at a particular locus, such as a single marker locus, or sequence polymorphisms at multiple loci along a chromosomal segment in a given genome. The former can also be referred to as "marker haplotypes" or "marker alleles", while the latter can be referred to as "long-range haplotypes".

[00124] A "heterotic group" comprises a set of genotypes that perform well when crossed with genotypes from a different heterotic group (Hallauer et al. (1998) Corn breeding, p. 463-564. In G. F. Sprague and J. W. Dudley (ed) Corn and corn improvement). Inbred lines are classified into heterotic groups, and are further subdivided into families within a heterotic group, based on several criteria such as pedigree, molecular marker-based associations, and performance in hybrid combinations (Smith et al. (1990) Theor. Appl. Gen. 80:833-840). The two most widely used heterotic groups in the United States are referred to as "Iowa Stiff Stalk Synthetic" (BSSS) and "Lancaster" or "Lancaster Sure Crop" (sometimes referred to as NSS, or non-Stiff Stalk).

[00125] The term "heterozygous" means a genetic condition wherein different alleles reside at corresponding loci on homologous chromosomes.

[00126] The term "homozygous" means a genetic condition wherein identical alleles reside at corresponding loci on homologous chromosomes.

[00127] "Hybridization" or "nucleic acid hybridization" refers to the pairing of complementary RNA and DNA strands as well as the pairing of complementary DNA single strands.

[00128] The term "hybridize" means to form base pairs between complementary regions of nucleic acid strands.

[00129] An "IBM genetic map" refers to any of following maps: IBM, IBM2, IBM2 neighbors, IBM2 FPC0507, IBM2 2004 neighbors, IBM2 2005 neighbors, IBM2 2005 neighbors frame, or IBM2 2008 neighbors. IBM genetic maps are based on a B73 X Mo17 population in which the progeny from the initial cross were random rate for multiple generations prior to constructing recombinant inbred lines for mapping. Newer versions reflect the addition of genetic and BAC mapped clones as well as enhanced map refinement due to the incorporation of information obtained from other genetic maps.

[00130] The term "indel" refers to an insertion or deletion, wherein one line may be referred to as having an insertion relative to a second line, or the second line may be referred to as having a deletion relative to the first line.

[00131] The term "introgression" or "introgressing" refers to the transmission of a desired allele of a genetic locus from one genetic background to another. For example, introgression of a desired allele at a specified locus can be transmitted to at least one progeny via a sexual cross between two parents of the same species, where at least one of the parents has the desired allele in its genome. Alternatively, for example, transmission of an allele can occur by recombination between two donor genomes, e.g., in a fused protoplast, where at least one of the donor protoplasts has the desired allele in its genome. The desired allele can be, e.g., a selected allele of a marker, a QTL, a transgene, or the like. In any case, offspring comprising the desired allele can be repeatedly backcrossed to a line having a desired genetic background and selected for the desired allele, to result in the allele becoming fixed in a selected genetic background. For example, the chromosome 1 locus described herein may be

introgressed into a recurrent parent that has problematic green snap. The recurrent parent line with the introgressed gene or locus then has increased culturability and transformability.

[00132] As used herein, the term "linkage" is used to describe the degree with which one marker locus is associated with another marker locus or some other locus (for example, a culturability and transformability locus). The linkage relationship between a molecular marker and a phenotype is given as a "probability" or "adjusted probability". Linkage can be expressed as a desired limit or range. For example, in some embodiments, any marker is linked (genetically and physically) to any other marker when the markers are separated by less than 50, 40, 30, 25, 20, or 15 map units for cM). In some aspects, it is advantageous to define a bracketed range of linkage, for example, between 10 and 20 cM, between 10 and 30 cM, or between 10 and 40 cM. The more closely a marker is linked to a second locus, the better an indicator for the second locus that marker becomes. Thus, "closely linked loci" such as a marker locus and a second locus display an inter-locus recombination frequency of 10% or less, preferably about 9% or less, still more preferably about 8% or less, yet more preferably about 7% or less, still more preferably about 6% or less, yet more preferably about 5% or less, still more preferably about 4% or less, yet more preferably about 3% or less, and still more preferably about 2% or less. In highly preferred embodiments, the relevant loci display a recombination frequency of about 1% or less, e.g., about 0.75% or less, more preferably about 0.5% or less, or yet more preferably about 0.25% or less. Two loci that are localized to the same chromosome, and at such a distance that recombination between the two loci occurs at a frequency of less than 10 (e.g., about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.75%, 0.5%, 0.25%, or less) are also said to be "proximal to" each other. Since one cM is the distance between two markers that show a 1% recombination frequency, any marker is closely linked (genetically and physically) to any other marker that is in close proximity, e.g., at or less than 10 cM distant. Two closely linked markers on the same chromosome can be positioned 9, 8, 7, 6, 5, 4, 3, 2, 1, 0.75, 0.5 or 0.25 cM or less from each other.

[00133] The term "linkage disequilibrium" refers to a non-random segregation of genetic loci or traits for both). In either case, linkage disequilibrium implies that the relevant loci are within sufficient physical proximity along a length of a chromosome so that they segregate together with greater than random (i.e., non-random) frequency (in the case of

co-segregating traits, the loci that underlie the traits are in sufficient proximity to each other). Markers that show linkage disequilibrium are considered linked. Linked loci cosegregate more than 50% of the time, e.g., from about 51% to about 100% of the time. In other words, two markers that cosegregate have a recombination frequency of less than 50% (and by definition, are separated by less than 50 cM on the same chromosome.) As used herein, linkage can be between two markers, or alternatively between a marker and a phenotype. A marker locus can be "associated with" (linked to) a trait, e.g., decreased green snap. The degree of linkage of a molecular marker to a phenotypic trait is measured, e.g. as a statistical probability of co-segregation of that molecular marker with the phenotype.

[00134] Linkage disequilibrium is most commonly assessed using the measure r^2 , which is calculated using the formula described by Hill, W. G. and Robertson, A, Theor Appl. Genet 38:226-231 (1988). When $r^2=1$, complete LD exists between the two marker loci, meaning that the markers have not been separated by recombination and have the same allele frequency. Values for r^2 above 1/3 indicate sufficiently strong LD to be useful for mapping (Ardlie et al., Nature Reviews Genetics 3:299-309 (2002)). Hence, alleles are in linkage disequilibrium when r^2 values between pairwise marker loci are greater than or equal to 0.33, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0.

[00135] As used herein, "linkage equilibrium" describes a situation where two markers independently segregate, i.e., sort among progeny randomly. Markers that show linkage equilibrium are considered unlinked (whether or not they lie on the same chromosome).

[00136] The "logarithm of odds (LOD) value" or "LOD score" (Risch, Science 255:803-804 (1992)) is used in interval mapping to describe the degree of linkage between two marker loci, A LOD score of three between two markers indicates that linkage is 1000 times more likely than no linkage, while a LOD score of two indicates that linkage is 100 times more likely than no linkage. LOD scores greater than or equal to two may be used to detect linkage.

[00137] A "locus" is a position on a chromosome where a gene or marker is located.

[00138] "Maize" refers to a plant of the *Zea mays* L. ssp. *mays* and is also known as "corn".

[00139] The term "maize plant" includes: whole maize plants, maize plant cells, maize plant protoplast, maize plant cell or maize tissue cultures from which maize plants can be regenerated, maize plant calli, and maize plant cells that are intact in maize plants or parts of maize plants, such as maize seeds, maize cobs, maize flowers, maize cotyledons, maize leaves, maize stems, maize buds, maize roots, maize root tips, and the like.

[00140] A "marker" is a nucleotide sequence or encoded product thereof (e.g., a protein) used as a point of reference. For markers to be useful at detecting recombinations, they need to detect differences, or polymorphisms, within the population being monitored. For molecular markers, this means differences at the DNA level due to polynucleotide sequence differences (e.g. SSRs, RFLPs, FLPs, SNPs). The genomic variability can be of any origin, for example, insertions, deletions, duplications, repetitive elements, point mutations, recombination events, or the presence and sequence of transposable elements. Molecular markers can be derived from genomic or expressed nucleic acids (e.g., ESTs) and can also refer to nucleic acids used as probes or primer pairs capable of amplifying sequence fragments via the use of PCR-based methods. A large number of maize molecular markers are known in the art, and are published or available from various sources, such as the Maize GDB Internet resource and the Arizona Genomics Institute Internet resource run by the University of Arizona.

[00141] Markers corresponding to genetic polymorphisms between members of a population can be detected by methods well-established in the art. These include, e.g., DNA sequencing, PCR-based sequence specific amplification methods, detection of restriction fragment length polymorphisms (RFLP), detection of isozyme markers, detection of polynucleotide polymorphisms by allele specific hybridization (ASH), detection of amplified variable sequences of the plant genome, detection of self-sustained sequence replication, detection of simple sequence repeats (SSRs), detection of single nucleotide polymorphisms (SNPs), or detection of amplified fragment length polymorphisms (AFLPs). Well established methods are also known for the detection of expressed sequence tags (ESTs) and SSR markers derived from EST sequences and randomly amplified polymorphic DNA (RAPD).

[00142] A "marker allele", alternatively an "allele of a marker locus", can refer to one of a plurality of polymorphic nucleotide sequences found at a marker locus in a population that is polymorphic for the marker locus.

[00143] "Marker assisted selection (MAS)" is a process by which phenotypes are selected based on marker genotypes.

[00144] "Marker assisted counter-selection" is a process by which marker genotypes are used to identify plants that will not be selected, allowing them to be removed from a breeding program or planting.

[00145] A "marker haplotype" refers to a combination of alleles at a marker locus, e.g. PZA01216 allele 1.

[00146] A "marker locus" is a specific chromosome location in the genome of a species when a specific marker can be found. A marker locus can be used to track the presence of a second linked locus, e.g., a linked locus that encodes or contributes to expression of a phenotypic trait. For example, a marker locus can be used to monitor segregation of alleles at a locus, such as a QTL or single gene, that are genetically or physically linked to the marker locus.

[00147] A "marker probe" is a nucleic acid sequence or molecule that can be used to identify the presence of a marker locus, e.g., a nucleic acid probe that is complementary to a marker locus sequence, through nucleic acid hybridization. Marker probes comprising 30 or more contiguous nucleotides of the marker locus ("all or a portion" of the marker locus sequence) may be used for nucleic acid hybridization. Alternatively, in some aspects, a marker probe refers to a probe of any type that is able to distinguish (i.e. genotype) the particular allele that is present at a marker locus.

[00148] The term "molecular marker" may be used to refer to a genetic marker, as defined above, or an encoded product thereof (e.g., a protein) used as a point of reference when identifying a linked locus. A marker can be derived from genomic nucleotide sequences or from expressed nucleotide sequences (e.g., from a spliced RNA, a cDNA, etc.), or from an encoded polypeptide. The term also refers to nucleic acid sequences complementary to or flanking the marker sequences, such as nucleic acids used as probes or primer pairs capable of amplifying the marker sequence.

[00149] A "molecular marker probe" is a nucleic acid sequence or molecule that can be used to identify the presence of a marker locus, e.g., a nucleic acid probe that is complementary to a marker locus sequence. Alternatively, in some aspects, a marker probe refers to a probe of any type that is able to distinguish (i.e., genotype) the particular allele that

is present at a marker locus. Nucleic acids are "complementary" when they specifically hybridize in solution, e.g., according to Watson-Crick base pairing rules. Some of the markers described herein are also referred to as hybridization markers when located on an indel region, such as the non-collinear region described herein. This is because the insertion region is, by definition, a polymorphism vis a via a plant without the insertion. Thus, the marker need only indicate whether the indel region is present or absent. Any suitable marker detection technology may be used to identify such a hybridization marker, e.g., SNP technology is used in the examples provided herein.

[00150] "Nucleotide sequence", "polynucleotide", "nucleic acid sequence", and "nucleic acid fragment" are used interchangeably and refer to a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. A "nucleotide" is a monomeric unit from which DNA or RNA polymers are constructed, and consists of a purine or pyrimidine base, a pentose, and a phosphoric acid group. Nucleotides (usually found in their 5'-monophosphate form) are referred to by their single letter designation as follows: "A" for adenylate or deoxyadenylate (for RNA or DNA, respectively), "C" for cytidylate or deoxycytidylate, "G" for guanylate or deoxyguanylate, "U" for uridylate, "T" for deoxythymidylate, "R" for purines (A or G), "Y" for pyrimidines (C or T), "K" for G or T, "H" for A or C or T, "I" for inosine, and "N" for any nucleotide.

[00151] The terms "phenotype", or "phenotypic trait" or "trait" refers to one or more traits of an organism. The phenotype can be observable to the naked eye, or by any other means of evaluation known in the art, e.g., microscopy, biochemical analysis, or an electromechanical assay. In some cases, a phenotype is directly controlled by a single gene or genetic locus, i.e., a "single gene trait". In other cases, a phenotype is the result of several genes.

[00152] A "physical map" of the genome is a map showing the linear order of identifiable landmarks (including genes, markers, etc.) on chromosome DNA. However, in contrast to genetic maps, the distances between landmarks are absolute (for example, measured in base pairs or isolated and overlapping contiguous genetic fragments) and not based on genetic recombination.

[00153] A "plant" can be a whole plant, any part thereof, or a cell or tissue culture derived from a plant. Thus, the term "plant" can refer to any of: whole plants, plant

components or organs (e.g., leaves, stems, roots, etc.), plant tissues, seeds, plant cells, and/or progeny of the same. A plant cell is a cell of a plant, taken from a plant, or derived through culture from a cell taken from a plant.

[00154] “Plant tissue culture” refers to a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture relies on the fact that many plant cells have the ability to regenerate a whole plant. Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation.

[00155] A "polymorphism" is a variation in the DNA that is too common to be due merely to new mutation. A polymorphism must have a frequency of at least 1% in a population. A polymorphism can be a single nucleotide polymorphism, or SNP, or an insertion/deletion polymorphism, also referred to herein as an "indel".

[00156] The "probability value" or "p-value" is the statistical likelihood that the particular combination of a phenotype and the presence or absence of a particular marker allele is random. Thus, the lower the probability score, the greater the likelihood that a phenotype and a particular marker will cosegregate. In some aspects, the probability score is considered "significant" or "nonsignificant". In some embodiments, a probability score of 0.05 ($p=0.05$, or a 5% probability) of random assortment is considered a significant indication of co-segregation. However, an acceptable probability can be any probability of less than 50% ($p=0.5$). For example, a significant probability can be less than 0.25, less than 0.20, less than 0.15, less than 0.1, less than 0.05, less than 0.01, or less than 0.001.

[00157] The term "progeny" refers to the offspring generated from a cross.

[00158] A "progeny plant" is generated from a cross between two plants.

[00159] A "reference sequence" is a defined sequence used as a basis for sequence comparison. The reference sequence is obtained by genotyping a number of lines at the locus, aligning the nucleotide sequences in a sequence alignment program (e.g. Sequencher), and then obtaining the consensus sequence of the alignment.

[00160] “Regeneration” is the process of growing a plant from a plant cell (e.g., plant protoplast, callus or explant).

[00161] The "Stiff Stalk" heterotic group represents a major heterotic group in the northern U.S. and Canadian corn growing regions. It can also be referred to as the Iowa Stiff Stalk Synthetic for BSSS) heterotic group.

[00162] The term "transformation" refers to a process of introducing a DNA sequence or construct (e.g., a vector or expression cassette) into a cell or protoplast in which that exogenous DNA is incorporated into a chromosome or is capable of autonomous replication.

[00163] The phrase "under stringent conditions" refers to conditions under which a probe or polynucleotide will hybridize to a specific nucleic acid sequence, typically in a complex mixture of nucleic acids, but to essentially no other sequences. Stringent conditions are sequence-dependent and will be different in different circumstances.

[00164] Longer sequences hybridize specifically at higher temperatures. Generally, stringent conditions are selected to be about 5-10°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength pH. The T_m is the temperature (under defined ionic strength, pH, and nucleic acid concentration) at which 50% of the probes complementary to the target hybridize to the target sequence at equilibrium (as the target sequences are present in excess, at T_m, 50 of the probes are occupied at equilibrium), Stringent conditions will be those in which the salt concentration is less than about 1.0 M sodium ion, typically about 0.01 to 1.0 M sodium ion concentration (or other salts) at pH 7.0 to 8.3, and the temperature is at least about 30° C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g. greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. For selective or specific hybridization, a positive signal is at least two times background, preferably 10 times background hybridization. Exemplary stringent hybridization conditions are often: 50% formamide, 5xSSC, and 1% SDS, incubating at 42° C, or, 5xSSC, 1% SDS, incubating at 65°C, with wash in 0.2xSSC, and 0.1% SDS at 65°C. For PCR, a temperature of about 36°C is typical for low stringency amplification, although annealing temperatures may vary between about 32°C and 48°C, depending on primer length. Additional guidelines for determining hybridization parameters are provided in numerous references.

[00165] Sequence alignments and percent identity calculations may be determined using a variety of comparison methods designed to detect homologous sequences including, but not limited to, the MEGALIGN.RTM. program of the LASERGENE.RTM. bioinformatics computing suite (DNASTAR.RTM. Inc., Madison, Wis.). Unless stated otherwise, multiple alignment of the sequences provided herein were performed using the Clustal V method of alignment (Higgins and Sharp, CABIOS. 5:151-153 (1989)) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10), Default parameters for pairwise alignments and calculation of percent identity of protein sequences using the Clustal V method are KTUPLE=1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For nucleic acids these parameters are KTUPLE=2, GAP PENALTY=5, WINDOW=4 and DIAGONALS SAVED=4. After alignment of the sequences, using the Clustal V program, it is possible to obtain "percent identity" and "divergence" values by viewing the "sequence distances" table on the same program; unless stated otherwise, percent identities and divergences provided and claimed herein were calculated in this manner.

[00166] A "vector" is a DNA molecule capable of replication in a host cell and/or to which another DNA segment can be operatively linked so as to bring about replication of the attached segment. A plasmid is an exemplary vector.

[00167] Before describing the present invention in detail, it should be understood that this invention is not limited to particular embodiments. It also should be understood that the terminology used herein is for the purpose of describing particular embodiments, and is not intended to be limiting. As used herein and in the appended claims, terms in the singular and the singular forms "a", "an" and "the", for example, include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to "plant", "the plant" or "a plant" also includes a plurality of plants. Depending on the context, use of the term "plant" can also include genetically similar or identical progeny of that plant. The use of the term "a nucleic acid" optionally includes many copies of that nucleic acid molecule.

[00168] Genetic Mapping

[00169] It has been recognized for quite some time that specific genetic loci correlating with particular phenotypes, such as increased culturability and transformability,

can be mapped in an organism's genome. The plant breeder can advantageously use molecular markers to identify desired individuals by detecting marker alleles that show a statistically significant probability of co-segregation with a desired phenotype, manifested as linkage disequilibrium. By identifying a molecular marker or clusters of molecular markers that cosegregate with a trait of interest, the breeder is able to rapidly select a desired phenotype by selecting for the proper molecular marker allele (a process called marker-assisted selection, or MAS).

[00170] A variety of methods well known in the art are available for detecting molecular markers or clusters of molecular markers that cosegregate with a trait of interest, such as increased culturability and transformability. The basic idea underlying these methods is the detection of markers, for which alternative genotypes (or alleles) have significantly different average phenotypes. Thus, one makes a comparison among marker loci of the magnitude of difference among alternative genotypes (or alleles) or the level of significance of that difference. Trait genes are inferred to be located nearest the marker(s) that have the greatest associated genotypic difference.

[00171] Two such methods used to detect trait loci of interest are: 1) Population based association analysis and 2) Traditional linkage analysis. In a population-based association analysis, lines are obtained from pre-existing populations with multiple founders, e.g. elite breeding lines. Population-based association analyses rely on the decay of linkage disequilibrium (LD) and the idea that in an unstructured population, only correlations between genes controlling a trait of interest and markers closely linked to those genes will remain after so many generations of random mating. In reality, most pre-existing populations have population substructure. Thus, the use of a structured association approach helps to control population structure by allocating individuals to populations using data obtained from markers randomly distributed across the genome, thereby minimizing disequilibrium due to population structure within the individual populations (also called subpopulations). The phenotypic values are compared to the genotypes (alleles) at each, marker locus for each line in the subpopulation. A significant marker-trait association indicates the dose proximity between the marker locus and one or more genetic loci that are involved in the expression of that trait.

[00172] The same principles underlie traditional linkage analysis; however, LD is generated by creating a population from a small number of founders. The founders are selected to maximize the level of polymorphism within the constructed population, and polymorphic sites are assessed for their level of cosegregation with a given phenotype. A number of statistical methods have been used to identify significant marker-trait associations. One such method is an interval mapping approach (Lander and Botstein, *Genetics* 121:185-199 (1989), in which each of many positions along a genetic map (say at 1 cM intervals) is tested for the likelihood that a gene controlling a trait of interest is located at that position. The genotype/phenotype data are used to calculate for each test position a LOD score (log of likelihood ratio). When the LOD score exceeds a threshold value, there is significant evidence for the location of a gene controlling the trait of interest at that position on the genetic map (which will fall between two particular marker loci).

[00173] As described above, methods well known in the art are available for detecting molecular markers or clusters of molecular markers that cosegregate with a trait of interest, such as increased culturability and transformability. However, certain experimental processes, such as the acts of culture, transformation and regeneration, apply a selection pressure on the genetically segregating population of plants so that only the plants containing the genetic regions for the trait of interest are able to survive and become BC₁ plants. Subsequently, if a genetic locus is important for the trait of interest, then an allele carried by one of the parents would be selected. Therefore, when the genome is scanned after the selection pressure is applied, alleles that are important for the trait of interest occur at a frequency of greater than 50%. Markers showing a significant deviation to greater than 50%, represent loci showing positive effects of selection, and can be identified using a *Chi*-square test ($p < 0.05$). Such methods for molecular marker detection are described within.

[00174] Markers Associated with Increased Culturability and Transformability.

[00175] Markers associated with increased culturability and transformability are identified herein. The methods involve detecting the presence of at least one marker allele associated with the enhancement of the germplasm of a maize plant. The marker locus can be selected from any of the marker loci provided in Table 1, including PZA01216.1, DAS-PZ-7146, DAS-PZ-12685, magi17761, Mo17-100177, DAS-PZ-5617, DAS-PZ-2343, PZA03203-2, Mo17-100291, PZA03409, DAS-PZ-19188, DAS-PZ-2043, DAS-PZ-20570,

PZA02965, Mo17-14519, DAS-PZ-12236, magi52178, and DAS-PZ-366, and any other marker linked to these markers (linked markers can be determined from the Maize GDB resource).

[00176] The genetic elements or genes located on a contiguous linear span of genomic DNA on a single chromosome are physically linked. The markers *asg62* and *magi87535*, both highly associated with culturability and transformability, delineate a culturability and transformability QTL. Any polynucleotide that assembles to the contiguous DNA between and including *asg62* and *magi87535*, or a nucleotide sequence that is 95% identical to *asg62* based on the Clustal V method of alignment, and *magi87535*, or a nucleotide sequence that is 95% identical to *magi87535* based on the Clustal V method of alignment, can house marker loci that are associated with culturability and transformability. Sequences of publicly available markers can be found using the Maize GDB resource.

[00177] Furthermore, *npi386a* and *gpm174b*, both highly associated with culturability and transformability, delineate a culturability and transformability QTL. Any polynucleotide that assembles to the contiguous DNA between and including *npi386a*, or a nucleotide sequence that is 95% identical to *npi386a* based on the Clustal V method of alignment, and *gpm174b*, or a nucleotide sequence that is 95% identical to *gpm174b* based on the Clustal V method of alignment, can house marker loci that are associated with culturability and transformability. Sequences of publicly available markers can be found using the Maize GDB resource.

[00178] Furthermore, *agrr37b* and *nfa104*, both highly associated with culturability and transformability, delineate a culturability and transformability QTL. Any polynucleotide that assembles to the contiguous DNA between and including *agrr37b*, or a nucleotide sequence that is 95% identical to *agrr37b* based on the Clustal V method of alignment, and *nfa104*, or a nucleotide sequence that is 95% identical to *nfa104* based on the Clustal V method of alignment, can house marker loci that are associated with culturability and transformability. Sequences of publicly available markers can be found using the Maize GDB resource.

[00179] Furthermore, *umc156a* and *pco061578*, both highly associated with culturability and transformability, delineate a culturability and transformability QTL. Any polynucleotide that assembles to the contiguous DNA between and including *umc156a*, or a

nucleotide sequence that is 95% identical to umc156a based on the Clustal V method of alignment, and pco061578, or a nucleotide sequence that is 95% identical to pco061578 based on the Clustal V method of alignment, can house marker loci that are associated with culturability and transformability. Sequences of publicly available markers can be found using the Maize GDB resource.

[00180] Furthermore, php20608a and idp6638, both highly associated with culturability and transformability, delineate a culturability and transformability QTL. Any polynucleotide that assembles to the contiguous DNA between and including php20608a, or a nucleotide sequence that is 95% identical to php20608a based on the Clustal V method of alignment, and idp6638, or a nucleotide sequence that is 95% identical to idp6638 based on the Clustal V method of alignment, can house marker loci that are associated with culturability and transformability. Sequences of publicly available markers can be found using the Maize GDB resource.

[00181] Furthermore, bnl4.36 and umc1482, both highly associated with culturability and transformability, delineate a culturability and transformability QTL. Any polynucleotide that assembles to the contiguous DNA between and including bnl4.36, or a nucleotide sequence that is 95% identical to bnl4.36 based on the Clustal V method of alignment, and umc1482, or a nucleotide sequence that is 95% identical to umc1482 based on the Clustal V method of alignment, can house marker loci that are associated with culturability and transformability. Sequences of publicly available markers can be found using the Maize GDB resource.

[00182] Furthermore, umc126a and idp8312, both highly associated with culturability and transformability, delineate a culturability and transformability QTL. Any polynucleotide that assembles to the contiguous DNA between and including umc126a, or a nucleotide sequence that is 95% identical to umc126a based on the Clustal V method of alignment, and idp8312, or a nucleotide sequence that is 95% identical to idp8312 based on the Clustal V method of alignment, can house marker loci that are associated with culturability and transformability. Sequences of publicly available markers can be found using the Maize GDB resource.

[00183] Furthermore, bnl9.11a and gpm609a, both highly associated with culturability and transformability, delineate a culturability and transformability QTL. Any

polynucleotide that assembles to the contiguous DNA between and including bnl9.11a, or a nucleotide sequence that is 95% identical to bnl9.11a based on the Clustal V method of alignment, and gpm609a, or a nucleotide sequence that is 95% identical to gpm609a based on the Clustal V method of alignment, can house marker loci that are associated with culturability and transformability. Sequences of publicly available markers can be found using the Maize GDB resource.

[00184] Furthermore, wx1 and bnlg1209, both highly associated with culturability and transformability, delineate a culturability and transformability QTL. Any polynucleotide that assembles to the contiguous DNA between and including wx1, or a nucleotide sequence that is 95% identical to wx1 based on the Clustal V method of alignment, and bnlg1209, or a nucleotide sequence that is 95% identical to bnlg1209 based on the Clustal V method of alignment, can house marker loci that are associated with culturability and transformability. Sequences of publicly available markers can be found using the Maize GDB resource.

[00185] A common measure of linkage is the frequency with which traits cosegregate. This can be expressed as a percentage of cosegregation (recombination frequency) or in centiMorgans (cM). The cM is a unit of measure of genetic recombination frequency. One cM is equal to a 1% chance that a trait at one genetic locus will be separated from a trait at another locus due to crossing over in a single generation (meaning the traits segregate together 99% of the time). Because chromosomal distance is approximately proportional to the frequency of crossing over events between traits, there is an approximate physical distance that correlates with recombination frequency.

[00186] Marker loci are themselves traits and can be assessed according to standard linkage analysis by tracking the marker loci during segregation. Thus, one cM is equal to a 1% chance that a marker locus will be separated from another locus, due to crossing over in a single generation.

[00187] Other markers linked to the markers listed in Table 2 can be used to predict culturability and transformability in a maize plant. This includes any marker within 50 cM of PZA01216.1, DAS-PZ-7146, DAS-PZ-12685, magi17761, Mo17-100177, DAS-PZ-5617, DAS-PZ-2343, PZA03203-2, Mo17-100291, PZA03409, DAS-PZ-19188, DAS-PZ-2043, DAS-PZ-20570, PZA02965, Mo17-14519, DAS-PZ-12236, magi52178, and DAS-PZ-

366, the markers associated culturability and transformability. The closer a marker is to a gene controlling a trait of interest, the more effective and advantageous that marker is as an indicator for the desired trait. Closely linked loci display an inter-locus cross-over frequency of about 10% or less, preferably about 9% or less, still more preferably about 8% or less, yet more preferably about 7% or less, still more preferably about 6% or less, yet more preferably about 5% or less, still more preferably about 4% or less, yet more preferably about 3% or less, and still more preferably about 2% or less. In highly preferred embodiments, the relevant loci (e.g., a marker locus and a target locus) display a recombination frequency of about 1% or less, e.g., about 0.75% or less, more preferably about 0.5% or less, or yet more preferably about 0.25% or less. Thus, the loci are about 10 cM, 9 cM, 8 cM, 7 cM, 6 cM, 5 cM, 4 cM, 3 cM, 2 cM, 1 cM, 0.75 cM, 0.5 cM or 0.25 cM or less apart. Put another way, two loci that are localized to the same chromosome, and at such a distance that recombination between the two loci occurs at a frequency of less than 10% (e.g., about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.75%, 0.5%, 0.25%, or less) are said to be "proximal to" each other.

[00188] Although particular marker alleles can show cosegregation with increased culturability and transformability, it is important to note that the marker locus is not necessarily responsible for the expression of the culturability and transformability phenotype. For example, it is not a requirement that the marker polynucleotide sequence be part of a gene that imparts increased culturability and transformability (for example, be part of the gene open reading frame). The association between a specific marker allele and the increased culturability and transformability phenotype is due to the original "coupling" linkage phase between the marker allele and the allele in the ancestral maize line from which the allele originated. Eventually, with repeated recombination, crossing over events between the marker and genetic locus can change this orientation. For this reason, the favorable marker allele may change depending on the linkage phase that exists within the resistant parent used to create segregating populations. This does not change the fact that the marker can be used to monitor segregation of the phenotype. It only changes which marker allele is considered favorable in a given segregating population.

[00189] The term "chromosomal interval" designates any and all intervals defined by any of the markers set forth in this invention. Chromosomal intervals that

correlate with culturability and transformability are provided. One interval, located on chromosome 1, comprises and is flanked by asg62 and magi87535. A subinterval of chromosomal interval asg62 and magi87535 is PZA01216.1 and magi17761. Another interval, located on chromosome 4, comprises and is flanked by npi386a and gpm174b, and includes Mo17-100177. Another interval, located on chromosome 4, comprises and is flanked by agr37b and nfa104. A subinterval of agr37b and nfa104 is DAS-PZ-5617 and DAS-PZ-19188. Another interval, located on chromosome 4, comprises and is flanked by umc156a and pco061578, and includes DAS-PZ-2043. Another interval, located on chromosome 4, comprises and is flanked by php20608a and idp6638, and includes DAS-PZ-20570. Another interval, located on chromosome 5, comprises and is flanked by bnl4.36 and umc1482, and includes PZA02965. Another interval, located on chromosome 5, comprises and is flanked by umc126a and idp8312. A subinterval of chromosomal interval umc126a and idp8312 is Mo17-14519 and DAS-PZ-12236. Another interval, located on chromosome 8, comprises and is flanked by bnl9.11a and gpm609a, and includes magi52178. Another interval, located on chromosome 9, comprises and is flanked by wx1 and bnlg1209, and includes DAS-PZ-366.

[00190] A variety of methods well known in the art are available for identifying chromosomal intervals. The boundaries of such chromosomal intervals are drawn to encompass markers that will be linked to the gene controlling the trait of interest. In other words, the chromosomal interval is drawn such that any marker that lies within that interval (including the terminal markers that define the boundaries of the interval) can be used as a marker for culturability and transformability. The interval described above encompasses a cluster of markers that cosegregate with culturability and transformability. The clustering of markers occurs in relatively small domains on the chromosomes, indicating the presence of a gene controlling the trait of interest in those chromosome regions. The interval was drawn to encompass the markers that cosegregate with culturability and transformability. The interval encompasses markers that map within the interval as well as the markers that define the termini. For example, asg62 and magi87535 define a chromosomal interval encompassing a cluster of markers that cosegregate with culturability and transformability in the Stiff Stalk subpopulation. A second example includes the subinterval, PZA01216.1 and magi17761, which define a chromosomal interval encompassing a cluster of markers that cosegregate

with culturability and transformability in the Stiff Stalk subpopulation. An interval described by the terminal markers that define the endpoints of the interval will include the terminal markers and any marker localizing within that chromosomal domain, whether those markers are currently known or unknown.

[00191] Chromosomal intervals can also be defined by markers that are linked to (show linkage disequilibrium with) a marker of interest, and is a common measure of linkage disequilibrium (LD) in the context of association studies. If the r^2 value of LD between any chromosome 1 marker locus lying within the interval of asg62 and magi87535, the subinterval of PZA01216.1 and magi17761, or any other subinterval of asg62 and magi87535, and an identified marker within that interval that has an allele associated with increased culturability and transformability is greater than 1/3 (Ardlie et al. Nature Reviews Genetics 3:299-309 (2002)), the loci are linked. Likewise the same is applied to any marker within any interval described herein.

A marker of the invention can also be a combination of alleles at marker loci, otherwise known as a haplotype. The skilled artisan would expect that there might be additional polymorphic sites at marker loci in and around the chromosome 1, 4, 5, 8, and 9 markers identified herein, wherein one, or more polymorphic sites is in linkage disequilibrium (LD) with an allele associated with increased culturability and transformability. Two particular alleles at different polymorphic sites are said to be in LD if the presence of the allele at one of the sites tends to predict the presence of the allele at the other site on the same chromosome (Stevens, Mol. Diag. 4:309-17 (1999)).

[00192] Marker Assisted Selection

[00193]

Molecular markers can be used in a variety of, plant breeding applications (e.g. see Staub et al. (1996) Hortscience 729-741; Tanksley (1983) Plant Molecular Biology Reporter 1: 3-8). One of the main areas of interest is to increase the efficiency of backcrossing and introgressing genes using marker-assisted selection (MAS). A molecular marker that demonstrates linkage with a locus affecting a desired phenotypic trait provides a useful tool for the selection of the trait in a plant population. This is particularly true where the phenotype is hard to assay, e.g. many disease resistance traits, or, occurs at a late stage in plant development, e.g. kernel characteristics. Since DNA marker assays are less laborious

and take up less physical space than field or greenhouse phenotyping, much larger populations can be assayed, increasing the chances of finding a recombinant with the target segment from the donor line moved to the recipient line. The closer the linkage, the more useful the marker, as recombination is less likely to occur between the marker and the gene causing the trait, which can result in false positives. Having flanking markers decreases the chances that false positive selection will occur as a double recombination event would be needed. The ideal situation is to have a marker in the gene itself, so that recombination cannot occur between the marker and the gene. Such a marker is called a 'perfect marker'.

[00194] When a gene is introgressed by MAS, it is not only the gene that is introduced but also the flanking regions (Gepts. (2002). *Crop Sci*; 42: 1780-1790). This is referred to as "linkage drag," In the case where the donor plant is highly unrelated to the recipient plant, these flanking regions carry additional genes that may code for agronomically undesirable traits. This "linkage drag" may also result in reduced yield or other negative agronomic characteristics even after multiple cycles of backcrossing into the elite maize line. This is also sometimes referred to as "yield drag." The size of the flanking region can be decreased by additional backcrossing, although this is not always successful, as breeders do not have control over the size of the region or the recombination breakpoints (Young et al, (1998) *Genetics* 120:579-585). In classical breeding it is usually only by chance that recombinations are selected that contribute to a reduction in the size of the donor segment (Tanksley et al. (1989). *Biotechnology* 7: 257-264). Even after 20 backcrosses in backcrosses of this type, one may expect to find a sizeable piece of the donor chromosome still linked to the gene being selected. With markers however, it is possible to select those rare individuals that have experienced recombination near the gene of interest. In 150 backcross plants, there is a 95% chance that at least one plant will have experienced a crossover within 1 cM of the gene, based on a single meiosis map distance. Markers will allow unequivocal identification of those individuals. With one additional backcross of 300 plants, there would be a 95% chance of a crossover within 1 cM single meiosis map distance of the other side of the gene, generating a segment around the target gene of less than 2 cM based on a single meiosis map distance. This can be accomplished in two generations with markers, while it would have required on average 100 generations without markers (See Tanksley et al., *supra*). When the exact location of a gene is known, flanking markers surrounding the gene can be utilized to

select for recombinations in different population sizes. For example, in smaller population sizes, recombinations may be expected further away from the gene, so more distal flanking markers would be required to detect the recombination.

[00195] The availability of integrated linkage maps of the maize genome containing increasing densities of public maize markers has facilitated maize genetic mapping and MAS. See, e.g. the IBM2 Neighbors maps, which are available online on the Maize GDB website.

[00196] The key components to the implementation of MAS are: (i) Defining the population within which the marker-trait association will be determined, which can be a segregating population, or a random or structured population: (ii) monitoring the segregation or association of polymorphic markers relative to the trait, and determining linkage or association using statistical methods: (iii) defining a set of desirable markers based on the results of the statistical analysis, and (iv) the use and/or extrapolation of this information to the current set of breeding germplasm to enable marker-based selection decisions to be made. The markers described in this disclosure, as well as other marker types such as SSRs and FLPs, can be used in marker assisted selection protocols.

[00197] SSRs can be defined as relatively short runs of tandemly repeated DNA with lengths of 6 bp or less (Tautz (1989) *Nucleic Acid Research* 17: 6463-6471; Wang et al. (1994) *Theoretical and Applied Genetics*, 88:1-6) Polymorphisms arise due to variation in the number of repeat units, probably caused by slippage during DNA replication (Levinson and Gutman (1987) *Mol Biol Evol* 4: 203-221). The variation in repeat length may be detected by designing PCR primers to the conserved non-repetitive flanking regions (Weber and May (1989) *Am J Hum Genet.* 44:388-396), SSRs are highly suited to mapping and MAS as they are multi-allelic, codominant, reproducible and amenable to high throughput automation (Rafalski et al. (1996) *Generating and using DNA markers in plants. In Non-mammalian genomic analysis: a practical guide. Academic press*, pp 75-135).

[00198] Various types of SSR markers can be generated, and SSR profiles from resistant lines can be obtained by gel electrophoresis of the amplification products. Scoring of marker genotype is based on the size of the amplified fragment. An SSR service for maize is available to the public on a contractual basis by DNA Landmarks in Saint-Jean-sur-Richelieu, Quebec, Canada.

[00199] Various types of FLP markers can also be generated. Most commonly, amplification primers are used to generate fragment length polymorphisms. Such FLP markers are in many ways similar to SSR markers, except that the region amplified by the primers is not typically a highly repetitive region. Still, the amplified region, or amplicon, will have sufficient variability among germplasm, often due to insertions or deletions, such that the fragments generated by the amplification primers can be distinguished among polymorphic individuals, and such indels are known to occur frequently in maize (Bhatramakki et al. (2002). *Plant Mol Biol* 48, 539-547; Rafalski (2002b), supra).

[00200] SNP markers detect single base pair nucleotide substitutions. Of all the molecular marker types, SNPs are the most abundant, thus having the potential to provide the highest genetic map resolution (Bhatramakki et al. 2002 *Plant Molecular Biology* 48:539-547). SNPs can be assayed at an even higher level of throughput than SSRs, in a so-called 'ultra-high-throughput' fashion, as they do not require large amounts of DNA and automation of the assay may be straight-forward. SNPs also have the promise of being relatively low-cost systems. These three factors together make SNPs highly attractive for use in MAS. Several methods are available for SNP genotyping, including but not limited to, hybridization, primer extension, oligonucleotide ligation, nuclease cleavage, minisequencing and coded spheres. Such methods have been reviewed in: Gut (2001) *Hum Mutat* 17 pp, 475-492; Shi (2001) *Clin Chem* 47, pp. 164-172; Kwok (2000) *Pharmacogenomics* 1, pp. 95-100; Bhatramakki and Rafalski (2001) *Discovery and application of single nucleotide polymorphism markers in plants*. In: R, J Henry, Ed, *Plant Genotyping: The DNA Fingerprinting of Plants*, CABI Publishing, Wallingford. A wide range of commercially available technologies utilize these and other methods to interrogate SNPs including Masscode™ (Qiagen), Invader® (Third Wave Technologies), SnapShot® (Applied Biosystems), Taqman® (Applied Biosystems) and Beadarrays™ (Illumina).

[00201] A number of SNPs together within a sequence, or across linked sequences, can be used to describe a haplotype for any particular genotype (Ching et al. (2002), *BMC Genet.* 3:19 pp Gupta et al. 2001, Rafalski (2002b), *Plant Science* 162:329-333). Haplotypes can be more informative than, single SNPs and can be more descriptive of any particular genotype. For example, single SNP may be allele 'T' for a specific line or variety with increased culturability and transformability, but the allele 'T' might also occur in

the maize breeding population being utilized for recurrent parents. In this case, a haplotype, e.g. a combination of alleles at linked SNP markers, may be more informative. Once a unique haplotype has been assigned to a donor chromosomal region, that haplotype can be used in that population or any subset thereof to determine whether an individual has a particular gene. See, for example, WO2003054229. Using automated high throughput marker detection platforms known to those of ordinary skill in the art makes this process highly efficient and effective.

[00202] The sequences listed in Table 2 can be readily used to obtain additional polymorphic SNPs (and other markers) within the QTL interval listed in this disclosure. Markers within the described map region can be hybridized to BACs or other genomic libraries, or electronically aligned with genome sequences, to find new sequences in the same approximate location as the described markers.

[00203] In addition to SSR's, FLPs and SNPs, as described above, other types of molecular markers are also widely used, including but not limited to markers developed from expressed sequence tags (ESTs), SSR markers derived from EST sequences, randomly amplified polymorphic DNA (RAPD), and other nucleic acid based markers.

[00204] Isozyme profiles and linked morphological characteristics can, in some cases, also be indirectly used as markers. Even though they do not directly detect DNA differences, they are often influenced by specific genetic differences. However, markers that detect DNA variation are far more numerous and polymorphic than isozyme or morphological markers (Tanksley (1983) Plant Molecular Biology Reporter 1:3-8).

[00205] Sequence alignments or contigs may also be used to find sequences upstream or downstream of the specific markers listed herein. These new sequences, close to the markers described herein, are then used to discover and develop functionally equivalent markers. For example, different physical and/or genetic maps are aligned to locate equivalent markers not described within this disclosure but that are within similar regions. These maps may be within the maize species, or even across other species that have been genetically or physically aligned with maize, such as rice, wheat, barley or sorghum.

[00206] In general, MAS uses polymorphic markers that have been identified as having a significant likelihood of co-segregation with culturability and transformability. Such markers are presumed to map near a gene or genes that give the plant its culturability

and transformability phenotype, and are considered indicators for the desired trait, or markers. Plants are tested for the presence of a desired allele in the marker, and plants containing a desired genotype at one or more loci are expected to transfer the desired genotype, along with a desired phenotype, to their progeny. The means to identify maize plants that have increased culturability and transformability by identifying plants that have a specified allele at any one of marker loci described herein, including PZA01216.1, DAS-PZ-7146, DAS-PZ-12685, magi17761, Mo17-100177, DAS-PZ-5617, DAS-PZ-2343, PZA03203-2, Mo17-100291, PZA03409, DAS-PZ-19188, DAS-PZ-2043, DAS-PZ-20570, PZA02965, Mo17-14519, DAS-PZ-12236, magi52178, and DAS-PZ-366 are presented herein.

[00207] The interval presented herein finds use in MAS to select plants that demonstrate increased culturability and transformability. Any marker that maps within the chromosome 1 interval defined by and including asg62 and magi87535 can be used for this purpose. In addition, haplotypes comprising alleles at one or more marker loci within the chromosome 1 interval defined by and including asg62 and magi87535 can be used to introduce increased culturability and transformability into maize lines or varieties.

[00208] Any marker that maps within the chromosome 4 interval defined by and including npi386a and gpm174b can be used for this purpose. In addition, haplotypes comprising alleles at one or more marker loci within the chromosome 4 interval defined by and including npi386a and gpm174b can be used to introduce increased culturability and transformability into maize lines or varieties.

[00209] Any marker that maps within the chromosome 4 interval defined by and including agr37b and nfa104 can be used for this purpose. In addition, haplotypes comprising alleles at one or more marker loci within the chromosome 4 interval defined by and including agr37b and nfa104 can be used to introduce increased culturability and transformability into maize lines or varieties.

[00210] Any marker that maps within the chromosome 4 interval defined by and including umc156a and pco061578 can be used for this purpose. In addition, haplotypes comprising alleles at one or more marker loci within the chromosome 4 interval defined by and including umc156a and pco061578 can be used to introduce increased culturability and transformability into maize lines or varieties.

[00211] Any marker that maps within the chromosome 4 interval defined by and including php20608a and idp6638 can be used for this purpose. In addition, haplotypes comprising alleles at one or more marker loci within the chromosome 4 interval defined by and including php20608a and idp6638 can be used to introduce increased culturability and transformability into maize lines or varieties.

[00212] Any marker that maps within the chromosome 5 interval defined by and including bnl4.36 and umc1482 can be used for this purpose. In addition, haplotypes comprising alleles at one or more marker loci within the chromosome 5 interval defined by and including bnl4.36 and umc1482 can be used to introduce increased culturability and transformability into maize lines or varieties.

[00213] Any marker that maps within the chromosome 5 interval defined by and including umc126a and idp8312 can be used for this purpose. In addition, haplotypes comprising alleles at one or more marker loci within the chromosome 5 interval defined by and including umc126a and idp8312 can be used to introduce increased culturability and transformability into maize lines or varieties.

[00214] Any marker that maps within the chromosome 8 interval defined by and including bnl9.11a and gpm609a can be used for this purpose. In addition, haplotypes comprising alleles at one or more marker loci within the chromosome 8 interval defined by and including bnl9.11a and gpm609a can be used to introduce increased culturability and transformability into maize lines or varieties.

[00215] Any marker that maps within the chromosome 9 interval defined by and including wx1 and bnlg1209 can be used for this purpose. In addition, haplotypes comprising alleles at one or more marker loci within the chromosome 9 interval defined by and including wx1 and bnlg1209 can be used to introduce increased culturability and transformability into maize lines or varieties.

[00216] Any allele or haplotype that is in linkage disequilibrium with an allele associated with increased culturability and transformability can be used in MAS to select plants with increased culturability and transformability.

EXAMPLES

[00217] The following examples are offered to illustrate, but not to limit, the appended claims. It is understood that the examples and embodiments described herein are for illustrative purposes only and that persons skilled in the art will recognize various reagents or parameters that can be altered without departing from the spirit of the invention or the scope of the appended claims.

[00218] **Example 1: Plant material**

[00219] Initial crosses were made between the highly transformable line A188 and Dow AgroSciences (DAS) elite inbred lines D046358 and SLB24. The F₁ plants from these populations were then backcrossed to the elite lines, and immature BC₁ (Backcross 1) embryos were harvested, cultured, and regenerated into BC₁ plants. Leaf tissue from the regenerated plants was collected and DNA was extracted using the Biocel 1800 robotic platform (Agilent Technologies, Santa Clara, CA) and the Qiagen MagAttract protocol (Qiagen, Valencia, CA).

[00220] **Example 2: Embryo collection**

[00221] Ears from the D046358 and SLB24 populations were surface-sterilized by immersion in a 20% solution of sodium hypochlorite (5%) and two drops of Tween 20, for 20-30 minutes, followed by three rinses in sterile water. Immature zygotic embryos (1.0–2.0 mm) were aseptically dissected from each ear and randomly distributed into micro-centrifuge tubes for *Agrobacterium* infection. Embryos were pooled in the cases where multiple ears were available from the same line.

[00222] **Example 3: Screening for Regeneration Ability** Embryos from the different lines and crosses were screened for their ability to regenerate in culture. A small number of embryos (5-30) from each of the ears used for transformation were plated on two types of media (ZM00002234 or ZM00001341, Table 1.5 or 1.4) lacking the selective agent. Cultures were incubated in the dark for 14 days at 28°C. Proliferated embryos were subcultured on the same type of media and incubated in the dark for another 14 days at 28°C. Embryogenic callus was transferred to regeneration media (ZM00002388, Table 1.8) and incubated under 16/8 hours (h) light/dark with light intensity of 80-100 micromoles per second per meter squared ($\mu\text{mol m}^{-2} \text{s}^{-1}$) for 10-14 days at 28°C. Calli with shoots initiated were transferred to a second type of regeneration media (ZM00002242, Table 1.10) and incubated under 16/8 h light/dark with light intensity of 80-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 10-14 days at

28°C. Regeneration frequency was estimated as the number of embryos that regenerated at least one shoot divided by the number of embryos plated.

[00223] Example 4: Transformation

[00224] Example 4.1: *Agrobacterium* strain and construct

[00225] *Agrobacterium tumefaciens* strain LBA4404 carrying the super binary vector pDAB1405 was used for all the transformation experiments. The pDAB1405 construct contains *GFP* gene v.2 under the control of ZmUbi1 promoter and its intron and *PAT* gene v.3 under the control of OsActin1 promoter and its intron. The two genes and promoters were flanked by RB7 MARs sequences (Figure 1).

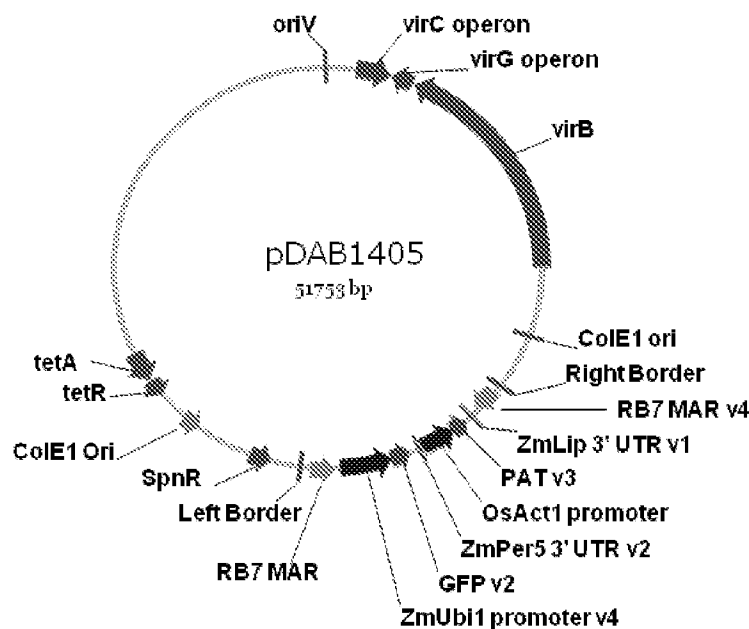


Figure 1. A map for pDAB1405 which was used in the transformation experiments.

[00226] Example 4.2: *Agrobacterium* culture initiation

[00227] Glycerol stocks of the superbinary vector pDAB1405 in the host *Agrobacterium tumefaciens* strain LBA4404 were obtained from the DAS Research Culture Collection. Streaked plates were made using AB minimal medium (AT00002172, Table 1.13) containing 100 mg/L spectinomycin, 250 mg/L streptomycin, and 10 mg/L tetracycline, and grown at 20°C for 3-4 days. A single colony was then picked and streaked onto YEP plates (AT0002170, Table 1.14) containing the same antibiotics and incubated at 28°C for 1-2 days.

[00228] **Example 4.3: *Agrobacterium* co-cultivation**

[00229] On the experiment day, *Agrobacterium* colonies were taken from the YEP plate, suspended in 7-10 ml of infection medium (ZM00002231, Table 1.1) in a 50 ml tube, and the cell density was adjusted to $OD_{550} = 1.2-1.4$ using a spectrophotometer. The *Agrobacterium* cultures were placed on a rotary shaker at 100 revolutions per minute (rpm) while embryo dissection was performed. Immature zygotic embryos between 1.5-2.0 mm in size were isolated from the sterilized maize kernels and placed in 1 ml of the infection medium (about 30-130 embryos in a 2.0 ml Eppendorf tube), followed by one wash with the same medium. The *Agrobacterium* suspension (1.0 ml) was added to each tube; the tubes were inverted for 20 times, and then allowed to sit for 5 minutes at room temperature. The embryos were then transferred onto co-cultivation media (ZM00002232 or ZM00001358, Table 1.2 or 1.3). The embryos were then oriented with the scutellum facing up using a microscope. After a 3 day co-cultivation at 20°C, transient expression of the green fluorescence protein (GFP) transgene was observed to validate *Agrobacterium* infection.

[00230] **Example 4.4: Callus selection and regeneration of transgenic events**

[00231] Following the co-cultivation period, embryos were transferred to resting media (ZM00002234 or ZM00001341, Table 1.5 or 1.4) containing the antibiotic cefotaxime, and incubated in the dark for 7 days at 28°C. Embryos were then transferred onto Selection 1 media (ZM00002240 or ZM00002180, Table 1.6 or 1.7) containing 3 mg/L Bialaphos as the selective agent for the introduced *pat* gene, and incubated in the dark for 14 days at 28°C. Proliferating embryogenic calli expressing GFP were cut under the stereomicroscope into smaller pieces (2-3 mm), transferred onto selection media containing 3 mg/L Bialaphos and incubated in the dark for another 10-14 days at 28°C. All the pieces cut

from one embryo were circled with a black marker and considered as one event. This selection step allowed the callus to further proliferate and enhanced the uniformity of stable GFP expression. The callus selection period lasted for approximately four to six weeks. Proliferating, embryogenic calli expressing GFP were transferred onto Regeneration 1 media (ZM00002254, Table 1.9) containing 3 mg/L Bialaphos and cultured in the dark for 7-10 days at 28°C. This period was essential for the maturation of embryogenic callus.

[00232] Embryogenic calli with shoots initiated were transferred onto Regeneration 2 media (ZM00002242 or ZM00002255, Table 1.10 or 1.11) without Bialaphos. The cultures were incubated under 16/8 h light/dark with light intensity of 80-100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ for 10-14 days at 28°C. Small shoots with primary roots were then transferred to shoot elongation and rooting media (ZM00002238, Table 1.12) in Magenta boxes and incubated under 16/8 h light/dark for 7-10 days at 28°C. Putative plantlets were confirmed for GFP expression and then scored as transgenic events.

[00233] Table 1. Media formulations

1.1. Infection

ID	pH	Ingredient	Conc.	Units
ZM00002231	5.2	MS BASAL SALTS	4.33	g/L
		CHU N6 VITAMIN SOLUTION (1000X)	1	mL/L
		SUCROSE	68.5	g/L
		D(+)-GLUCOSE	36	g/L
		2,4-D 10 MG/ML STOCK	150	$\mu\text{L/L}$

1.2. Co-cultivation

ID	pH	Ingredient	Conc.	Units
ZM00002232	5.8	MS BASAL SALTS	4.33	g/L
		2,4-D 10 MG/ML STOCK	200	$\mu\text{L/L}$
		L-PROLINE	700	mg/L
		MES	500	mg/L
		CHU N6 VITAMIN SOLUTION (1000X)	1	mL/L
		SUCROSE	20	g/L
		MYO-INOSITOL	100	mg/L

GLUCOSE	10	g/L
AGAR	7	g/L
ACETOSYRINGONE 200MM	200	μM

1.3. Co-cultivation

ID	pH	Ingredient	Conc.	Units
ZM00001358	5.8	MS BASAL SALT	4.33	g/L
		L-PROLINE	700	mg/L
		MYO-INOSITOL	100	mg/L
		CASEIN ENZYMATIC		
		HYDROLYSATE	100	mg/L
		SUCROSE	30	g/L
		DICAMBA 50 MG/ML	3.3	mg/L
		GELRITE 714246	2.3	g/L
		SILVER NITRATE	15	mg/L
		ISU MODIFIED MS VITAMIN (1000X)	1	mg/L
		ACETOSYRINGONE 100 MM	100	μM
		L-CYSTEINE	300	mg/L

1.4. Resting

ID	pH	Ingredient	Conc.	Units
ZM00001341	5.8	MS BASAL SALT	4.33	g/L
		L-PROLINE	700	mg/L
		MES	500	mg/L
		MYO-INOSITOL	100	mg/L
		CASEIN ENZYMATIC		
		HYDROLYSATE	100	mg/L
		SUCROSE	30	g/L
		DICAMBA 50 MG/ML	3.3	mg/L
		GELRITE 714246	2.3	g/L
		SILVER NITRATE	15	mg/L
		ISU MODIFIED MS VITAMIN (1000X)	1	mg/L
		CEFOTAXIME 250 MG/ML	250	mg/L

1.5. Resting

ID	pH	Ingredient	Conc.	Units
----	----	------------	-------	-------

ZM00002234	5.8	MS BASAL SALTS	4.33	g/L
		2,4-D 10 MG/ML STOCK	150	μL/L
		L-PROLINE	700	mg/L
		MES	500	mg/L
		CHU N6 VITAMIN SOLUTION (1000X)	1	mL/L
		SUCROSE	30	g/L
		MYO-INOSITOL	100	mg/L
		AGAR	7	g/L
		CARBENICILLIN 250 MG/ML	200	mg/L
		SILVER NITRATE	0.85	mg/L

1.6. 3 mg/L Bialaphos selection medium

ID	pH	Ingredient	Conc.	Units
ZM00002240		MS BASAL SALTS	4.33	g/L
		2,4-D 10 MG/ML STOCK	150	μL/L
		L-PROLINE	700	mg/L
		MES	500	mg/L
		CHU N6 VITAMIN SOLUTION (1000X)	1	mL/L
		SUCROSE	30	g/L
		MYO-INOSITOL	100	mg/L
		AGAR	7	g/L
		CARBENICILLIN 250 MG/ML	200	mg/L
		SILVER NITRATE	0.85	mg/L
		BIALAPHOS 5.0 MG/ML	1.5	mg/L

1.7. 3 mg/L Bialaphos selection medium

ID	pH	Ingredient	Conc.	Units
ZM00002180	5.8	MS BASAL SALT	4.33	g/L
		L-PROLINE	700	mg/L
		MES	500	mg/L
		MYO-INOSITOL	100	mg/L
		CASEIN ENZYMATIC HYDROLYSATE	100	mg/L
		SUCROSE	30	g/L
		DICAMBA 1 MG/ML STOCK	3.3	mL/L
		GELRITE 714246	3	g/L
		SILVER NITRATE	15	mg/L

ISU MODIFIED MS VITAMIN (1000X)	1	mg/L
CEFOTAXIME 250 MG/ML	250	mg/L
BIALAPHOS 5.0 MG/ML	3	mg/L

1.8. Regeneration 1 KD

ID	pH	Ingredient	Conc.	Units
ZM00002388	5.8	MS BASAL SALTS WITH VITAMINS	4.33	g/L
		MES	500	mg/L
		L-PROLINE	500	mg/L
		SUCROSE	30	g/L
		AGAR	6.5	g/L
		2,4-D 1 MG/ML STOCK	0.25	mL/L
		KINETIN 1 MG/ML STOCK	0.5	mL/L
		CEFOTAXIME 250 MG/ML	150	mg/L

1.9. Regeneration 1 + 3 mg/L Bialaphos

ID	pH	Ingredient	Conc.	Units
ZM00002254	5.8	MS BASAL SALT	4.33	g/L
		ISU MODIFIED MS VITAMIN (1000X)	1	mg/L
		SUCROSE	60	g/L
		MYO-INOSITOL	100	mg/L
		GELRITE 714246	2.5	g/L
		BIALAPHOS 5.0 MG/ML	3	mg/L
		CEFOTAXIME 250 MG/ML	250	mg/L

1.10. Regeneration 2

ID	pH	Ingredient	Conc.	Units
ZM00002242	5.8	MS BASAL SALTS WITH VITAMINS	4.43	g/L
		MES	500	mg/L
		SUCROSE	30	g/L
		AGAR	7	g/L

1.11. Regeneration 2

ID	pH	Ingredient	Conc.	Units
ZM00002255		MS BASAL SALT	4.33	g/L
		ISU MODIFIED MS VITAMIN (1000X)	1	mg/L

SUCROSE	30	g/L
MYO-INOSITOL	100	mg/L
GELRITE 714246	2.5	g/L
CEFOTAXIME 250 MG/ML	250	mg/L

1.12. Shoot elongation

ID	pH	Ingredient	Conc.	Units
ZM00002238	5.8	MS BASAL SALTS WITH VITAMINS	2.2	g/L
		SUCROSE	20	g/L
		AGAR	6	g/L

1.13. AB + Antibiotics

ID	pH	Ingredient	Conc.	Units
AT00002172	7	GLUCOSE	5	g/L
		BACTO AGAR	15	g/L
		AB MINIMAL BUFFER-MICRO	1000	mg/L
		AB MINIMAL SALTS-MICRO	1000	mg/L
		SPECTINOMYCIN 100MG/ML	100	mg/L
		STREPTOMYCIN 100 MG/ML	250	mg/L
		TETRACYCLINE 10 MG/ML	10	mg/L

1.14. YEP + Antibiotics

ID	pH	Ingredient	Conc.	Units
AT00002170	7	BACTO-PEPTONE	10	g/L
		YEAST EXTRACT	10	g/L
		SODIUM CHLORIDE	5	g/L
		BACTO AGAR	15	g/L
		STREPTOMYCIN 250MG/ML AI-MICRO	250	mg/L
		TETRACYCLINE 10 MG/ML	10	mg/L
		SPECTINOMYCIN 100MG/ML AI-MICRO	100	mg/L

[00234] Example 5: Genotyping the BC₁ plants

[00235] For the SLB24 population, 173 BC₁ plants were regenerated from independent cultures, while 117 BC₁ plants were regenerated for the D046358 population. The KBioscience Competitive Allele Specific PCR system, or KASPar™ (KBiosciences, Hertfordshire, UK), was used to genotype DNA from leaf tissue from the regenerated plants using 256 and 235 polymorphic markers for the SLB24 and D046358 populations, respectively. The KASPar™ system is comprised of two components (1) the SNP specific assay (a combination of three unlabelled primers), and (2) the universal Reaction Mix, which contains all other required components including the universal fluorescent reporting system and a specially developed Taq polymerase. The three primers, allele-specific 1 (A1), allele-specific 2 (A2), and common (C1), or reverse, were designed using the assay design algorithm of the workflow manager, Kraken (KBiosciences, Hertfordshire, UK).

[00236] An Assay Mix of the 3 primers was made, consisting of 12 µM each of A1 and A2 and 30 µM of C1. The universal 1536 Reaction Mix was diluted to 1x. A volume of 2.0 µl of DNA diluted 1:20 from MagAttract extracted DNA was dispensed into PCR plates using a liquid handling robot and dried for 2 hours at 65°C. Next, 1.3 µl of 1x KASP 1536 Reaction Mix was added to the PCR plates. Plates were sealed using a Fusion heat sealer (KBioscience, Hertfordshire, UK). Thermal cycling was completed in the Hydrocycler water bath thermal cycler (Kbioscience, Hertfordshire, UK) with the following conditions: initial denaturation and hot-start enzyme activation at 94°C for 15 minutes followed by 10 cycles of denaturation at 94°C for 20 seconds and touchdown over 65-57°C for 60 seconds (dropping 0.8°C per cycle). This was followed by 29 cycles of denaturation at 94°C for 20 seconds and 57°C annealing for 60 seconds.

KASPar™ uses the fluorophores FAM and VIC for distinguishing genotypes. The passive reference dye ROX is also used to allow normalization of variations in signal caused by differences in well-to-well liquid volume. In Kraken, the FAM and VIC data are plotted on the *x*- and *y*- axes, respectively. Genotypes can then be determined according to sample clusters.

[00237] Example 6: Statistical analysis

[00238] The acts of culture, transformation and regeneration applied a selection pressure on the genetically segregating population of embryos so that only embryos

containing the genetic regions important for tissue culture, transformation and regeneration were able to survive and become BC₁ plants. Subsequently, if a genetic locus is important for culture, transformation or regeneration, then an allele carried by one of the parents would be selected. Therefore, when the genome is scanned after culture and plant regeneration, alleles that are important for culturability and transformability occur at a frequency of greater than 50%. Markers showing a significant deviation to greater than 50%, represent loci showing positive effects of selection, and were identified using a *Chi*-square test ($p < 0.05$).

[00239] *Chi*-square analysis results revealed nine genetic regions associated with increased culturability and transformability (Table 2). These genetic regions were located on chromosomes 1, 4, 5, 8, and 9 and represent novel regions not previously reported as associated with culturability and transformability. Bin boundaries were determined using the IBM2 2008 Neighbors maps on the Maize GDB website.

[00240] Table 2. Genetic regions important for culture and transformation of the BC₁ populations.

Chromosome	Bin	Chromosome interval from Maize GDB (bp)	Bin boundaries from Maize GDB IBM2 2008 Neighbors maps
1	1.07	198854443 - 228254155	asg62 (CBM) - magi87535
4	4.04	21583117 - 32251206	npi386a (CBM) - gpm174b
4	4.05	32251206 - 151082840	agrr37b (CBM) - nfa104
4	4.06	151082840 - 171036627	umc156a (CBM) - pco061578
4	4.10	236702403 - 239441082	php20608a (CBM) - idp6638
5	5.04	80804839 - 172395871	bnl4.36 (CBM) - umc1482
5	5.06	195305700 - 204605586	umc126a (CBM) - idp8312
8	8.02	10072194 - 21148079	bnl9.11a (CBM) - gpm609a
9	9.03	23256783 - 100740934	wx1 (CBM) - bnlgl209

Core Bin Marker (CBM)

[00241] **Example 7: Marker framework and use for marker assisted selection**

[00242] A set of common markers can be used to establish a framework for identifying markers in the chromosome interval. Table 3 shows markers that are in consistent position relative to one another on the B73 reference genome, version 2. Physical locations of the DAS proprietary markers were determined using the DAS proprietary GBrowser. The

physical locations of public markers were determined using the B73 reference genome, version 2 on the publicly available Maize GDB website.

[00243] Closely linked markers associated with the trait of interest that have alleles in linkage disequilibrium with a favorable allele at that locus may be effectively used to select for progeny plants with increased culturability and transformability. Thus, the markers described in herein, such as those listed in Table 3, as well as other markers genetically or physically mapped to the same chromosomal interval, may be used to select for maize plants with increased culturability and transformability. Typically, a set of these markers will be used (e.g. 2 or more, 3 or more, 4 or more, 5 or more) in the regions associated with the trait of interest. Optionally, a marker within the actual gene and/or locus may be used. Exemplary primers for amplifying and detecting genomic regions associated with increased culturability and transformability are shown in Table 4.

[00244] Table 3: SNP markers associated with increased culturability and transformability and their favorable, or donor, allele.

Chrom	Bin	Markers within Bin	SEQ ID NO	Physical position from Maize GDB (bp)	SNP	Donor Allele
1	1.07	PZA01216.1	*	203587826	T/C	C
		DAS-PZ-7146	1	206859812	A/T	A
		DAS-PZ-12685	2	207466563	A/G	G
		magi17761	*	210759949	A/C	A
4	4.04	Mo17-100177	3	30929128	A/C	C
4	4.05	DAS-PZ-5617	4	38286840	A/C	C
		DAS-PZ-2343	5	43590080	T/C	C
		PZA03203-2	*	82892983	A/G	G
		Mo17-100291	6	124897440	A/T	A
		PZA03409	*	129818270	A/C	A
		DAS-PZ-19188	7	146008071	A/T	A
4	4.06	DAS-PZ-2043	8	157093697	A/G	G
4	4.10	DAS-PZ-20570	9	237494592	C/G	G
5	5.04	PZA02965	*	164124177	A/G	A
5	5.06	Mo17-14519	10	195791958	T/C	C
		DAS-PZ-12236	11	203305499	T/C	C

8	8.02	magi52178	*	18215760	T/C	T
9	9.03	DAS-PZ-366	12	62289718	C/G	C

* Sequence of public markers found at Maize GDB

[00245] Table 4: Exemplary primers for amplifying and detecting genomic regions associated with increased culturability and transformability.

Chrom	Bin	Markers within Bin	Allele specific primer 1 (SEQ ID NO)	Allele specific primer 2 (SEQ ID NO)	Reverse primer (SEQ ID NO)
1	1.07	PZA01216.1	13	14	15
		DAS-PZ-7146	16	17	18
		DAS-PZ-12685	19	20	21
		magi17761	22	23	24
4	4.04	Mo17-100177	25	26	27
4	4.05	DAS-PZ-5617	28	29	30
		DAS-PZ-2343	31	32	33
		PZA03203-2	34	35	36
		Mo17-100291	37	38	39
		PZA03409.1	40	41	42
		DAS-PZ-19188	43	44	45
4	4.06	DAS-PZ-2043	46	47	48
4	4.10	DAS-PZ-20570	49	50	51
5	5.04	PZA02965-14	52	53	54
5	5.06	Mo17-14519	55	56	57
		DAS-PZ-12236	58	59	60
8	8.02	magi52178	61	62	63
9	9.03	DAS-PZ-366	64	65	66

References:

Armstrong CL, Romero-Severson J, Hodges TK. 1992. Improved tissue culture response of an elite maize inbred through backcross breeding, and identification of chromosomal regions important for regeneration by RFLP analysis. *Theor Appl Genet* 84:755-762.

Lowe BA and Chomet PS. 2010. Methods and compositions for production of maize lines with increased transformability. United States Patent US 7759545 B2.

Zhao ZY, Smith OS, Li B, Bhattaramakki D, Shu GG. 2008. Marker assisted selection for transformation traits in maize. United States Patent Application US 2008/0078003 A1.

CLAIMS

We claim:

1. A method of identifying a maize plant that displays increased culturability and/or transformability, the method comprising:
 - a) detecting in germplasm of the maize plant at least nine marker loci, wherein at least one marker locus is located within each chromosomal interval (i) – (ix):
 - (i) comprising and flanked by asg62 and magi87535;
 - (ii) comprising and flanked by npi386a and gpm174b;
 - (iii) comprising and flanked by agr37b and nfa104;
 - (iv) comprising and flanked by umc156a and pco061578;
 - (v) comprising and flanked by php20608a and idp6638;
 - (vi) comprising and flanked by bnl4.36 and umc1482;
 - (vii) comprising and flanked by umc126a and idp8312;
 - (viii) comprising and flanked by bnl9.11a and gpm609a; and,
 - (ix) comprising and flanked by wx1 and bnl1209; and
 - b) at least one allele is associated with increased culturability and/or transformability.
2. The method of claim 1, wherein at least one marker locus is selected from each of the groups (i) – (ix) consisting of:
 - (i) PZA01216.1, DAS-PZ-7146, DAS-PZ-12685, and magi17761;
 - (ii) Mo17-100177;
 - (iii) DAS-PZ-5617, DAS-PZ-2343, PZA03203-2, Mo17-100291, PZA03409, and DAS-PZ-19188;
 - (iv) DAS-PZ-2043;
 - (v) DAS-PZ-20570;
 - (vi) PZA02965;
 - (vii) Mo17-14519 and DAS-PZ-12236;
 - (viii) magi52178; and,
 - (ix) DAS-PZ-366.
3. The method of claim 1, wherein the maize plant belongs to the Stiff Stalk heterotic group.

4. A maize plant identified by the method of claim 1.

5. A method of identifying a maize plant that displays increased culturability and/or transformability, the method comprising detecting in germplasm of the maize plant a haplotype comprising alleles at one or more marker loci, wherein:

a) one or more marker loci are located within each chromosomal interval (i) – (ix):

- (i) comprising and flanked by asg62 and magi87535;
- (ii) comprising and flanked by np1386a and gpm174b;
- (iii) comprising and flanked by agr37b and nfa104;
- (iv) comprising and flanked by umc156a and pco061578;
- (v) comprising and flanked by php20608a and idp6638;
- (vi) comprising and flanked by bn14.36 and umc1482;
- (vii) comprising and flanked by umc126a and idp8312;
- (viii) comprising and flanked by bn19.11a and gpm609a; and,
- (ix) comprising and flanked by wx1 and bnlg1209; and

b) the haplotype is associated with increased culturability and/or transformability.

6. The method of claim 5, wherein the one or more marker loci are located within each chromosomal interval (i) – (ix):

- (i) comprising and flanked by asg62 and magi87535;
- (ii) comprising and flanked by np1386a and gpm174b;
- (iii) comprising and flanked by agr37b and nfa104;
- (iv) comprising and flanked by umc156a and pco061578;
- (v) comprising and flanked by php20608a and idp6638;
- (vi) comprising and flanked by bn14.36 and umc1482;
- (vii) comprising and flanked by umc126a and idp8312;
- (viii) comprising and flanked by bn19.11a and gpm609a; and,
- (ix) comprising and flanked by wx1 and bnlg1209.

7. The method of claim 5, wherein the maize plant belongs to the Stiff Stalk heterotic group.

8. A maize plant identified by the method of claim 5, wherein the maize plant comprises within its germplasm a haplotype associated with increased culturability and/or transformability, wherein the haplotype comprises alleles at one or more marker loci located within each chromosomal interval (i) – (ix):

- (i) comprising and flanked by asg62 and magi87535;
- (ii) comprising and flanked by np1386a and gpm174b;
- (iii) comprising and flanked by agr37b and nfa104;
- (iv) comprising and flanked by umc156a and pco061578;
- (v) comprising and flanked by php20608a and idp6638;
- (vi) comprising and flanked by bn14.36 and umc1482;
- (vii) comprising and flanked by umc126a and idp8312;
- (viii) comprising and flanked by bn19.11a and gpm609a; and,
- (ix) comprising and flanked by wx1 and bnlg1209.

9. A method of marker assisted selected comprising:

a) obtaining a first maize plant having at least nine marker loci, wherein at least one marker locus is located within each chromosomal interval (i) – (ix):

- (i) comprising and flanked by asg62 and magi87535;
- (ii) comprising and flanked by np1386a and gpm174b;
- (iii) comprising and flanked by agr37b and nfa104;
- (iv) comprising and flanked by umc156a and pco061578;
- (v) comprising and flanked by php20608a and idp6638;
- (vi) comprising and flanked by bn14.36 and umc1482;
- (vii) comprising and flanked by umc126a and idp8312;
- (viii) comprising and flanked by bn19.11a and gpm609a; and,
- (ix) comprising and flanked by wx1 and bnlg1209; and, the allele of the marker locus is associated with increased culturability and/or transformability;

b) crossing the first maize plant to a second maize plant;

c) evaluating the progeny for the at least one allele; and

d) selecting progeny plants that possess the at least one allele.

10. The method of claim 9, wherein at least one marker locus is selected from each of the groups (i) – (ix) consisting of:

- (i) PZA01216.1, DAS-PZ-7146, DAS-PZ-12685, and magi17761;
- (ii) Mo17-100177;
- (iii) DAS-PZ-5617, DAS-PZ-2343, PZA03203-2, Mo17-100291, PZA03409, and DAS-PZ-19188;
- (iv) DAS-PZ-2043;
- (v) DAS-PZ-20570;
- (vi) PZA02965;
- (vii) Mo17-14519 and DAS-PZ-12236;
- (viii) magi52178; and,
- (ix) DAS-PZ-366.

11. The method of claim 9, wherein the maize plant belongs to the Stiff Stalk heterotic group.

12. A maize progeny plant selected by the method of claim 9 wherein the plant has at least one allele of a marker locus wherein at least one marker locus is located within each chromosomal interval (i) – (ix):

- (i) comprising and flanked by asg62 and magi87535;
- (ii) comprising and flanked by np1386a and gpm174b;
- (iii) comprising and flanked by agr37b and nfa104;
- (iv) comprising and flanked by umc156a and pco061578;
- (v) comprising and flanked by php20608a and idp6638;
- (vi) comprising and flanked by bn14.36 and umc1482;
- (vii) comprising and flanked by umc126a and idp8312;
- (viii) comprising and flanked by bn19.11a and gpm609a; and,
- (ix) comprising and flanked by wx1 and bnlg1209.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/054858

A. CLASSIFICATION OF SUBJECT MATTER

INV. A01H5/10 C07K14/415 G01N33/53 A01H1/04 C12Q1/68
G01N33/00

ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

A01H C07K G01N C12Q

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

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

EP0-Internal, BIOSIS, Sequence Search, EMBASE, MEDLINE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2008/034045 A2 (PIONEER HI BRED INT [US]; DU PONT [US]; ZHAO ZUO-YU [US]; SMITH OSCAR) 20 March 2008 (2008-03-20) cited in the application claims 1-4, 9-12, 14-17; pages 1-10; Examples 1-8; Tables 7A, 8-10 ----- -/--	1-12

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

19 January 2016

Date of mailing of the international search report

09/02/2016

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Kurz, Birgit

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2015/054858

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ARMSTRONG C L ET AL: "Improved tissue culture response of an elite maize inbred through backcross breeding and identification of chromosomal regions important for regeneration by RFLP analysis", THEORETICAL AND APPLIED GENETICS, SPRINGER, BERLIN, DE, vol. 84, 1 January 1992 (1992-01-01), pages 755-762, XP002982155, ISSN: 0040-5752, DOI: 10.1007/BF00224181 cited in the application abstract; Figures 1 and 2; page 756-758 -----	1-12
X	US 2008/072344 A1 (WANG NING [US] ET AL) 20 March 2008 (2008-03-20) paragraphs [0002], [0024]-[0033], [0138]-[0142]; Tables 1-3 -----	1-12
X	WO 03/103377 A2 (MONSANTO TECHNOLOGY LLC [US]; LOWE BRENDA A [US]; CHOMET PAUL [US]) 18 December 2003 (2003-12-18) cited in the application pages 1-5, 36, 39-43; Examples 1, 8; Tables 1, 2, 9, 12; claims 1-10, 15, 16 -----	1-12
A	HOISINGTON D A ET AL: "From theory to practice: Marker-assisted selection in maize", BIOTECHNOLOGY IN AGRICULTURE AND FORESTRY, SPRINGER VERLAG, BERLIN, DE, 1 January 2005 (2005-01-01), pages 335-352, XP009099731, ISSN: 0934-943X, DOI: 10.1007/3-540-26538-4_20 the whole document, in particular pages 335, 337, 338, 342-347 -----	1-12

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2015/054858

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008034045	A2	20-03-2008	BR PI0716747 A2 17-09-2013
			CA 2664337 A1 20-03-2008
			CN 101662931 A 03-03-2010
			EP 2066169 A2 10-06-2009
			US 2008078003 A1 27-03-2008
			WO 2008034045 A2 20-03-2008

US 2008072344	A1	20-03-2008	US 2008072344 A1 20-03-2008
			US 2009300789 A1 03-12-2009

WO 03103377	A2	18-12-2003	AT 461617 T 15-04-2010
			AU 2003243391 A1 22-12-2003
			BR 0311618 A 13-06-2006
			CA 2488332 A1 18-12-2003
			CA 2855445 A1 18-12-2003
			EP 1513393 A2 16-03-2005
			US 2004016030 A1 22-01-2004
			US 2010287653 A1 11-11-2010
			US 2014310834 A1 16-10-2014
			WO 03103377 A2 18-12-2003
