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(12) United States Patent

Kaeppler et al.

(54) EXTENDING JUVENILITY IN GRASSES

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- (22) Filed: Apr. 7, 2017

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- (58) Field of Classification Search NoneSee application file for complete search history.

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(Continued)

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(57) **ABSTRACT**

The present invention relates to compositions and methods for modulating the juvenile to adult developmental growth transition in plants, such as grasses (e.g. maize). In particular, the invention provides methods for enhancing agronomic properties in plants by modulating expression of GRMZM2G362718, GRMZM2G096016, or homologs thereof. Modulation of expression of one or more additional genes which affect juvenile to adult developmental growth transition such as Glossy15 or Cg1, in conjunction with such modulation of expression is also contemplated. Nucleic acid constructs for down-regulation of GRMZM2G362718 and/ or GRMZM2G096016 are also contemplated, as are transgenic plants and products produced there from, that demonstrate altered, such as extended juvenile growth, and display associated phenotypes such as enhanced yield, improved digestibility, and increased disease resistance. Plants described herein may be used, for example, as improved forage or feed crops or in biofuel production.

33 Claims, 12 Drawing Sheets

Specification includes a Sequence Listing.

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FIG. 1.



FIG. 2

AT5G55390.1 GRMZM2G362718_P01	MTFVDDDEEEDFSVPQSASNYYFEDDDKEPVSFARLPIQWSVEEKVDGS-GLGFYLRGRS MFDDDDDDGVDPQIEDVNRYYFEDGEEKPVCFSILPFQFGEDDSEAVFLRKDVFLCGFV :.***:: :***** :::**.*: **:*:. ::.
AT5G55390.1 GRMZM2G362718_P01	DNGLLPLHKLVKAWRYDLSNFQPEISVLTKDNIWIKLEEPRKSYGELIRTVLVTLHSIQF -DKNLPVYKEVVAWKIRLDSEHPNIYVLSIEHKWIKLLKPRKCYGDIVRSTLITVQMLHF : **::* * **: * :*:* **: :. **** :***.**:::*:.*::*:
AT5G55390.1 GRMZM2G362718_P01	LRRNPQASEKALWEKLTRSLRSYDVKPSQNDLVDHIGLIAEAAKRDRNLANSKFILAFLT FGRGEQRSSNHLWDHLDEVFGKYNPKPVEDDLNKHHTLIKLFVEKDQTLMKSKILQRLIE : * * *.: **::* . : .*: ** ::**:.* ** .::*:.* ::
AT5G55390.1 GRMZM2G362718_P01	KKPTKRRLPDEDNAKDDFIVGDEDTYVASDEDELDDEDDDFF NGFKRTKKALGMEAQSIVSDGWRARKNDDNNYGNKDDSGDDCDGDGSSDDGDGSSDDDVT : .: * **: *
AT5G55390.1 GRMZM2G362718_P01	ESVCAICDNGGEILCCEGSCLRSFHATKKDGEDSLCDSLGFNKMQVEAIQKYFCPNCEHK DQICALCDDGGHLLSCDGPCKRSFHPTKKDGRESKCESLHYTSAEVKRIGTYLCANCKNK :.:**:**:**.:*.:*.*:* * **** *****.:* *:** : :*: * .*:* *:**
AT5G55390.1 GRMZM2G362718_P01	IHQCFICKNLGSSDNSSGAAEVFQCVSATCGYFYHPHCVTRRLRLGNKEESEALERQII- QHQCFRCGELEPSHGPNAKVFQCNQASCGYFYHPKCIAQLLDPNATDGELERRIMS **** * :* *. *:**** .*:********:*::: * ***:*:
AT5G55390.1 GRMZM2G362718_P01	AGEYTCPLHKCSVCENGEVKTDSNLQFAVCRRCPKSYHRKCLPREISFEDIEDEDILTRA GMSFPCPIHWCFKCGHMENKAQRALQLAVCRRCPRAYHRECLPRDLSFGTKDK-DGNQRA : **:* * * . * *:: **:*******::***:***
AT5G55390.1 GRMZM2G362718_P01	WDGLLHNRVLIYCQEHEIDEELLTPVRDHVKFPFTEEQKVFVKEQRRILESHVGRDKARL WKLSDTIFIYCLDHEIDKDTGTTSRNHIKFPATPEYTKTKGLGNSKGRM *. * : ::*** :****:: * *:*:*** * *. :**:
AT5G55390.1 GRMZM2G362718_P01	KVKDPNLQDTCGKASKNSFRSSFPSSKDGFSTKKHGLVSSVPD- TGKRRKNKRRKNTDQSTKPTDLPNRLCGAESEQADNVGAKSTLPQI . * : ** *::: : : * *::*:
AT5G55390.1 GRMZM2G362718_P01	HSRKRKDIDPSIKHKMVPQKSQKMMEDSREAGKNKLGVKEARDAGKSKISLGERL VVEPHCAAKHLKGDPQIAKQGVA-ARQNGAETMKGHENQ : *: . **.* :: *. **.*
AT5G55390.1 GRMZM2G362718_P01	FSYTQEPNPVKPGRVIPVDSKHNKTDSIASKEPGSEIPTLDNDSQRRLLAVMKKATEEIT FGISFCVASTETEKRVTCLAQRGTC *.: : : : : :: :: ::
AT5G55390.1 GRMZM2G362718_P01	MGTILKKFKIQSTMSTHSTRNVVDKTITMGKVEGSVQAIRTALKKLEEGGNIEDAKAVCE LGTQYDGPSTKGNYDCSVQDTPMDDDVELDNVACI :** . **: * : *** :::* :*

FIG. 2 (continued)

AT5G55390.1 GRMZM2G362718_P01	PEVLSQILKWKDKLKVYLAPFLHGARYTSFGRHFTNPEKLQQIVDRLHWYADDGDMIVDF IAVDKYVNGRGKTQEDYTRKEAAQRK-DSSE : ::.* *:*. * *: *
AT5G55390.1 GRMZM2G362718_P01	CCGSNDFSCLMNAKLEETGKKCLYKNYDLFPAKNNFNFERKDWMTVSKDELEPGSKL NQGQNDALELDNLRMEMQADERPLEPGNKRDRK *.** * * :: ****.*
AT5G55390.1 GRMZM2G362718_P01	IMGLNPPFGVNASLANKFITKALEFRPKILILIVPPETERLDKKKSSYVLIWEDKT WQKNVYGLGSASGQKETLSRRENPRSDRGMVHSND : ** * : :*: *:*
AT5G55390.1 GRMZM2G362718_P01	FLSGNSFYLPGSVNEEDKQLEDWNLVPPPLSLUSRSDFAAKHKKIAEKHCHLSRDVGSSK SKTIYYRKGGTEVDNVDDHPLEKQDHQDTSSDGSKKRSRPVDNASGGNR *.:**: :: ::* *** ::*
AT5G55390.1 GRMZM2G362718_P01	LKIVEEEANASLHPLGASDGMCDDIPMEKDELEVAECVNKILVSEKIDTVETVARVHQSD -PYLDENKKRNLREDGRYA-HYEDWRSERNTAADTSGYKAQSE-EKPVWTNTRTGSRE ::*: : .*: * :* :*: .*: ** .** :*:
AT5G55390.1 GRMZM2G362718_P01	HLSRRSQLKKEGKTKDYSGRKLGKSMDSNNVDWKSNDMEEDQGELSRAPESIKVKIPEMT HSLDRQRIECGDSYRGTYNNRQRHEWLHPHASGNSSRIGWDDR * *.:::* * . :: :* . :* . ::.
AT5G55390.1 GRMZM2G362718_P01	SDWQSPVRSSPDDIYAVCTSISTTTPQRSHEAVEASLPAITRTKSNLGKNIREHGCKVQG RQWSSSRSPFPSAEFGGDRSCSRAHPRGSKYRTGGRHDHPQYLG :*.* *. :. * * : *: *: *: *: *: *: *: *: *:
AT5G55390.1 GRMZM2G362718_P01	TGKPEVSRDRPSSVRTSREDIYTVRPSPENTGQKPFEAFEPSYGASLSHFDDG LGTPQHGTSRPHHTMGWDRDTFHDHQHGRRPPHHTMGWDRAPFRDHQHGEYDDS *.*:*** : ** * : **
AT5G55390.1 GRMZM2G362718_P01	LAAKYGGFGGGYRMPDPPFLPDQFPLRNGPNEMFDFRGYSDLDRGIGQREYPQQYGGHLD RYGEYDATDNGPDSAHRPYTAAGVAGRSAPSYQL-AGGYGEGSRAWR .:*. * . * * **. : **. *.*.
AT5G55390.1 GRMZM2G362718_P01	PMLAPPPPPNLMDNAFPLQQRYAPHFDQMNYQRMSSFPPQPPLQPSGHNLLNPHDFPLPP PVTDKYAPWPLP- *: ::***
AT5G55390.1 GRMZM2G362718_P01	PPPSDFEMSPRGFAPGPNPNYPYMSRSGGWIND

FIG. 3

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRMZM2G362718_P01 Rice|LOC_0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRM2M2G362718_P01 Rice|LOC_0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRMZM2G362718_P01 Rice|LOC_0808g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRMZM2G362718_P01 Rice|LOC_0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRMZM2G362718_P01 Rice|LOC 0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRMZM2G362718_P01 Rice|LOC 0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRM2M2G362718_P01 Rice|LOC_0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRMZM2G362718_P01 Rice|LOC_0s08g24946.1|13108.m23057|protein -MSDDDDGVDPEIEDVNGYYFEDGEGEPVCFSILPFQFGENDNEADFSRKNVFLHGFVDQ NFDDDDDGVDPQIEDVNRYYFEDGEEKPVCFSILPFQFGEDDSEAVFLRKDVFLCGFVDK -MMSSDDDLEPQLKAVENYYFVDDNDVPVSFDVLPFQFDAAEGVASF-KKDVYLRGFTDG : ...** ::*::: *: *** * : ***** : **.*

SP-HVYKEVVAUKIL------NL-PVYKEVVAUKIRLDSEHPNIYVLSIEHKUIKLLKPRKCYGDIVRSTLITVQMLHFFG GLQKVYKQVVAUKLVLDGDSPEIAVLSTEGSUIALLKPRPSYEETIRSVLITVEMLHFVR ***:*****:

FERTKKV-----KNDDNNYGN-----KDDSGDDCDGDGSSDDGDG FKRTKKALGMEAQSIVSDG-URAR---KNDDNNYGN-----KDDSGDDCDGDGSSDDGDG IMEKTNEVGSNNLDNKREPDIKQEPDIKQEPVAAGDEMEEIVEEGIPDAPSNDDDDDEED : ...:

SSDDDVTDQICALCDDGGHLLSCDGPCKRSFHPTKKDGRESKCESLHYTSAEVKRIGTYL EEDGDLFDSVCAICDNGGELLCCEGPCNRSFHAKIRDGEDSYCATLGYTKAEVKALKNFV

-NPRAWKLSKTIFFYCLDHEIDKDTRTASRNHIKFPATPECTK----TKELGNRKGRNT -NQRAWKLSDTIFIYCLDHEIDKDTGTTSRNHIKFPATPEYTK----TKGLGNSKGRNT IITRAWELSKRILIYCLDHEIDLDIGTPPRDHIKFPHVEKSAYSAKKKVKELAEKKRRIC ****:**. *::********* * * *:****** . :: . * *:: * *:

FIG. 3 (continued)

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRMZM2G362718_P01 Rice|LOC 0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRMZM2G362718_P01 Rice|LOC 0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRMZM2G362718_P01 Rice|LOC_0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRMZM2G362718_P01 Rice|LOC_0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRMZM2G362718_P01 Rice|LOC_0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRMZM2G362718_P01 Rice|LOC_0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRM2M2G362718_P01 Rice|LOC_0s08g24946.1|13108.m23057|protein

Sorghum|Sb02g003420.1_Sb02g003430.1 Maize|GRM2M2G362718_P01 Rice|LOC_0s08g24946.1|13108.m23057|protein

GKRR-----KNTDQSTEPTEL---SNRLYGAESEQADNVGAKSTSPQIVVEPHCAAKVLK GKRRKNKRRKNTDQSTKPTDL---PNRLCGAESEQADNVGAKSTLPQIVVEPHCAAKHLK DDSY-----VSEPLQKRAKLNEKFNAKGDKSKKAGVKSEFEEVLESEKKKTRSLK ::*: *. *:.::.:.*.** :::..: :: ** GDPQIEQSIIGV---AGSQNGAETMNGHEKQFG-----IS-CVARTETEKRVTY GDPQIAK--QGV---AARQNGAETNKGHENQFG-----ISFCVASTETEKRVTC KRTQPEEPLVECAAAAANNANRPVKEREKELGTSSLDMGKIPLSSFPIVDSETEKRISA * : *. :*. . :: :*:::* * :. :*****:: LAQKG-----KDFEL LAQKG-----KDFEL LAQRG-----DDVEL CARGESTKGNYDCSVQDTPND----DDVEL LVEKEVSSLTVADISRRCVIPSTYACSGRQIDKIVVRGKLERSIQAVKAALQKLENGGAV *.:: ;* * * .: : *:*. 1 D--NVAYR-----IMEDKYANGREET--QEDYTRKETAHRKDSSENQGQN D--WACI-----IAVDKYVNGRGKT--QEDYTRKEAAQRKDSSENQGQN DDAKAVCESEVLRQLTRUHNKLRVYLAPFIHGNRYTSFGRHFTKKEK------; ; , * * . . . * * . . . * * * :.. DVLELD--NLWVEIQAD--GSPLEPGNKRYK--EENAYGLGSASGHEKET--SSSRRENV DALELD--NLRMENQAD--ERPLEPGNKRDRKWQKNVYGLGSASGQKE----TLSRRENP -LIEIAEKLHWYVQPGDHKSNNVDPETRPRR--VNMLRGFGALSQFMKEKLDKVGKRCNF .* ::*.: : : *:*: * : ..:* *

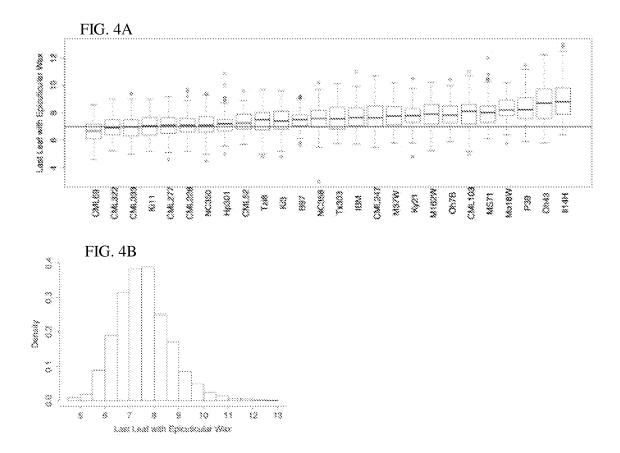
QSDRGNVPNNDSKTIDYRKG-GTTLDNNVYDH3---SEGSYPCQGECS---HSKCN----RSDRGNVHSNDSKTIYYRKG-GTEVDNVD-DHP---LE-------KNYD-VIQPKNS--FSFEKRDWNTVRQKELPHGSKLINGLNPPFGPKAMLANKFIDKALT :. :: ::* : :* : :* : *

GR-DAHYEDRRTERNTAADTSRYKCRDKIQLDRREPELVGRNTRARSSEHSPERQRMERD GR-YAHYEDWRSERNTAADTSGYKAQSE------EKPVUTNTRTGSREHSLDRQRIECG PLYLUSRPDWTQKHKRIAEQHGHTKANV--FSHNEEDLVYLFEDRATQNHDVNNKNYTSG

* ::: *: :. . . * ::*.:..

FIG. 3 (continued)

Sorghum|Sb02g003420.1 Sb02g003430.1 -----GSYPGT-YNRRRYESL-H-----NFNPPRSGCDDRRQLSPCQSSFPL Maize|GRMZN2G362718_P01 -----DSYRGT-YNNRQRHEWLH---PHASGNSSRIGUDDRRQUSSSRSPFPS Rice|LOC 0s08g24946.1|13108.m23057|protein -NGNFTAEKPVQADAFPPEKLVEVAYEEMKVASNRSSNYQSDQISVHDERD---AHSDLPM 11 1 11 : : . .*.*: .:* :* Sorghum|Sb02g003420.1 Sb02g003430.1 PEFCGDHSH--L-Y--PRDS---TIGRH-----NPHRYLG----I------Maize|GRMZM2G362718 PO1 AEFGGDRSC--S-RAHPRGSKYRTGGRH-----DHPQYLG----LGTPQHGTSRPH Rice|LOC 0s08g24946.1|13108.m23057|protein -SRHNSMKAKEVSNSSRDRRKSDKTGHEADSDNSILPSDSRNFLHKSGNLEPPISS----R : .:* Sorghum|Sb02g003420.1 Sb02g003430.1 -----PQYGP Maize|GRMZN2G362718 PO1 HTMGWDRDTFHDHQHGRRPPHHTMGWDRAPFRDHQHGEYDDSRYGEYDATDNGPDSAHRP Rice|LOC 0s08g24946.1|13108.m23057|protein SGYTLERLRYHDNHFDHLVGEHSSSS-----LQMPIFEDSYFRSV-----NE Sorghum|Sb02q003420.1 Sb02q003430.1 YMAASAAGHSAVCYRLAGGYGEGSRASRPVTDW--YAPHLD-----Maize|GRMZN2G362718 PO1 YTAAGVAGRSAPSYQLAGGYGEGSRAWRPVTDK--YAPVPL------Rice|LOC_0s08g24946.1|13108.m23057|protein YGVASVENN---IALSTDNVGAGSRMYSPDPELNGYAVDPTVNAYGSVSGGTGGSFYRRQ * .*.. : * *** * : ** Sorghum|Sb02g003420.1 Sb02g003430.1 -----RTNCQPRSQIDLQ-----Maize|GRMZM2G362718 PO1 _____p_____p_____ Rice|LOC 0s08g24946.1|13108.m23057|protein NLEDYTMDSSESAQMNPVPGRDVQEYARTYYGHNRDEVPQTAINTPSMDIRTHIRMYGRH Sorghum|Sb02g003420.1 Sb02g003430.1 Maize|GRMZN2G362718 PO1 Rice|LOC_0s08g24946.1|13108.m23057|protein IRDDHTQTTMNPPANDIRAQIRNYGQHATSDHQHASRYSSGSPDARFEQQPSFTSYGMPS Sorghum|Sb02g003420.1 Sb02g003430.1 -LQASRPVTDKYAPQLELTNYPPRSQSDL-----QYCTTTI*-----Maize|GRMZM2G362718 PO1 Rice|LOC_0s08g24946.1|13108.m23057|protein LGSTGRSMMDRYSPSIDETSYRTGQRGPYNASDFRRDRHPDDMNFALHNQYPYPHPGSSG Sorghum|Sb02g003420.1 Sb02g003430.1 ____ Maize|GRMZN2G362718 PO1 ----Rice|LOC 0s08g24946.1|13108.m23057|protein -GWHD



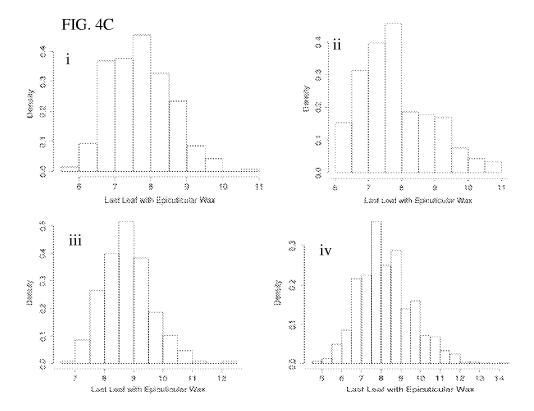
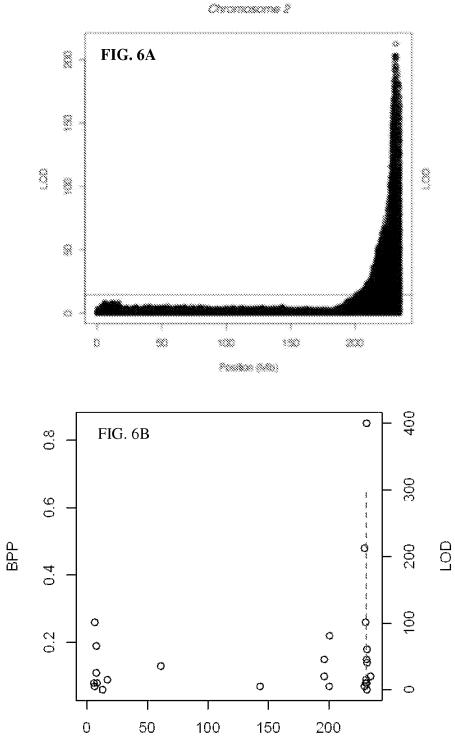


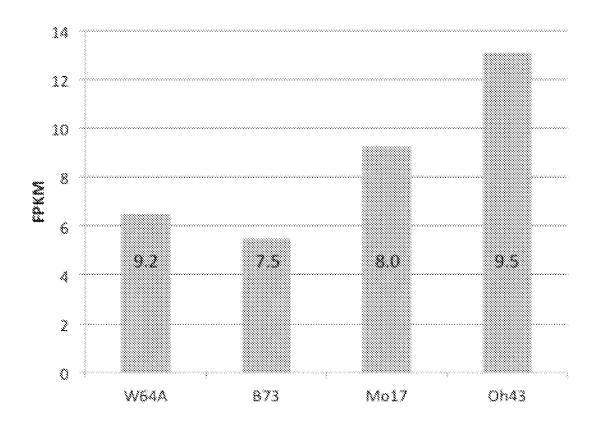
FIG. 5

		AtV			- Style	01078	200012	
No. of lines	3	875	31	12	243	227	573	\$09
Year	20084	2039 ³	2008 ² 2030 ²	2009 ² 2011 ²	2010 ¹ 2011 ³	2010, 2011,	20092 20102	2010;
Location	W3A	ARL	84M	ABL	8 886	844	ARL	WW.
			A81	WAA				
Plot size/ Plants	3	/42	2/2	\$2	1/15	1/15	2/42	1/15
Traits Measured	Transition DAP DAS	Transition Node # Plant Height	Transiti Node # Plant M DAP		Transition	Transition	Transition Node # Flont Height GDD	Transition



Chromosome 2 Position (Mb)

FIG. 7



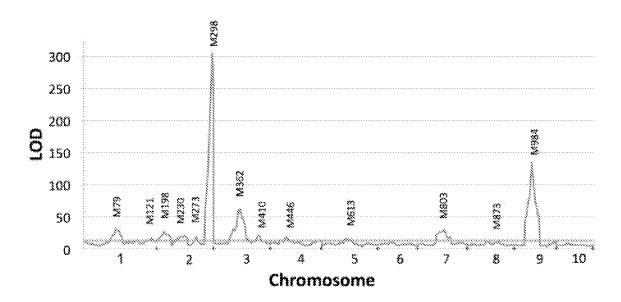


FIG. 8

EXTENDING JUVENILITY IN GRASSES

This application is a divisional of U.S. application Ser. No. 13/834,114, filed Mar. 15, 2013 (pending), which application claims the benefit of U.S. Provisional Appl. Ser. No. ⁵ 61/651,540 filed May 24, 2012, the entire disclosures of which are incorporated herein by reference.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under DE-FC02-07ER64494 awarded by the U.S. Department of Energy. The Government has certain rights in the invention.

INCORPORATION OF SEQUENCE LISTING

The sequence listing that is contained in the file named "WARF103US_ST25.txt", which is 116,564 bytes (measured in MS-WINDOWS) and was created on Mar. 15, ²⁰ 2013, is filed herewith by electronic submission and incorporated herein by reference.

BACKGROUND OF THE INVENTION

Field of the Invention

The invention relates to methods and compositions for altering the juvenile phase of growth of plants.

Background of the Invention

Juvenile and adult vegetative tissues in grasses differ 30 dramatically in anatomy, biochemical composition, and in their ability to withstand biotic and abiotic stresses. Juvenile plants cannot flower and are capable of only vegetative growth. Juvenile leaf tissue further has inherent resistance to specific abiotic stresses such as cold and drought, is gener- 35 ally less recalcitrant when used for processing for biofuels, and may be more digestible when used as feed. Researchers have identified certain parameters such as age, leaf number, and certain growth conditions as playing a role in the maturation of juvenile plant tissue to adult plant tissue. 40 However, the genetic triggers controlling the transition between juvenile and adult tissue in plants has not been well understood. Therefore, increasing the proportion of the plant that is juvenile has potential benefit for improving the yield and processing ability of plant biomass, among other agro- 45 nomic traits.

SUMMARY OF THE INVENTION

In one aspect the invention provides a polynucleotide 50 molecule comprising a sequence selected from the group consisting of: (a) a sequence encoding a polypeptide at least 85% identical to SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:17; wherein the polypeptide regulates juvenile to adult phase change in grass 55 plant leaves; (b) a sequence comprising SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:16; (c) a sequence hybridizing to (b) under wash conditions of 0.15 M NaCl and 70° C. for 10 minutes, wherein the sequence encodes a protein that regulates juvenile to adult 60 phase change in grass plant leaves; (d) a sequence comprising at least 85% sequence identity over its full length to the full length of SEQ ID NO:2 or SEQ ID NO:16, wherein the sequence encodes a protein that regulates juvenile to adult phase change in grass plant leaves; and (e) a sequence 65 complementary to (a), (b), (c) or (d), further wherein the polynucleotide molecule is operably linked to a heterolo-

gous promoter functional in plants. In a particular embodiment the polynucleotide molecule comprises the coding sequence of SEQ ID NO:2. In another embodiment the polynucleotide molecule comprises the coding sequence of SEQ ID NO:16.

Other embodiments of the invention provide a recombinant vector comprising such a polynucleotide molecule. In certain embodiments, the invention provides the recombinant vector, further comprising an additional polynucleotide sequence which, after being transcribed, regulates the timing of the juvenile to adult phase change in a plant. Thus, in particular embodiments the recombinant vector may comprise an additional polynucleotide sequence which encodes all or part of a sequence selected from the group consisting of: Glossy15, Cg1, a homolog of either thereof, and/or a sequence complementary thereto.

In some embodiments the recombinant vector further comprises at least one additional sequence chosen from the group consisting of: a regulatory sequence such as a pro-20 moter, a selectable marker, a leader sequence and a terminator. The additional sequence may be a heterologous sequence. In some embodiments the promoter is a tissuespecific promoter. In a particular embodiment the promoter directs expression in leaf tissue. In certain embodiments the 25 recombinant vector may be defined as an isolated expression cassette.

In other embodiments, the recombinant vector comprises a first sequence selected from the group consisting of: (a) a sequence encoding a polypeptide at least 85% identical to SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:17; wherein the polypeptide regulates juvenile to adult phase change in grass plant leaves; (b) a sequence comprising SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8; or SEQ ID NO:16; (c) a sequence hybridizing to (b) under wash conditions of 0.15 M NaCl and 70° C. for 10 minutes, wherein the sequence encodes a protein that regulates juvenile to adult phase change in grass plant leaves; (d) a sequence comprising at least 85% sequence identity over its full length to the full length of SEQ ID NO:2 or SEQ ID NO:16, wherein the sequence encodes a protein that regulates juvenile to adult phase change in grass plant leaves; and (e) a sequence complementary to (a), (b), (c) or (d), or a fragment thereof; and a second sequence comprising the reverse complement of the first sequence, wherein the expression of the construct in a plant down regulates the expression of a coding sequence and/or encoded polypeptide in the plant. Some embodiments of the invention provide the recombinant vector further comprising an additional polynucleotide sequence which, after being transcribed, regulates the timing of the juvenile to adult phase change in a plant.

Another aspect of the invention is a transgenic plant or seed comprising a recombinant vector comprising a polynucleotide molecule comprising a sequence selected from the group consisting of: (a) a sequence encoding a polypeptide at least 85% identical to SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:17; wherein the polypeptide regulates juvenile to adult phase change in grass plant leaves; (b) a sequence comprising SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:16; (c) a sequence hybridizing to (b) under wash conditions of 0.15 M NaCl and 70° C. for 10 minutes, wherein the sequence encodes a protein that regulates juvenile to adult phase change in grass plant leaves; (d) a sequence comprising at least 85% sequence identity over its full length to the full length of SEQ ID NO:2 or SEQ ID NO:16, wherein the sequence encodes a protein that regulates juve-

nile to adult phase change in grass plant leaves; and (e) a sequence complementary to (a), (b), (c) or (d), further wherein the polynucleotide molecule is operably linked to a heterologous promoter functional in plants. In yet other embodiments, the transgenic plant may comprise a recom-5 binant vector as described above, comprising an additional polynucleotide sequence which, after being transcribed, regulates the timing of the juvenile to adult phase change in the plant.

Yet another aspect of the invention is a transgenic plant or 10 seed comprising a first sequence selected from the group consisting of: (a) a sequence encoding a polypeptide at least 85% identical to SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:17; wherein the polypeptide regulates juvenile to adult phase change in grass 13 plant leaves; (b) a sequence comprising SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, or SEQ ID NO:16; (c) a sequence hybridizing to (b) under wash conditions of 0.15 M NaCl and 70° C. for 10 minutes, wherein the sequence encodes a protein that regulates juvenile to adult 20 phase change in grass plant leaves; (d) a sequence comprising at least 85% sequence identity over its full length to the full length of SEQ ID NO:2 or SEQ ID NO:16, wherein the sequence encodes a protein that regulates juvenile to adult phase change in grass plant leaves; and (e) a sequence 25 complementary to (a), (b), (c) or (d), or a fragment thereof; and a second sequence comprising the reverse complement of the first sequence, wherein the expression of the construct in a plant down regulates the expression of a coding sequence and/or encoded polypeptide in the plant. 30

In some embodiments the transgenic plant may further be defined as a monocotyledonous plant. In particular embodiments the transgenic plant is further defined as a member of the Poaceae. In more particular embodiments the transgenic plant is further defined as a member of the Panicoideae or 35 the Pooideae. In yet more particular embodiments the transgenic plant may further be defined as maize, rice, sorghum, or switchgrass.

The invention also provides a seed or cell of such a transgenic plant wherein the seed or cell comprises recom- 40 binant vector.

In certain embodiments the plant is a plant wherein the last leaf with epicuticular wax is produced later during plant development relative to that found in an otherwise isogenic plant lacking the recombinant vector.

In another aspect, the invention provides a method of altering the timing of juvenile to adult phase change in a plant, the method comprising modulating the expression of GRMZM2G362718 or GRMZM2G90616, or a homolog of either thereof, in the plant. Other contemplated embodi- 50 ments of such methods further comprise modulating the expression of at least a second gene which regulates the timing of the juvenile to adult phase change in a plant. In particular embodiments the second gene is selected from the group consisting of Glossy15 and Cg1. Thus in some 55 embodiments the method comprises expressing a recombinant vector or construct, as defined above, in the plant. In certain embodiments, the timing of the juvenile to adult phase change is extended (delayed) relative to a wild type plant (i.e. an otherwise essentially isogenic plant not com- 60 prising such a recombinant construct). In some embodiments the method comprises mutagenizing GRMZM2G362718 or GRMZM2G90616 or a homolog thereof.

In certain embodiments of the method, the timing of 65 juvenile to adult phase in the plant is extended relative to a wild type plant. In particular embodiments, the timing of

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juvenile to adult phase change is calculated by a method comprising counting the last leaf displaying epicuticular wax.

In some embodiments of the method, the plant exhibits a trait selected from the group consisting of: an increase of at least one in the numbering of the last leaf which displays epicuticular wax or which does not contain abaxial trichomes; an altered proportion of juvenile, transitional, or adult leaves; enhanced yield of vegetative tissue; enhanced digestibility of vegetative tissue; enhanced resistance to a plant pest; and enhanced resistance to a plant disease. In certain embodiments of the method, the plant has altered development or morphology when compared to a wild type plant, further wherein the plant displays a trait selected from the group consisting of: enhanced digestibility, enhanced abiotic stress tolerance, and improved utility for biofuel production.

Yet another aspect of the invention provides a method of producing plant biomass, the method comprising: (a) obtaining a plant comprising a recombinant vector as described above; and (b) preparing biomass from said plant or a part thereof. In certain embodiments the method further comprises producing biofuel from the biomass. The method may also comprise producing food or feed from the biomass.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 depicts a maize transition leaf, with areas of juvenile tissue, and other areas of adult tissue.

FIG. **2** depicts a CLUSTAL protein alignment of GRMZM2G362718 with *Arabidopsis* homolog AT5G55390.1.

FIG. **3** shows a CLUSTAL protein alignment of GRMZM2G362718 with homologs from sorghum and rice.

FIG. 4 depicts bar plots showing variation of transition leaf numbering. (A) Variation for transition leaf by NAM family, labeled by the non-B7 parent on top. The horizontal line at leaf 7 represents the average transition leaf for B73; (B) Phenotypic distribution of the last leaf with epicuticular wax in the NAM population. Leaf number distribution ranged from leaf 4.5 to leaf 13.25; (C) Phenotypic variation for transition leaf in the IBM, NYH, OWRI, and Wisconsin diversity panel populations (plots i-iv, respectively).

FIG. **5** depicts a summary table of studies providing phenotypic data from defined mapping populations.

FIG. **6** shows genome wide association results with 1.6 million polymorphic markers across the NAM population. (A) Position of significant QTL found on the long arm of chromosome 2.; (B) sub sampling analysis confirming location of QTL on chromosome 2. Dashed line represents F-test log(1/P) in the final joint linkage model. Vertical position of points represents bootstrap posterior probability (BPP) of the SNP.

FIG. 7 depicts RNA sequence expression data of GRMZM2G362718 for four maize inbred lines that are parents of RIL mapping populations (Oh43×W64A; B73×M017; B73×Oh43). The inbred's transition phenotype is displayed numerically within the bar.

FIG. 8 depicts LOD scores for detecting the presence of QTL located on any of chromosomes 1-10. Stepwise regression with covariates was used in joint QTL mapping of all NAM populations with a threshold value of 12.26 (Buckler et al., *Science* 325:714-718, 2009).

BRIEF DESCRIPTION OF THE SEQUENCES

- SEQ ID NO:1 GRMZM2G362718 genomic nucleotide sequence from Z. mays B73.
- SEQ ID NO:2 GRMZM2G362718 nucleotide coding 5 sequence from Z. mays B73, with UTR.
- SEQ ID NO:3 GRMZM2G362718 predicted protein sequence from Z. mays B73.
- SEQ ID NO:4 GRMZM2G362718 nucleotide coding sequence from Z. mays Mo17.
- SEQ ID NO:5 GRMZM2G362718 predicted protein sequence from Z. mays Mo17.
- SEQ ID NO:6 GRMZM2G362718 nucleotide coding sequence from Z. mays Oh43.
- SEQ ID NO:7 GRMZM2G362718 predicted protein 15 sequence from Z. mays Oh43.
- SEQ ID NO:8 GRMZM2G362718 nucleotide coding sequence from Z. mays W64A.
- SEQ ID NO:9 GRMZM2G362718 predicted protein sequence from Z. mays W64A.
- SEQ ID NO:10 Predicted protein sequence of AT5G55390.1 from Arabidopsis thaliana.
- SEQ ID NO:11 Predicted protein sequence of Os08g24946.1 from Oryza sativa.
- SEQ ID NO:12 Predicted protein sequence of 25 Sb02g003420.1 from Sorghum bicolor.
- SEQ ID NO:13 Predicted protein sequence of Bradi4g27190.1 from Brachypodium distachyon.
- SEQ ID NO:14 Glossy15 nucleotide coding sequence from Z. mays W64A (GenBank U41466).
- SEQ ID NO:15 Glossy15 predicted protein sequence from Z. mays W64A.
- SEQ ID NO:16 GRMZM2G096016 nucleotide coding sequence from Z. mays.
- SEQ ID NO:17 GRMZM2G096016 predicted protein 35 sequence from Z. mays.
- SEQ ID NO:18 Cg1 nucleotide coding sequence for miR156 transcripts.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides a gene, and methods for its use, to modulate the transition of plant tissue from the juvenile to the adult phase of growth. By modulate is meant to either 45 hasten or delay such transition. A plant or product comprising a recombinant DNA construct comprising such a gene may exhibit improved properties relating to, for instance, biofuel production and/or processing, use as animal feed, and resistance to a plant pest or plant disease, and is also an 50 aspect of the invention. Seed of such a plant is also an aspect of the invention. Thus, for instance, one or more agronomic traits of a grass, such as a member of the Poaceae including corn, sorghum, rice, and switchgrass, among others, may be enhanced. Such traits may include one or more of: improved 55 vegetative yield; reduced recalcitrance during biofuel processing; improved resistance to a plant pest such as European Corn Borer; improved resistance to a plant disease such as a rust disease; enhanced cold tolerance; enhanced digestibility of an animal feed ingredient such as plant vegetative 60 tissue; and improved nutritional content of plant vegetative tissue.

GRMZM2G362718 is a gene of previously unknown function in corn (maize) which was identified through chromosomal mapping of juvenile plant tissue, and appar- 65 ently functions as a trigger of juvenile to adult growth phase change. Predicted protein alignments (e.g. FIGS. 2-3) show

that this gene encodes a protein with some similarity to the enhanced downy mildew 2-transcription factor (EDM2) of Arabidopsis, rice, Brachypodium, and sorghum (displaying approximately 52.9, 56.7, 42.9, 63.2, percent similarity, respectively). Modulating, such as disrupting, the expression of GRMZM2G362718 may alter, such as extend, the temporal duration during which a plant is in a juvenile phase of growth. Homologs of GRMZM2G362718 exist in other plant species such as Arabidopsis, rice (Oryza sativa), Brachypodium, and sorghum (Sorghum bicolor), among others; see exemplary sequence database accession numbers AT5G55390.1, Os08g24946.1, Bradi4g27190.1, and Sb02g003420.1, respectively (SEQ ID NOs: 10-13), so this effect may be seen in other plants, e.g. monocotyledonous plants such as grass plants (e.g. members of the Poaceae such as maize, rice sorghum, or switchgrass), as well as dicotyledonous plants.

An additional genome wide association analysis, using transcript presence/absence as the dependent variable, iden-20 tified GRMZM2G096016 (LOC100285984; Maize Genome Sequencing Project; MaizeSequence.org; Schnable et al. Science, 326:1112, 2009) on chromosome 2 as also being associated with a change in the timing of production of the last juvenile leaf, e.g. when vegetative phase change was scored by identifying the last leaf with epicuticular wax. Although close in proximity (~24.5 Kb) to the first candidate gene underlying this QTL (i.e. GRMZM2G362718), GRMZM2G096016, which encodes a predicted nuclear transcription factor Y-subunit A-10, is not in linkage disequilibrium with EDM2. Thus, in particular embodiments, the invention provides methods and compositions for moduexpression of GRMZM2G362718 and/or lating GRMZM2G096016, each found on maize chromosome 2, or homologs thereof, in order to alter the timing of vegetative phase change in maize, rice, sorghum, switchgrass, or other plants.

MicroRNAs play an important role in regulating the timing of plant developmental transitions. By regulating transcripts of developmental genes, miRNAs control some 40 aspects of leaf morphology, polarity and floral organ identity, and some stress responses (Willmann and Poethig, Curr. Opin. Plant Biol. 8:548-552, 2005) as well as the timing of juvenile to adult vegetative phase change. The maize and Arabidopsis signaling pathway and miRNA expression cascade are similar (Nonogaki, Plant Cell Physiol. 51:1840-1846, 2010). In maize, the Corngrass1 (Cg1) mutant retains juvenile traits resulting in initiation of tillers at each leaf axil causing a bush-like appearance. This phenotype is due to the ectopic overexpression of two tandem miR156 genes (Chuck et al., Nature Genetics 39:544-549, 2007; Chuck et al., PNAS 108:17550-17555, 2011;GenBank: GQ905502.1). miR156 targets SBP-domain transcription factors-teosinte glume architecture1 (tga1) in maize and SPL13 in Arabidopsis. SPB transcription factors up regulate miR172 in both species and miR172 targets AP2-like transcription factors such as glossy15 in maize and SCHN-ARCHSAPFEN (SNZ) in Arabidopsis. Glossy15 maintains expression of juvenile traits in the leaf epidermis and suppresses adult traits. Mutants of glossy15 (Gl15) show premature vegetative phase change to the adult state (Evans et al., Devel. 120:1971-1981, 1994). In Cg1 mutants of maize, the overexpression of miR156 causes a decrease in tga1 and miR172 (Chuck, 2007, ibid), which cause an increase in expression of Glossy15.

In further embodiments, the invention provides methods and compositions for modulating the expression of one or more additional genes involved in regulating the juvenile to

adult growth phase change, in conjunction with modulating GRMZM2G362718 expression of and/or GRMZM2G096016, or homologs thereof. Thus, for instance, the expression of Glossy15 (Gl15; GRMZM2G160730), or Cg1, or a homolog thereof, may be 5 modulated along with modulation of expression of GRMZM2G362718 and/or GRMZM2G096016, or a homolog thereof, in a plant.

I. NUCLEIC ACIDS, POLYPEPTIDES AND PLANT TRANSFORMATION CONSTRUCTS

Certain embodiments of the current invention concern polynucleotide sequences comprising a GRMZM2G362718 coding sequence, or a GRMZM2G096016 coding sequence. 15 Exemplary coding sequences for use with the invention include SEQ ID NO: 2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, and SEQ ID NO:16, encoding the polypeptides of SEQ ID NO: 3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, and SEQ ID NO:17, respectively. Constructs may 20 also be designed that are complementary to all or part of the promoter and other control regions, exons, introns or even exon-intron boundaries of a gene.

Other contemplated constructs may be designed which, in addition to a GRMZM2G362718 coding sequence, 25 the following: Algorithm: Needleman and Wunsch (1970); GRMZM2G096016 coding sequence, or homolog thereof, also comprise all or part of a Glossy15 or Cg1 and/or other coding sequence, wherein such additional sequence also modulates the juvenile to adult growth phase change. Thus for instance, such constructs, in addition to comprising all or 30 part of a GRMZM2G362718 coding sequence, or homolog thereof, may further comprise, for instance, a Glossy15 coding sequence, or homolog thereof. Exemplary coding sequences for use with the invention therefore include SEQ ID NO:14, encoding the polypeptide of SEQ ID NO:15, and 35 SEQ ID NO:18.

The invention provides a nucleic acid sequence identical over its entire length to each coding sequence provided herein. The invention further provides a nucleic acid sequence displaying at least 85%, 90%, 95%, or 99% 40 identity over its entire length to a the full length, or a fragment, of the coding sequence provided herein. The invention also provides the coding sequence for the polypeptide or a fragment thereof, as well as the coding sequence for the polypeptide or a fragment thereof in a reading frame 45 with other coding sequences, such as those encoding a leader or secretory sequence, a pre-, pro-, or prepro-protein sequence. The nucleic acid can also include non-coding sequences, including for example, but not limited to, noncoding 5' and 3' sequences, such as the transcribed, untrans- 50 lated sequences, termination signals, ribosome binding sites, sequences that stabilize mRNA, introns, polyadenylation signals, and additional coding sequence that encodes additional amino acids. For example, a marker sequence can be included to facilitate the purification of a fused polypeptide. 55 Nucleic acids of the present invention also include nucleic acids comprising a structural gene and the naturally associated sequences that control gene expression.

"Identity," as is well understood in the art, is a relationship between two or more polypeptide sequences or two or more 60 polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as determined by the match between strings of such sequences. Methods to determine "identity" are 65 designed to give the largest match between the sequences tested. Moreover, methods to determine identity are codified

in publicly available programs. "Identity" can be readily calculated by known methods. Computer programs can be used to determine "identity" between two sequences these programs include but are not limited to, GCG; suite of BLAST programs, three designed for nucleotide sequences queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries (BLASTP and TBLASTN). The BLASTX program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., 10 et al., NCBI NLM NIH, Bethesda, Md. 20894; Altschul, S.,

et al., J. Mol. Biol. 215:403-410, 1990). The well known Smith Waterman algorithm can also be used to determine identity.

Parameters for polypeptide sequence comparison include the following: Algorithm: Needleman and Wunsch (J. Mol. Biol. 48:443-453, 1970); Comparison matrix: BLOSUM62 from Hentikoff and Hentikoff, (PNAS 89:10915-10919, 1992); Gap Penalty: 12; and Gap Length Penalty: 4. A program which can be used with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison Wis. The above parameters along with no penalty for end gap may serve as default parameters for peptide comparisons.

Parameters for nucleic acid sequence comparison include Comparison matrix: matches=+10; mismatches=0; Gap Penalty: 50; and Gap Length Penalty: 3. A program which can be used with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison Wis. The above parameters may serve as the default parameters for nucleic acid comparisons.

The present inventors have identified chromosomal regions responsible for such growth, and in particular a specific candidate gene termed GRMZM2G362718 that may trap a plant in a juvenile phase of growth. Marker assisted breeding as well as methods of genetic modification may thus be used to introduce or introgress this gene, a modified version of this gene, or the described linkage group, into a plant to alter the timing of the juvenile to adult growth transition to achieve agronomic improvement. In certain embodiments of the invention, the process for producing such plants or lines comprises introducing a recombinant copy of GRMZM2G362718 or GRMZM2G096016, or a variant thereof into a plant. In other embodiments, the method comprises introgressing at least one chromosomal locus mapping to QTL bounded by markers mmc2184 and mmp183 on maize chromosome 2 into a plant. In other embodiments the function of a gene controlling the juvenile to adult phase change may be disrupted, allowing for enhanced juvenile growth, such as by delaying the juvenile to adult growth phase transition.

Vectors used for plant transformation may include, for example, plasmids, cosmids, YACs (yeast artificial chromosomes), BACs (bacterial artificial chromosomes) or any other suitable cloning system, as well as fragments of DNA there from. Thus when the term "vector" or "expression vector" is used, all of the foregoing types of vectors, as well as nucleic acid sequences isolated there from, are included. It is contemplated that utilization of cloning systems with large insert capacities will allow introduction of large DNA sequences comprising more than one selected gene. In accordance with the invention, this could be used to introduce genes corresponding to an entire biosynthetic pathway into a plant. Introduction of such sequences may be facilitated by use of bacterial or yeast artificial chromosomes (BACs or YACs, respectively), or even plant artificial chromosomes.

II. ANTISENSE AND RNAI CONSTRUCTS

A polynucleotide construct of the present invention may comprise a DNA for expression of an antisense RNA, siRNA or miRNA, which modulates expression of a 5 GRMZM2G362718 or GRMZM2G096016 coding sequence. By "modulates expression" is meant an increase or a decrease in such expression. Techniques for RNAi are well known in the art. Antisense and RNAi treatments represent one way of altering agronomic characteristics in 10 accordance with the invention (e.g., by down regulation of a GRMZM2G362718 and/or GRMZM2G096016 coding sequence). In particular, constructs comprising a GRMZM2G362718 coding sequence, including fragments thereof (or a GRMZM2G096016 coding sequence or fragments thereof), in antisense orientation, or combinations of sense and antisense orientation, may be used to decrease or effectively eliminate the expression of a GRMZM2G362718 or GRMZM2G096016 coding sequence in a plant and to alter agronomic characteristics (e.g., leaf morphology or 20 disease resistance). Accordingly, each of these may be used to "knock-out" the function of a GRMZM2G362718 or GRMZM2G096016 coding sequence or homologous sequences thereof.

III. GENETIC TRANSFORMATION

Suitable methods for transformation of plant or other cells for use with the current invention are believed to include virtually any method by which DNA can be introduced into 30 a cell, such as by direct delivery of DNA such as by PEG-mediated transformation of protoplasts. These methods and their use are well known in the art.

After effecting delivery of exogenous DNA to recipient cells, the next steps generally concern identifying the trans- 35 formed cells for further culturing and plant regeneration. In order to improve the ability to identify transformants, one may desire to employ a selectable or screenable marker gene with a transformation vector prepared in accordance with the invention. In this case, one would then generally assay the 40 embryo rescue due to cessation of seed development and potentially transformed cell population by exposing the cells to a selective agent or agents, or one would screen the cells for the desired marker gene trait.

Cells that survive the exposure to the selective agent, or cells that have been scored positive in a screening assay, 45 may be cultured in media that supports regeneration of plants. In an exemplary embodiment, MS media may be modified by including further substances such as growth regulators. Examples of such growth regulators are dicamba and 2,4-D. However, other growth regulators may be 50 employed, including NAA, NAA+2,4-D or picloram. Media improvement in these and like ways has been found to facilitate the growth of cells at specific developmental stages. Tissue may be maintained on a basic media with growth regulators until sufficient tissue is available to begin 55 plant regeneration efforts, or following repeated rounds of manual selection, until the morphology of the tissue is suitable for regeneration, then transferred to media conducive to maturation of embryoids. Cultures are transferred as needed on this medium. Shoot development will signal the 60 time to transfer to medium lacking growth regulators.

The transformed cells, identified by selection or screening and cultured in an appropriate medium that supports regeneration, will then be allowed to mature into plants. Developing plantlets are transferred to soilless plant growth mix, 65 and hardened, e.g., in an environmentally controlled chamber, for example, at about 85% relative humidity, 600 ppm

CO₂, and 25-250 microeinsteins m-2 s-1 of light. Plants may be matured in a growth chamber or greenhouse. Plants can be regenerated from about 6 wk to 10 months after a transformant is identified, depending on the initial tissue. During regeneration, cells are grown on solid media in tissue culture vessels. Illustrative embodiments of such vessels are petri dishes and Plant Cons. Regenerating plants can be grown at a suitable temperature, for instance about 19 to 28° C. After the regenerating plants have reached the stage of shoot and root development, they may be transferred to a greenhouse for further growth and testing.

To confirm the presence of the exogenous DNA or "transgene(s)" in the regenerating plants, a variety of assays may be performed. Such assays include, for example, "molecular biological" assays, such as Southern and northern blotting and PCRTM; "biochemical" assays, such as detecting the presence of a protein product, e.g., by immunological means (ELISAs and western blots) or by enzymatic function; plant part assays, such as leaf or root assays; and also, by analyzing the phenotype of the whole regenerated plant.

Very frequently the expression of a gene product is determined by evaluating the phenotypic results of its expression. These assays also may take many forms including but not limited to analyzing changes in the chemical 25 composition, morphology, or physiological properties of the plant. Morphological changes may include ones known to demonstrate juvenile characteristics in plant vegetative tissues, such as presence or absence of wax production, or trichome formation. Most often changes in response of plants or plant parts to imposed treatments are evaluated under carefully controlled conditions termed bioassays.

The present invention provides for a seed of a plant capable of producing a plant having enhanced juvenile growth. In one aspect, the plant can be an open-pollinated variety, a hybrid parent inbred line, or a male sterile line. In another aspect, the invention provides seed of a plant capable of producing a plant having enhanced juvenile growth.

Seeds on transformed plants may occasionally require premature senescence of plants. To rescue developing embryos, they are excised from surface-disinfected seeds 10-20 days post-pollination and cultured. An embodiment of media used for culture at this stage comprises MS salts, 2% sucrose, and 5.5 g/l agarose. In embryo rescue, large embryos (defined as greater than 3 mm in length) are germinated directly on an appropriate media. Embryos smaller than that may be cultured for 1 wk on media containing the above ingredients along with 10-5M abscisic acid and then transferred to growth regulator-free medium for germination.

In yet another aspect, tissue culture of the plants described herein relates to the culture of protoplasts, calli, or plant cells, that are isolated from, or present in, intact parts of the plants described herein.

Once plants are produced which display an enhanced, e.g. extended, juvenile phase of growth, the plants themselves can be cultivated in accordance with conventional procedures, including via tissue culture and by sexual reproduction. The seeds resulting from sexual reproduction can be recovered and planted or otherwise grown as a means of propagation. Plants may also be obtained through asexual reproduction. Protoplast or propagules (e.g., cuttings, scions or rootstocks) can be recovered from plants or parts thereof and may be employed to propagate additional plants.

The present invention also provides for and includes a container of seeds.

One aspect of the invention relates to vegetative tissues, including tissues harvested, dried, or otherwise processed, biomass produced by a plant having a genome that comprises at least one genetic locus giving rise to an enhanced juvenile phase of growth.

The present invention also provides progeny of plants displaying extended juvenile growth. As used herein, progeny include not only, without limitation, the products of any cross (be it a backcross or otherwise) between two plants, but all progeny whose pedigree traces back to the original 10 cross.

One embodiment of the present invention provides for a plant that contains a genetic marker linked to one or more locus allowing for extended juvenile growth. By "extended juvenile growth locus" or "enhanced juvenile growth locus" ¹⁵ is meant a locus that contributes to such extended or enhanced juvenile growth either alone or in combination with one more other locus.

IV. DEFINITIONS

As used herein, a "desirable trait" or "desirable traits" include, but are not limited to: increased vegetative growth, improved vegetative yield, improved digestibility when used as animal feed, and improved processing of biomass for 25 preparation of, for instance, biofuel, among others.

As used herein, "polymorphism" means the presence of one or more variations of a nucleic acid sequence at one or more loci in a population of one or more individuals. The variation may comprise but is not limited to one or more 30 base changes, the insertion of one or more nucleotides or the deletion of one or more nucleotides. A polymorphism may arise from random processes in nucleic acid replication, through mutagenesis, as a result of mobile genomic elements, from copy number variation and during the process 35 of meiosis, such as unequal crossing over, genome duplication and chromosome breaks and fusions. The variation can be commonly found, or may exist at low frequency within a population, the former having greater utility in general plant breeding and the latter may be associated with rare but 40 important phenotypic variation. Useful polymorphisms may include single nucleotide polymorphisms (SNPs), insertions or deletions in DNA sequence (Indels), simple sequence repeats of DNA sequence (SSRs) a restriction fragment length polymorphism, and a tag SNP. A genetic marker, a 45 gene, a DNA-derived sequence, a haplotype, a RNA-derived sequence, a promoter, a 5' untranslated region of a gene, a 3' untranslated region of a gene, microRNA, siRNA, a QTL, a satellite marker, a transgene, mRNA, dsRNA, a transcriptional profile, and a methylation pattern may comprise 50 polymorphisms. In addition, the presence, absence, or variation in copy number of the preceding may comprise a polymorphism.

As used herein, "genotype" is the actual nucleic acid sequence at a locus in an individual plant. As used herein, 55 "phenotype" means the detectable characteristics (e.g. number of juvenile leaves, or timing of production of leaves displaying adult morphological characteristics, such as the presence of waxes) of a cell or organism which can be influenced by genotype. 60

As used herein, linkage of two nucleic acid sequences, including a nucleic acid marker sequence and a nucleic acid sequence of a genetic locus imparting a desired trait may be genetic or physical or both. In one aspect of the invention, the nucleic acid marker and genetic locus conferring an 65 enhanced juvenile growth trait are genetically linked, and exhibit a LOD score of greater than 2.0, as judged by

interval mapping for the trait based on maximum likelihood methods described by Lander and Botstein, 1989 (Genetics, 121:185-199), and implemented in the software package MAPMAKER (e.g. Lander et al., Genomics 1:174-181, (1987); default parameters). Alternatively, other software such as QTL Cartographer v1.17 (Basten et al., Zmap-a QTL cartographer. In: Proceedings of the 5th World Congress on Genetics Applied to Livestock Production: Computing Strategies and Software, edited by C. Smith, J. S. Gavora, B. Benkel, J. Chesnais, W. Fairfull, J. P. Gibson, B. W. Kennedy and E. B. Burnside. Volume 22, pages 65-66. Organizing Committee, 5th World Congress on Genetics Applied to Livestock Production, Guelph, Ontario, Canada, 1994; and Basten et al., QTL Cartographer, Version 1.17. Department of Statistics, North Carolina State University, Raleigh, N.C., 2004) may be used. Mapping of QTLs is well-described (e.g. WO 90/04651; U.S. Pat. Nos. 5,492, 547, 5,981,832, 6,455,758; reviewed in Flint-Garcia et al. 2003 (Ann. Rev. Plant Biol. 54:357-374, the disclosures of 20 which are hereby incorporated by reference). In other embodiments, the marker and region conferring enhanced juvenile growth are genetically linked and exhibit a LOD score of greater than 3.0, or a LOD score of greater than 6.0, 9.0, 12.0, 15.0, or 18.0. In one embodiment, the marker and region contributing to such growth are genetically linked and exhibit a LOD score of between about 14 and about 20. When assigning the presence of a QTL, the LOD threshold score associated with a QTL analysis as described herein may be determined to be significant for instance at the 95% confidence level, or higher, such as at the 98% or 99% confidence level.

In another aspect, the nucleic acid marker is genetically linked at a distance of between about 0 and about 50 centimorgans (cM) to the locus of interest, e.g. a GRMZM2G362718 or GRMZM2G096016 coding sequence. In other embodiments, the distance between the nucleic acid marker and the locus of interest is between about 0 and about 35 cM, or between about 0 and about 25 cM, or between about 0 and about 15 cM, or between about 0 and about 10 cM, or between about 0 and about 5 cM, including less than about 4, 3, 2 or 1 cM.

As used herein, two nucleic acid molecules are said to be capable of hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure. Conventional stringency conditions are described by Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989) and by Haymes et al., Nucleic Acid Hybridization, A Practical Approach, IRL Press, Washington, D.C. (1985). Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. Thus, in order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

Appropriate stringency conditions which promote DNA
hybridization are known in the art, for example 6.0x sodium chloride/sodium citrate (SSC) at about 45° C., followed by a wash of 2.0xSSC at 50° C.; or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y. (1989), 6.3.1-6.3.6. In some embodiments, hybridization
conditions can be high, moderate or low stringency conditions. Preferred conditions include those using 50% formamide, 5.0xSSC, 1% SDS and incubation at 42° C. for 14

hours, followed by a wash using 0.2×SSC, 1% SDS and incubation at 65° C. Alternative wash conditions, such as of 0.15 M NaCl and 70° C. for 10 minutes may also be used.

The specificity of hybridization can be affected by posthybridization washes. For example, the salt concentration in 5 the wash step can be selected from a low stringency of about 2.0×SSC at 50° C. to a moderate stringency of about 1.0×SSC at 50° C. to a high stringency of about 0.2×SSC at 50° C.; or 0.15 M NaCl and 70° C. In addition, the temperature in the wash step can be increased from low 10 stringency conditions at room temperature, about 22° C., to moderate stringency conditions at about 50° C., to high stringency conditions at about 65° C. Both temperature and salt concentration may be varied, or either the temperature or the salt concentration may be held constant while the 15 other variable is changed. In some aspects, the wash step can be performed for 5, 10, 15, 20, 25, 30, or more minutes. In another aspect, the wash step is performed for about 20 minutes. In yet another aspect, the wash step can be repeated 1, 2, 3, 4, or more times using the selected salt concentration. 20 temperature, and time. In another aspect, the wash step is repeated twice.

A genetic marker profile of a plant may be predictive of the agronomic traits of a hybrid produced using that inbred. For example, if an inbred plant of known genetic marker 25 profile and phenotype is crossed with a second inbred of known genetic marker profile and phenotype it is possible to predict the phenotype of the F_1 hybrid based on the combined genetic marker profiles of the parent inbreds. Methods for prediction of hybrid performance from genetic marker 30 data are disclosed in U.S. Pat. No. 5,492,547, the disclosure of which is specifically incorporated herein by reference in its entirety. Such predictions may be made using any suitable genetic marker, for example, SSRs, INDELs, RFLPs, AFLPs, SNPs, ISSRs, or isozymes. 35

Additional markers, such as SSRs, AFLP markers, RFLP markers, RAPD markers, phenotypic markers, SNPs, isozyme markers, or microarray transcription profiles that are genetically linked to or correlated with the juvenile growth trait can be utilized (Walton, *Seed World* 22-29 (July, 40 1993); Burow and Blake, *Molecular Dissection of Complex Traits*, 13-29, Eds. Paterson, CRC Press, New York (1988)). Methods to isolate such markers and to design probes or primers useful in following the presence of such markers are known in the art. For example, locus-specific SSRs can be 45 obtained by screening a genomic library for SSRs, sequencing of "positive" clones, designing primers which flank the repeats, and amplifying genomic DNA with these primers. Likewise, SNP markers may be identified as well.

The genetic linkage of marker molecules to the loci 50 described herein can be established by a gene mapping model such as, without limitation, the flanking marker model, and the interval mapping, based on maximum like-lihood methods described by Lander and Botstein, 1989 (*Genetics*, 121:185-199), and implemented in the software 55 packages MAPMAKER (Whitehead Institute for Biomedical Research, Cambridge Mass., USA) or QTL Cartographer (North Carolina State University, Bioinformatics Research Center) or the like.

A maximum likelihood estimate (MLE) for the presence 60 of a marker is calculated, together with an MLE assuming no trait effect, to avoid false positives. A \log_{10} of an odds ratio (LOD) is then calculated as: LOD= \log_{10} (MLE for the presence of a trait (MLE given no linked trait)).

The LOD score essentially indicates how much more 65 likely the data are to have arisen assuming the presence of a resistance allele rather than in its absence. The LOD

threshold value for avoiding a false positive with a given confidence, say 95%, depends on the number of markers and the length of the genome. Graphs indicating LOD thresholds are set forth in Lander and Botstein (1989), and further described by Ars and Moreno-Gonzalez, *Plant Breeding*, Hayward, Bosemark, Romagosa (eds.) Chapman & Hall, London, pp. 314-331 (1993), and van Ooijen (*Heredity* 83:613-624, 1999).

Selection of appropriate mapping or segregation populations is important in trait mapping. The choice of appropriate mapping population depends on the type of marker systems employed (Tanksley et al., Molecular mapping plant chromosomes. Chromosome structure and function: Impact of new concepts J. P. Gustafson and R. Appels (eds.), Plenum Press, New York, pp. 157-173 (1988)). Consideration must be given to the source of parents (adapted vs. exotic) used in the mapping population. Chromosome pairing and recombination rates can be severely disturbed (suppressed) in wide crosses (adapted×exotic) and generally yield greatly reduced linkage distances. Wide crosses will usually provide segregating populations with a relatively large array of polymorphisms when compared to progeny in a narrow cross (adapted×adapted).

Advanced breeding lines are collected from breeding programs. These are tested for their phenotype (e.g. their disease score reactions, the presence of adult leaves, an alteration in the relative proportion of juvenile vs. adult tissues, or an alteration in the timing of production of adult tissues, among others), and genotyped for markers in the QTL intervals described herein. From these data, the smallest genetic interval is identified within each QTL containing the donor parent (DP) favorable allele among the tested lines.

Considerable genetic information can be obtained from a 35 completely classified F_2 population using a codominant marker system (Mather, Measurement of Linkage in Heredity: Methuen and Co., (1938)). An F_2 population is the first generation of self or sib pollination after the hybrid seed is produced. Usually a single F_1 plant is self or sib pollinated 40 to generate a population segregating for the nuclear-encoded genes in a Mendelian (1:2:1) fashion.

In contrast to the use of codominant markers, using dominant markers often requires progeny tests (e.g., F₃ or back cross self families) to identify heterozygous individuals. The information gathered can be equivalent to that obtained in a completely classified F₂ population. This procedure is, however, often prohibitive because of the cost and time involved in progeny testing. Progeny testing of F₂ individuals is often used in map construction where error is associated with single plant phenotyping, or when sampling the plants for genotyping affects the ability to perform accurate phenotyping, or where trait expression is controlled by a QTL. Segregation data from progeny test populations (e.g., F_3 or backcrossed or selfed families) can be used in trait mapping. Marker-assisted selection can then be applied to subsequent progeny based on marker-trait map associations (F_2, F_3) , where linkage has not been completely disassociated by recombination events (i.e., maximum disequilibrium).

Recombinant inbred lines (RILs) (genetically related lines; usually >F₅) can be used as a mapping population. RILs can be developed by selfing F2 plants, then selfing the resultant F3 plants, and repeating this generational selfing process, thereby increasing homozygosity. Information obtained from dominant markers can be maximized by using RILs because all loci are homozygous or nearly so. Under conditions of tight linkage (i.e., about <10% recombination), dominant and co-dominant markers evaluated in RIL populations provide more information per individual than either marker type in backcross populations (e.g. Reiter et al., 1992; *Proc. Natl. Acad. Sci.* (*U.S.A.*) 89:1477-1481). However, as the distance between markers becomes larger (i.e., 5 loci become more independent), the information in RIL populations decreases dramatically when compared to codominant markers.

Backcross populations can be utilized as mapping populations. A backcross population (BC) can be created by crossing an F₁ to one of its parents. Typically, backcross populations are created to recover the desirable traits (which may include most of the genes) from one of the recurrent parental (the parent that is employed in the backcrosses) while adding one or a few traits from the second parental, 15 which is often referred to as the donor. A series of backcrosses to the recurrent parent can be made to recover most of the recurrent parent's desirable traits. Thus a population is created consisting of individuals nearly like the recurrent parent, wherein each individual carries varying amounts or 20 a mosaic of genomic regions from the donor parent. Backcross populations can be useful for mapping dominant markers particularly if all loci in the recurrent parent are homozygous and the donor and recurrent parent have contrasting polymorphic marker alleles (Reiter et al., 1992; 25 Proc. Natl. Acad. Sci. (U.S.A.) 89:1477-1481).

Information obtained from backcross populations using either codominant or dominant markers is less than that obtained from completely classified F_2 populations because recombination events involving one, rather than two, gam- 30 etes are sampled per plant. Backcross populations, however, are more informative (at low marker saturation) when compared to RILs as the distance between linked loci increases in RIL populations (i.e., about 15% recombination). Increased recombination can be beneficial for resolution of 35 tight linkages, but may be undesirable in the construction of maps with low marker saturation.

Near-isogenic lines (NIL) created by many backcrosses to produce an array of individuals that are nearly identical in genetic composition except for the trait or genomic region 40 under interrogation can be used as a mapping population. In mapping with NILs, only a portion of the loci polymorphic between the parentals are expected to segregate in the highly homozygous NIL population. Those loci that are polymorphic in a NIL population, however, are likely to be linked to 45 the trait of interest.

Bulk segregant analysis (BSA) is a method developed for the rapid identification of linkage between markers and traits of interest (Michelmore, et al., 1991; *Proc. Natl. Acad. Sci.* (U.S.A.) 88:9828-9832). In BSA, two bulk DNA samples are 50 drawn from a segregating population originating from a single cross. These bulk samples contain individuals that are identical for a particular trait (e.g., resistant or susceptible to a particular pathogen) or genomic region but arbitrary at unlinked regions (i.e., heterozygous). Regions unlinked to 55 the target trait will not differ between the bulked samples of many individuals in BSA.

In another aspect, the present invention provides a method of producing a plant displaying enhanced juvenile growth comprising: (a) crossing a plant displaying such growth with ⁶⁰ a plant lacking such growth to form a segregating population; (b) screening the population for amount and/or duration of juvenile growth; and (c) selecting one or more members of the population having said enhanced or extended juvenile growth. ⁶⁵

For highly heritable traits, a choice of superior individual plants evaluated at a single location will be effective, whereas for traits with low heritability, selection should be based on statistical analyses (e.g., mean values) obtained from replicated evaluations of families of related plants. Popular selection methods commonly include pedigree selection, modified pedigree selection, mass selection, and recurrent selection. In a preferred embodiment a backcross or recurrent breeding program is undertaken.

The complexity of inheritance influences choice of the breeding method. Backcross breeding can be used to transfer one or a few favorable genes for a highly heritable trait into a desirable cultivar. This approach has been used extensively for breeding disease-resistant cultivars. Various recurrent selection techniques are used to improve quantitatively inherited traits controlled by numerous genes. The use of recurrent selection in self-pollinating crops depends on the ease of pollination, the frequency of successful hybrids from each pollination, and the number of hybrid offspring from each successful cross.

Breeding lines can be tested and compared to appropriate standards in environments representative of the commercial target area(s) for two or more generations. The best lines are candidates as parents for new commercial cultivars; those still deficient in traits may be used as parents for hybrids, or to produce new populations for further selection.

One method of identifying a superior plant is to observe its performance relative to other experimental plants and to a widely grown standard cultivar. If a single observation is inconclusive, replicated observations can provide a better estimate of its genetic worth. A breeder can select and cross two or more parental lines, followed by repeated self or sib pollinating and selection, producing many new genetic combinations.

The development of new plant lines requires the development and selection of varieties, the crossing of these varieties and selection of superior hybrid crosses. The hybrid seed can be produced by manual crosses between selected male-fertile parents or by using male sterility systems. Hybrids can be selected for certain single gene traits such as flower color, seed yield or herbicide resistance that indicate that the seed is truly a hybrid. Additional data on parental lines, as well as the phenotype of the hybrid, influence the breeder's decision whether to continue with the specific hybrid cross.

Pedigree breeding and recurrent selection breeding methods can be used to develop cultivars from breeding populations. Breeding programs combine desirable traits from two or more cultivars or various broad-based sources into breeding pools from which cultivars are developed by selfing and selection of desired phenotypes into parent lines. These lines are used to produce new cultivars. New cultivars can be evaluated to determine which have commercial potential.

Pedigree breeding is used commonly for the improvement of self-pollinating crops. Two parents who possess favorable, complementary traits are crossed to produce an F_1 . An F_2 population is produced by selfing one or several F_1 's. Selection of the best individuals in the best families is performed. Replicated testing of families can begin in the F_4 generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (i.e., F_6 and F_7), the best lines or mixtures of phenotypically similar lines are tested for potential release as new cultivars.

Backcross breeding and cross breeding have been used to transfer genes for a simply inherited, highly heritable trait into a desirable homozygous cultivar or inbred line, which is the recurrent parent. The source of the trait to be transferred is called the donor parent. The resulting plant

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obtained from a successful backcrossing program is expected to have the attributes of the recurrent parent (e.g., cultivar) and the desirable trait transferred from the donor parent. After the initial cross, individuals possessing the phenotype of the donor parent are selected and repeatedly crossed (backcrossed) to the recurrent parent. After multiple backcrossing generations with selection, the resulting line is expected to have the attributes of the recurrent parent (e.g., cultivar) and the desirable trait transferred from the donor parent.

Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several available reference books (e.g., Fehr, *Principles of Cultivar Development* Vol. 1, pp. 2-3 (1987)).

The present invention also provides for parts of the plants¹⁵ produced by a method of the present invention. Parts of grass plants, without limitation, include plant cells or parts of plant cells, seed, endosperm, meristem, flower, anther, ovule, pollen, fruit, flowers, stems, roots, stalks or leaves, scions, and root stocks. Plant parts also include the parts of²⁰ a fruit. In one embodiment of the present invention, the plant part is a seed.

In other aspects of the invention, the plants bearing one or more desirable traits in addition to enhanced juvenile growth may display a greater than 10%, or a greater than 30%, or a 25 greater than 60%, or a greater than 80% reduction in foliar symptoms of, for instance, European corn borer damage on the second leaf above the ear (Riedeman, et al., 2008; Crop Sci. 48:1723-1731), relative to a an otherwise isogenic control plant. Additionally, juvenile leaves from plants dis- 30 playing enhanced juvenile growth may comprise increased content of total uronosyl acids, arabinose, and galactose; decreased lignification, decreased neutral sugars, decreased glucose and xylose; decreased ester-linked monomers of p-coumaric acid, and decreased levels of ferulates, among 35 other changes. Such changes may, for instance, beneficially allow for improved efficiency for biofuel production or allow for enhanced feed digestibility or nutritional content.

V. EXAMPLES

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques 45 discovered by the inventor to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments ⁵⁰ which are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

Example 1

Genetic Structure of Juvenile to Adult Phase Change in Maize

Juvenile and adult vegetative tissues in grasses differ dramatically in anatomy, biochemical composition, and in 60 their ability to withstand biotic and abiotic stresses. A maize transition leaf, with juvenile tissue distinguished by the presence of epicuticular wax with a dull blueish appearance is shown in FIG. **1**. Dark glossy green portions of the leaf are adult tissue. 65

The molecular network controlling the process of developmental transition has been poorly understood. The present study utilizes the dramatic variation in the timing of juvenile to adult vegetative transition in different maize populations to identify genes and pathways controlling this fundamental biological process. This work evaluates structured populations and diverse collections of maize that have been characterized extensively for allelic variation, for instance at the GRMZM2G362718 locus, in order to provide a genetic basis for the extensive observed natural variation for developmental timing in plants such as maize. Exemplary phenotypic variation for timing of production of a transition leaf in the maize NAM population is provided in FIG. 4A-4C, with variation for transition leaf by NAM family, labeled by the non-B7 parent in FIG. 4A. The horizontal line at leaf 7 represents the average transition leaf for B73. Phenotypic distribution of the last leaf with epicuticular wax in the NAM population is shown in FIG. 4B. Transition leaf number distribution ranged from leaf 4.5 to leaf 13.25. Phenotypic variation for transition leaf in the IBM, NYH, OWRI, and Wisconsin diversity panel populations is shown in FIG. 4C, plots i-iv.

QTL discovery was accomplished by analysis of a collection of structured biparental mapping populations and a diversity panel of maize inbred lines (summarized in FIG. 5). These included the publicly available Nested Association Mapping (NAM) resource (Flint-Garcia et al Plant J 44(6): 1054-64, 2005) and the intermated B73×Mo17 (IBM) RIL mapping population (Lee et al., Plant Mol Biol 48(5-6):453-61, 2002). In addition, Oh43×W64A (OWRI) and Ny821× H99 (NyH) populations were evaluated. The diversity panel included a set of northern adapted inbreds described by Hansey et al (Bioenergy Res. 3:28-37, 2010) plus 512 lines released by CIMMYT (International Maize and Wheat Improvement Center; Texcoco, Mexico) that are of tropical, subtropical, and highland origin. In total, 5779 unique genotypes were evaluated in at least one location and season, with many of the materials replicated across years.

Example 2

Phenotypic Analysis

The primary trait that was scored to reflect the timing of juvenile to adult transition was the last leaf with juvenile wax (FIG. 1). Maize leaves, in order of emergence, can be fully juvenile, part juvenile and part adult (termed transition leaves), and fully adult. Since the earliest emerging juvenile leaves can senesce and become no longer visible at the time that the uppermost transition leaf can be scored, leaf 5 was marked at the young seedling stage (~V7) by punching a hole in the leaf with a leaf punch. At the ~V10 stage, a paper collar was secured around the stalk between leaf 8 and 9 to mark that internode before the punched leaf 5 fully senesced. The last leaf with juvenile wax was scored on 5 plants per plot with the exact node from which it emerged determined 55 by the position of the leaf collar. At flowering time or thereafter, the total number of leaves (nodes) was determined by counting 5 plants per plot. Node number is both a measure of the duration of plant development (highly correlated with flowering time), but also allowing for calculation of the proportion of nodes which were juvenile versus adult. Days to pollen shed and days to silk emergence were scored by visual assessment of the day that 50% or greater of the plants in a plot had visible pollen shed and visible silk emergence, respectively.

The following linear model was used for phenotypic analysis of the NAMs:

 $Y_{ik} \sim G + Y_k + e_{ik}$

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where Y is the last leaf with epicuticular wax of the ith genotype (G) in the kth year (Y) and μ is the overall mean with residual error e_{ik} . All effects were considered random.

Repeatability in the NAM, NyH, and OWRI populations were calculated as:

$$R^2 = \frac{\sigma^2(G)}{\sigma^2(E) + \sigma^2(G)}$$

where $\sigma^2(G)$ is the genotypic variance and $\sigma^2(E)$ is the error variance.

The following linear model was used for phenotypic analysis of the IBM, NyH and OWRI populations as well as 15 the Wisconsin Diversity Panel:

$$Y_{ijk} \sim \mu + G_i + R_{j(k)} + Y_k + Y_k \times G_i + e_{ijk}$$

where Y is the last leaf with epicuticular wax of the ith genotype (G) in the jth rep (R) within the kth year (Y) and μ is the overall mean. All effects were considered random.

Heritability on an entry mean basis was calculated in the IBM population and the WiDiv panel using the following formula:

$$H^{2} = \frac{\sigma^{2}(G)}{\frac{\sigma^{2}(E)}{ry} + \frac{\sigma^{2}(GY)}{r} + \sigma^{2}(G)}$$

where $\sigma^2(G)$ is the genotypic variance, $\sigma^2(GY)$ is the genotype by year variance and $\sigma^2(E)$ is the error variance.

Significant Pearson and Spearman rank correlations between years were calculated and allowed analysis of averages across years. Following correlation analysis, 35 means across years (and replications for the IBM, NyH, and OWRI populations) were used for QTL mapping. Phenotypic Pearson correlations were performed for transition and flowering time, node number, and internode length.

Example 3

QTL Analysis and Integration of QTL Results Across Materials

1. Nested Association Mapping (NAM) Population:

1106 single nucleotide polymorphisms (SNPs) markers on the 3875 NAM lines (Buckler et al., Science 325:714-718, 2009) were used for composite interval mapping with Windows QTL Cartographer v2.5 (Wang, http://statgen.nc- 50 su.edu/qtlcart/WQTLCart.htm, 2011). One thousand permutations were performed to determine an appropriate significance threshold.

QTL were then mapped in a combined analysis of all 25 NAM populations by joint stepwise regression of transition 55 leaf on the same 1106 SNP makers. Because stepwise regression cannot use individuals with missing marker data, an initial step was to impute missing markers. In the joint stepwise regression, a population and marker by population effect was fit. Using the SAS experimental procedure, GLM- 60 SELECT, covariates were determined by forward regression (p=0.0001) and SQL was subsequently used to calculate a likelihood ratio for all markers, as per Buckler et al (2009), to determine a genome-wide error rate of 12.26 by permutation. 65

The 1.6 million SNPs identified in the HapMap project were imputed in the offspring of the NAM RILs based on

founder genotypes. Genome wide association was conducted on top of the joint linkage mapping from above. First, residuals for each chromosome were calculated from the full joint linkage model and with the removal of any QTL located on that chromosome. Single marker analysis was then performed on the residuals across all 1.6 million SNPs to determine significance at each locus. A threshold was also set using 1000 permutation scans.

The last leaf with epicuticular wax varied in the NAM 10 RILs ranging from leaf 4.5 to leaf 13.25 with a repeatability of 0.72. The phenotypic distribution of the NAM families in FIG. 4 shows the trait centering near leaf 7, which is the average transition leaf of B73 and the common parent among the NAMs. Although node number is highly correlated with flowering time, transition leaf was not found to be correlated with flowering time, node number, or internode length with Pearson correlation coefficients of -0.18, -0.10, and 0.07 respectively in the NAM populations.

Through single-population composite interval mapping, 20 56 total OTL were detected across all NAM populations. A QTL on the long arm of chromosome two in bin ten was detected in 22 of the 25 NAM populations explaining between 5-55% of the variation. The LOD scores ranged from 6.4 to 32.9, while the significance threshold was 2.5. Similar QTL were detected with the joint-linkage composite interval mapping. The major QTL located on chromosome two had LOD scores of 303.9. The combined average additive effects of the three most significant QTL equate to almost a three-leaf difference in transition, or near 40% of the variation observed in the NAM population. Interestingly, the additive effect of all non-B73 alleles at the chromosome two QTL extends the juvenile wax phase compared to B73.

Using the genome-wide association scan, the most significant SNP is located at 234,407,421 on chromosome two (FIG. 6A) reaching a maximum LOD score of 212.4. FIG. 6B shows results of chromosome two from a similar genome-wide analysis using sub sampling. The results are in agreement with the single marker genome wide scan; the 40 most significant SNP is at position 234,407,421 on chromosome two.

2. Intermated B73×Mo17, Ny821×H99, and Oh43× W64A Populations:

1340 markers on the recombinant inbred lines of the IBM population (Lee et al Plant Mol Biol 48(5-6):453-61, 2002), 45 78 markers on the NyH RILs, and 169 markers on the OWRI RILS were used for composite interval mapping with Windows QTL Cartographer v2.5 (Wang, 2011). One thousand permutations were performed to determine an appropriate significance threshold. Updated genetic maps of these populations are developed with over 1480 SNP markers identified through genotyping-by-sequencing, and composite interval mapping of transition leaf is analyzed. The increased marker density improves the precision of QTL detection in these populations.

The last leaf with epicuticular wax ranged from leaf 5.4 to 11 in the IBM RILs, from 4.6 to 14.2 in the diversity panel with a heritability of 0.53, 0.6 respectively. The NyH population ranged in transition from leaf 6 to 11 and from leaf 6.9 to 12.2 in the OWRI population (FIG. 4).

The same QTL on chromosome two detected in 23 NAM populations was also detected in the IBM population, having a LOD score of 18.7. This QTL explains 16% of the variation in the IBM population.

Four QTL were detected in the NyH mapping population, one located on chromosome 2. The QTL on chromosome 2 is consistent with the chromosome 2 QTL detected in NAM

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and IBM. This QTL explains 11.6% of the variation observed in the NyH population.

3. Wisconsin Diversity Panel (WiDiv):

Over 100,000 SNPs have been identified in this diversity panel through genotyping-by-sequencing (Elshire et al PLoS 5 One 6(5): e19379, 2011). Association analysis including appropriate kinship and population structure matrices is performed; and genome-wide association analysis of transition leaf is analyzed on the WiDiv data set.

A summary of all QTL mapping results can be found in Table 1. Numbers indicate LOD score. Overlapping QTL based on the physical position of QTL support intervals are italicized. NAM QTL are presented from joint-linkage composite interval mapping (LOD threshold 12.26). IBM, NyH, 15 and OWRI results are from composite interval mapping (LOD threshold 2.5).

TABLE 1

Numbers indicate LOD score. C	ross all RIL mapping populations. Overlapping QTL based on physical ort intervals are italicized.
Mapping Population	LOD score of QTL on chromosome 2
NAM IBM NyH	21.3, 20.1, 18.2, <i>303.9</i> <i>18.7</i> <i>3.3</i>

Two common QTL were detected across multiple map- 30 ping populations. The QTL on the long arm of chromosome two was detected in NAM, IBM, and NyH populations. No previously known genes affecting vegetative phase change or miRNA targets are located in the chromosome two QTL peak.

The putative chromosome two peak was initially defined as covering a 1.1 Mb region containing over 50 predicted gene models (MaizeGDB; world wide web maizegdb.org). However, the most significant polymorphism from 1.6 million loci, was narrowed to a single SNP at position 234,407, 421 on chromosome two (AGP_v2). These results demonstrate that a major QTL on chromosome 2 underlies natural variation for this important developmental trait of juvenileadult transition.

Example 4

Candidate Gene GRMZM2G362718

The gene model nearest the most significant SNP on chromosome two is GRMZM2G362718 whose predicted protein contains a DNMT1 and PHD-finger domain. A protein BLAST shows this gene is highly similar to the enhanced downy mildew 2 (EDM2-encoding) transcription 55 factor of Arabidopsis, rice, Brachypodium, and sorghum (52.9, 56.7, 42.9, 63.2, percent similarity respectively).

Although the function of GRMZM2G362718 is unknown, several known functions of EDM2 in other species point to its potential significance in underlying the chromosome two 60 QTL. Mutations in EDM2 show a delay in flowering and elevated transcripts of the flowering suppressor FLC (Tsuchiya and Eulgem Plant. J. 62:518-528, 2010). These authors reported EDM2's function in regulating the vegetative to floral transition in an FLC-dependent manner; EDM2 also has a direct effect on the juvenile to adult vegetative phase change in Arabidopsis.

edm2 plants appear to skip the early juvenile phase of development by not producing the initial pair of rosette leaves. The effect of edm2-2 on trichome production was also examined by these authors. In wild-type Arabidopsis, juvenile leaves lack trichomes on the abaxial side, while adult leaves gradually produce an increasing number of trichomes. Mutant edm2-2 plants delay the onset of trichome production and, therefore, EDM2 seems to have a role in promoting the transition from the juvenile to adult vegetative phase (Tsuchiya and Eulgem BMC Plant Bio. 10:203-217, 2010). Further, Willmann and Poethig (Devel. 138:677-685, 2011) show FLC has both flowering-dependent and flowering-independent effects on vegetative transition. EDM2 does not appear to affect expression of the trans-acting siRNAs (HASTY, ZIPPY, SGS3, RDR6) or the other five genes (ARF3, ARF4, SPL3, At1g63130, At5g18040) of this pathway that have previously been shown to control vegetative phase change in Arabidopsis 20 (Peragine et al Genes Devel. 18:2368-2379, 2004). This suggests EDM2's role in vegetative phase change may be independent of the siRNA pathway, and GRMZM2G362718 may act similarly.

Sekhon et al (Plant J. 66:553-563, 2011) developed a ²⁵ maize B73 gene atlas showing gene expression levels across all 11 major organs at varying developmental time points (60 total tissue samples). The atlas shows some level of GRMZM2G362718 expression in all tissue sampled, such as a pooled leaf sample as well as in tissue at the base of stage two leaves and immature leaves (v9). Neighboring gene models 500 kb up and downstream of GRMZM2G362718 were therefore studied in the gene atlas to determine if any could be ruled out as candidates due to inappropriate tissue expression. All predicted neighboring gene models were either not present in the atlas data set or were expressed at some level in the shoot apical meristem.

RNA-seq expression levels on a subset of the Wisconsin diversity panel (Hansey et al PLos ONE 7(3):e33071, 2011) were thus used to determine if a relationship exists between expression of GRMZM2G362718 and timing of vegetative phase change. In this analysis, diverse inbreds were ordered from early to late transition and their gene expression pattern is plotted. Either categorical differences (i.e. as shown by groups of early or late transitioning inbreds have a shared expression level), or quantitative differences (i.e. via a progressive increase or decrease in expression level trending with timing of phase change) would indicate a relationship between the expression of GRMZM2G362718 and phenotype. Analysis of RNA-seq information is performed to demonstrate such differences.

Specific allelic contrasts between B73, Mo17, Oh43, and W64A show some association whereby later transitioning displayed higher expression plants levels of GRMZM2G362718 (FIG. 7). For example, B73 has an average transition leaf of 7.5 and an expression level of 5 fragments per kilobase per million reads (FPKM) compared to Oh43 which transitions at leaf 9.5 on average, and has an expression level of 13 FPKM. In this comparison, the later transition corresponds with a higher expression level. However the comparison is between plants with different GRMZM2G362718 alleles which may differ in function or activity, and thus correlating function and expression level may not be straightforward. It is also important to consider the tissue sampled (whole seedling) when making these comparisons; thus further expression analysis of the shoot apical meristem at various developmental time points is performed.

Example 5

Candidate Gene Glossy 15

QTL mapping performed with the NAM population 5 detected three major QTL located on chromosomes two, three, and nine, which had LOD scores of 303.9, 87.5 and 141.2 respectively (FIG. 8). The gene model nearest the most significant SNP on chromosome nine is Glossy 15 ("Gl15" (GRMZM2G160730); e.g. Moose and Sisco, Genes 10 Dev. 10:3018-3027, 1996). Glossy15 encodes an AP2-like transcription factor which is responsible for the expression of adult traits in the leaf epidermis. Additional mapping populations were also analyzed. Based on overlapping LOD confidence intervals, the QTL detected on chromosomes 2 15 and 9 after composite interval mapping of the IBM population are consistent with the QTL detected in the NAM population. For the Wisconsin Diversity Panel population a mixed linear model including relatedness and population structure was used to perform a genome wide association 20 study. After an experiment wide Bonferroni correction for multiple tests, one genomic region was significantly associated with changes in the production of the last juvenile leaf ("LJL") and was located on chromosome nine with an additive effect of -0.43, relative to the minor allele. The 25 most significant SNPs in this region are located within the gene Glossy15.

Glossy15 is thus a candidate gene, modulation of expression or activity of which can result in altering the timing of juvenile to adult phase change in plants. For instance, ³⁰ Glossy15 may be utilized in conjunction with GRMZM2G362718, and/or GRMZM2G096016 (see Example 6), to modulate, e.g. delay, the transition of a plant from a juvenile to an adult phase of growth.

Example 6

Candidate Gene GRMZM2G096016

Sequencing of whole seedling RNA was conducted from 40 a set of 503 diverse maize inbred lines to evaluate the maize seedling pan-transcriptome as a proxy to the maize pan genome. Using de novo assembly of reads unmapped to the B73 reference genome, 8,681 novel representative transcript assemblies (RTAs) were identified. Genomic Presence/Ab-45 sence Variation Analysis was performed, and pooled reads were cleaned using the fastx_clipper program within the FASTX toolkit. The minimum sequence length was set to 15

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ggatggattg gtctattcct tgttttgctt caagcggaat aaaacaatct ggtttagaat 240

bp after clipping using both IIlumina single end adapter sequences. Sequence reads were parsed into individual genotype files requiring a perfect match to the barcode and ApeKI cut site, and the barcode sequences were removed. Sequence reads were mapped to AGPv2 using Bowtie version 0.12.7 (Langmead, *Genome Biol.* 10:R25, 2009) requiring a unique alignment and allowing up to two mismatches. SAMtools version 0.1.7 (Li et al., *Bioinformatics* 25:2078-2079, 2009) was used to generate unfiltered pileup files. Representative genes/RTAs with at least two uniquely aligned reads were considered present at the genome level.

Sequence reads for each library were mapped to an AGPv2 formatted maize reference genome plus the 8,681 unfiltered RTAs using Bowtie version 0.12.7 (Langmead, 2009, ibid) and TopHat version 1.4.1 (Trapnell et al., Nature Protocols 7:562-578, 2012). Normalized gene expression levels were determined using Cufflinks version 1.3.0 (Trapnell, ibid). To characterize transcript presence/absence variation (PAV), sequence reads were also mapped to AGPv2 plus the 8,681 unfiltered RTAs requiring a unique alignment. A gene/RTA was then defined as expressed if the fragments per kilobase of exon model per million fragments mapped (FPKM) low confidence interval as described by Cufflinks was greater than zero. The 503 included inbred lines were clustered with hierarchical clustering using a Pearson correlation distance metric and average linkage using Multiple Experiment Viewer Software (MeV) version 4.5 (Saeed et al., Biotechniques 34:374-378, 2003).

Vegetative phase change was scored by identifying the last leaf with epicuticular wax in a subset of the 503 inbred lines. Significant natural variation for the last juvenile leaf was observed, ranging from leaf 3.45 to leaf 13.4. 186,733 SNPs were subjected to genome wide association analysis (GWAS) which was performed using a mixed linear model 35 accounting for both familial relatedness (Q) and population structure (K) (Yu et al., Nature Genetics 38:203-208, 2006). GWAS was also performed with transcript presence/absence state for all of the reference genes and RTAs for last juvenile leaf. The association analysis was done using the same mixed model as described above but instead of using a SNP as the dependent variable, transcript presence/absence was used as the genetic marker. In the presence/absence analysis, GRMZM2G096016 (GenBank EU975023.1) which encodes predicted nuclear transcription factor Y-subunit A-10, was found to be significantly associated with regulation of the timing of vegetative phase change transition, and may be utilized to modulate, e.g. delay, the transition of a plant from a juvenile to an adult phase of growth.

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Tyr Lys Gl 65	u Val Val A 7		Ile Arg Leu 75	Asp Ser Gl	u His Pro 80	
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Pro Arg Ly	s Cys Tyr G 100		Val Arg Ser 105	Thr Leu Il 11		

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Gln	Met	Leu 115	His	Phe	Phe	Gly	Arg 120	Gly	Glu	Gln	Arg	Ser 125	Leu	Asn	His
Leu	Trp 130	Asp	His	Leu	Asp	Glu 135	Val	Phe	Gly	Lys	Ser 140	Asn	Pro	Lys	Pro
Val 145	Glu	Asp	Asp	Leu	Met 150	Lys	His	His	Thr	Leu 155	Ile	Lys	Leu	Phe	Val 160
Glu	Lys	Asp	Gln	Thr 165	Leu	Met	Lys	Ser	Lys 170	Ile	Leu	Gln	Arg	Leu 175	Ile
Glu	Asn	Gly	Phe 180	Гла	Arg	Thr	Lys	Lys 185	Ala	Leu	Gly	Met	Glu 190	Ala	Gln
Ser	Ile	Val 195	Ser	Asp	Gly	Trp	Arg 200	Ala	Arg	Гүз	Asn	Asp 205	Asp	Asn	Asn
Tyr	Gly 210	Asn	ГЛа	Asp	Asp	Ser 215	Gly	Asp	Asp	Суз	Asp 220	Gly	Asp	Gly	Ser
Ser 225	Asp	Asp	Gly	Asp	Gly 230	Ser	Ser	Asp	Asp	Asp 235	Val	Thr	Asp	Gln	Ile 240
Сүз	Ala	Leu	Суз	Asp 245	Asp	Gly	Gly	His	Leu 250	Leu	Ser	Суз	Asp	Gly 255	Pro
Сүз	Lys	Arg	Ser 260	Phe	His	Pro	Thr	Lys 265	Lys	Aap	Gly	Arg	Glu 270	Ser	Lys
Cys	Glu	Ser 275	Leu	His	Tyr	Thr	Ser 280	Ala	Glu	Val	Гла	Arg 285	Ile	Gly	Thr
Tyr	Leu 290	Суз	Ala	Asn	Сув	Lys 295	Asn	Гла	Gln	His	Gln 300	Суз	Phe	Arg	Сув
Gly 305	Glu	Leu	Glu	Pro	Ser 310	His	Gly	Pro	Asn	Ala 315	Гла	Val	Phe	Gln	Сув 320
Asn	Gln	Ala	Ser	Сув 325	Gly	Tyr	Phe	Tyr	His 330	Pro	Lys	Суа	Ile	Ala 335	Gln
Leu	Leu	Asp	Pro 340	Asn	Ala	Thr	Asp	Gly 345	Glu	Leu	Glu	Arg	Arg 350	Ile	Met
Ser	Gly	Met 355	Ser	Phe	Pro	Суа	Pro 360	Ile	His	Trp	СЛа	Phe 365	Lys	Суз	Gly
His	Met 370	Glu	Asn	Гла	Ala	Gln 375	Arg	Ala	Leu	Gln	Leu 380	Ala	Val	Суз	Arg
Arg 385	Суз	Pro	Arg	Ala	Tyr 390	His	Arg	Glu	Сүз	Leu 395	Pro	Arg	Asp	Leu	Ser 400
	Gly	Thr	Lys	Asp 405		Asp	Gly	Asn	Gln 410		Ala	Trp	Lys	Leu 415	
Asp	Thr	Ile	Phe 420		Tyr	Суз	Leu	Asp 425		Glu	Ile	Asp	Lys 430		Thr
Gly	Thr	Thr 435		Arg	Asn	His	Ile 440		Phe	Pro	Ala	Thr 445		Glu	Tyr
Thr	Lys 450		Lys	Gly	Leu	Gly 455	Asn	Ser	Lys	Val	Arg 460		Thr	Gly	Lys
Arg 465		Lys	Asn	ГЛЗ	Arg 470		Lys	Asn	Thr	Asp 475		Ser	Thr	Lys	Pro 480
	Asp	Leu	Pro			Leu	Суз	Gly			Ser	Glu	Gln		
Asn	Val	Gly		485 Lys	Ser	Thr	Leu		490 Gln	Ile	Val	Val		495 Pro	His
Сув	Ala	Ala	500 Lys	His	Ser	Lys	Gly	505 Asp	Pro	Gln	Ile	Ala	510 Lys	Gln	Gly
Val	۵la	515 Ala	Ara	Glr	Asn	Glv	520 Ala	Glu	Thr	Met	Lve	525 Glv	Hig	Glu	Agn

Val Ala Ala Arg Gln Asn Gly Ala Glu Thr Met Lys Gly His Glu Asn

	530					535					540					
Gln 545	Phe	Gly	Ile	Ser	Phe 550	Суз	Val	Ala	Ser	Thr 555	Glu	Thr	Glu	Lys	Arg 560	
Val	Thr	Cys	Leu	Ala 565	Gln	Arg	Gly	Thr	Cys 570	Leu	Gly	Thr	Gln	Tyr 575	Asp	
Gly	Pro	Ser	Thr 580	Lys	Gly	Met	Tyr	Asp 585	Суз	Ser	Val	Gln	Asp 590	Thr	Pro	
Met	Asp	Asp 595	Asp	Val	Glu	Leu	Asp 600	Asn	Val	Ala	Суз	Ile 605	Ile	Ala	Val	
Asp	Lys 610	Tyr	Val	Asn	Gly	Arg 615	Gly	Lys	Thr	Gln	Glu 620	Asp	Tyr	Thr	Arg	
Lys 625	Glu	Ala	Ala	Gln	Arg 630	Lys	Aab	Ser	Ser	Glu 635	Asn	Gln	Gly	Gln	Asn 640	
Asp	Ala	Leu	Glu	Leu 645	Asp	Asn	Leu	Arg	Met 650	Glu	Met	Gln	Ala	Asp 655	Lys	
Arg	Pro	Leu	Glu 660	Pro	Gly	Asn	Lys	Arg 665	Asp	Arg	Lys	Trp	Gln 670	Lys	Asn	
Ala	Tyr	Gly 675	Leu	Gly	Ser	Ala	Ser 680	Gly	Gln	Lys	Glu	Thr 685	Leu	Ser	Arg	
Arg	Glu 690	Asn	Pro	Pro	Ser	Asp 695	Arg	Gly	Met	Val	His 700	Ser	Asn	Asp	Ser	
Lys 705	Thr	Ile	Tyr	Tyr	Arg 710	Lys	Gly	Gly	Thr	Glu 715	Val	Asp	Asn	Val	Asp 720	
Asp	His	Pro	Leu	Glu 725	Lys	Gln	Aab	His	Gln 730	Asp	Thr	Ser	Ser	Asp 735	Gly	
Ser	Lys	Lys	Arg 740	Ser	Arg	Pro	Val	Asp 745	Asn	Ala	Ser	Gly	Gly 750	Asn	Arg	
Pro	Tyr	Leu 755	Asp	Glu	Asn	Lys	Lys 760	Arg	Asn	Phe	Glu	Asp 765	Gly	Arg	Tyr	
Ala	His 770	Tyr	Glu	Asp	Trp	Arg 775	Ser	Glu	Arg	Asn	Thr 780	Ala	Ala	Asp	Thr	
Ser 785	Gly	Tyr	Lys	Ala	Gln 790	Ser	Glu	Glu	Lys	Pro 795	Val	Trp	Thr	Asn	Thr 800	
Arg	Thr	Gly	Ser	Arg 805	Glu	His	Ser	Leu	Asp 810	Arg	Gln	Arg	Ile	Glu 815	Суз	
Gly	Asp	Ser	Tyr 820	Arg	Gly	Thr	Tyr	Asn 825	Asn	Arg	Gln	Arg	His 830	Glu	Trp	
Leu	His	Pro 835	His	Ala	Ser	Gly	Asn 840	Ser	Ser	Arg	Ile	Gly 845	Trp	Asp	Asp	
Arg	Arg 850	Gln	Trp	Ser	Ser	Ser 855	Arg	Ser	Pro	Phe	Pro 860	Ser	Ala	Glu	Phe	
Gly 865	Gly	Asp	Arg	Ser	Cys 870	Ser	Arg	Ala	His	Pro 875	Arg	Gly	Ser	Lys	Tyr 880	
Arg	Thr	Gly	Gly	Arg 885	His	Asp	His	Pro	Gln 890	Tyr	Leu	Gly	Leu	Gly 895	Thr	
Pro	Gln	His	Gly 900	Thr	Ser	Arg	Pro	His 905	His	Thr	Met	Gly	Trp 910	Asp	Arg	
Asp	Thr	Phe 915	His	Asp	His	Gln	His 920	Gly	Arg	Arg	Pro	Pro 925	His	His	Thr	
Met	Gly 930	Trp	Asp	Arg	Ala	Pro 935	Phe	Arg	Asp	His	Gln 940	His	Gly	Glu	Tyr	
Asp 945	Asp	Ser	Arg	Tyr	Gly 950	Glu	Tyr	Asp	Ala	Thr 955	Asp	Asn	Gly	Pro	Asp 960	

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				325					330					335	
Arg	Gln	Ile	Ile 340	Ala	Gly	Glu	Tyr	Thr 345	Суз	Pro	Leu	His	Lys 350	Cys	Ser
Val	Суз	Glu 355	Asn	Gly	Glu	Val	Lys 360	Thr	Asp	Ser	Asn	Leu 365	Gln	Phe	Ala
Val	Cys 370	Arg	Arg	Суз	Pro	Lys 375	Ser	Tyr	His	Arg	Lys 380	Cys	Leu	Pro	Arg
Glu 385	Ile	Ser	Phe	Glu	Asp 390	Ile	Glu	Asp	Glu	Asp 395	Ile	Leu	Thr	Arg	Ala 400
Trp	Asp	Gly	Leu	Leu 405	His	Asn	Arg	Val	Leu 410	Ile	Tyr	Суз	Gln	Glu 415	His
Glu	Ile	Asp	Glu 420	Glu	Leu	Leu	Thr	Pro 425	Val	Arg	Asp	His	Val 430	Lys	Phe
Pro	Phe	Thr 435	Glu	Glu	Gln	Lys	Val 440	Phe	Val	Lys	Glu	Gln 445	Arg	Arg	Ile
Leu	Glu 450	Ser	His	Val	Gly	Arg 455	Asp	Lys	Ala	Arg	Leu 460	ГЛа	Val	Lys	Asp
Pro 465	Ala	Leu	Gln	Asp	Thr 470	Cys	Gly	Lys	Ala	Ser 475	Lys	Asn	Ser	Phe	Arg 480
Ser	Ser	Phe	Pro	Ser 485	Ser	Lys	Asp	Gly	Phe 490	Ser	Thr	Lys	Lys	His 495	Gly
Leu	Val	Ser	Ser 500	Val	Pro	Asp	His	Ser 505	Arg	Lys	Arg	Lys	Asp 510	Ile	Asp
Pro	Ser	Ile 515	Гла	His	Lys	Met	Val 520	Pro	Gln	Lys	Ser	Gln 525	Lys	Met	Met
Glu	Asp 530	Ser	Arg	Glu	Ala	Gly 535	Lys	Asn	ГЛа	Leu	Gly 540	Val	Lys	Glu	Ala
Arg 545	Asp	Ala	Gly	Гла	Ser 550	Lys	Ile	Ser	Leu	Gly 555	Glu	Arg	Leu	Phe	Ser 560
	Thr	Gln	Glu	Pro 565		Pro	Val	Lys	Pro 570		Arg	Val	Ile	Pro 575	
Asp	Ser	Lys	His 580		Lys	Thr	Asp	Ser 585		Ala	Ser	Lys	Glu 590	Pro	Gly
Ser	Glu	Ile 595		Thr	Leu	Asp	Asn 600		Ser	Gln	Arg	Arg 605		Leu	Ala
Val	Met 610		Lys	Ala	Thr	Glu 615	Glu	Ile	Thr	Met	Gly 620		Ile	Leu	Lys
Lys 625		Lys	Ile	Gln	Ser 630			Ser	Thr	His 635		Thr	Arg	Asn	Val 640
	Asp	Lys	Thr			Met	Gly	Lys			Gly	Ser	Val	Gln	
Ile	Arg	Thr		645 Leu	ГЛа	ГЛа	Leu		650 Glu	Gly	Gly	Asn		655 Glu	Asp
Ala	Lys		660 Val	Сув	Glu	Pro		665 Val	Leu	Ser	Gln		670 Leu	Lys	Trp
Lys	Asp	675 Lys	Leu	Гла	Val	Tyr	680 Leu	Ala	Pro	Phe	Leu	685 His	Gly	Ala	Arg
-	690	-		-		695					700		-	Gln	-
705				_	710					715		-			720
				725					730					Ile 735	
Asp	Phe	Сүз	Cys 740	Gly	Ser	Asn	Asp	Phe 745	Ser	Суз	Leu	Met	Asn 750	Ala	Lys

Leu	Glu	Glu 755	Thr	Gly	Lys	Lys	Cys 760	Leu	Tyr	Lys	Asn	Tyr 765		Leu	Phe
Pro	Ala 770	Lys	Asn	Asn	Phe	Asn 775	Phe	Glu	Arg	Lys	Asp 780	Trp	Met	Thr	Val
Ser 785	Lys	Asp	Glu	Leu	Glu 790	Pro	Gly	Ser	Lys	Leu 795	Ile	Met	Gly	Leu	Asn 800
Pro	Pro	Phe	Gly	Val 805	Asn	Ala	Ser	Leu	Ala 810	Asn	ГЛа	Phe	Ile	Thr 815	
Ala	Leu	Glu	Phe 820	Arg	Pro	Lys	Ile	Leu 825	Ile	Leu	Ile	Val	Pro 830		Glu
Thr	Glu	Arg 835	Leu	Asp	ГЛа	ГЛа	Lys 840	Ser	Ser	Tyr	Val	Leu 845		Trp	Glu
Asp	Lys 850	Thr	Phe	Leu	Ser	Gly 855	Asn	Ser	Phe	Tyr	Leu 860	Pro	Gly	Ser	Val
Asn 865	Glu	Glu	Asp	Lys	Gln 870	Leu	Glu	Asp	Trp	Asn 875	Leu	Val	Pro	Pro	Pro 880
Leu	Ser	Leu	Trp	Ser 885	Arg	Ser	Asp	Phe	Ala 890	Ala	ГÀа	His	Lys	Lys 895	Ile
Ala	Glu	Lys	His 900	Суз	His	Leu	Ser	Arg 905	Asp	Val	Gly	Ser	Ser 910		Leu
Lys	Ile	Val 915	Glu	Glu	Glu	Ala	Asn 920	Ala	Ser	Leu	His	Pro 925	Leu	Gly	Ala
Ser	Asp 930	Gly	Met	Суз	Asp	Asp 935	Ile	Pro	Met	Glu	Lys 940	Asp	Glu	Leu	Glu
Val 945	Ala	Glu	Суз	Val	Asn 950	Lys	Ile	Leu	Val	Ser 955	Glu	Lys	Ile	Asp	Thr 960
Val	Glu	Thr	Val	Ala 965	Arg	Val	His	Gln	Ser 970	Asp	His	Leu	Ser	Arg 975	Arg
Ser	Gln	Leu	Lys 980	Lys	Glu	Gly	Lys	Thr 985	Lys	Asp	Tyr	Ser	Gly 990		Lys
Leu	Gly	Lys 995	Ser	Met	Asp	Ser	Asn 1000		n Val	l Asj	o Trj	р Ly 10		er A	sn Asp
Met	Glu 1010		ı Asl	9 Glr	n Gly	/ Glu 101		eu Se	er Ai	rg Al		ro 020	Glu	Ser	Ile
Lys	Val 1025		s Ile	e Pro	o Glu	u Met 103		nr Se	er As	ар Ті		ln 035	Ser	Pro	Val
Arg	Ser 1040		r Pro	o Asl	o Aar) Ile 104		yr Al	La Va	al Cy		nr 050	Ser	Ile	Ser
Thr	Thr 1055		r Pro	o Glr	n Arg	g Sei 100		is G	Lu A	La Va		lu 065	Ala	Ser	Leu
Pro	Ala 1070		e Thi	r Arg	g Thi	107 107		er As	sn Le	eu Gi)80 Va	Asn	Ile	Arg
Glu	His 1085	-	7 Cys	з Цуз	8 Val	l Glr 109		Ly Tł	nr G	Ly Ly		ro 095	Glu	Val	Ser
Arg	Asp 1100		g Pro	Sei	: Sei	7 Va 110		rg Tł	nr Se	er Ai	-	lu . 110	Asp	Ile	Tyr
Thr	Val	-	g Pro	Sei	r Pro			∍n Tł	nr G	Ly G		ys 125	Pro	Phe	Glu
	1115	5				112	.0								
Ala	1115 Phe 1130	Glu	ı Pro	Sei	т Туз		/ A.	la S€	er Le	eu Se		is 140	Phe	Asp .	Asp

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Pro	Asp 1160		> Pro	> Phe	e Leu	1 Prc 116		ap	Gln	Phe	e Pr		eu 170	Arg	Asn	Gly
Pro	Asn 1175		ı Met	: Phe	e Asp	> Phe 118		rg	Gly	Тут	s Se		sp 185	Leu	Asp	Arg
Gly	Ile 1190	-	/ Glr	n Arg	g Glu	1 Tyr 119		ro	Gln	Glr	і Ту		ly 200	Gly	His	Leu
Asp	Pro 1205		: Leu	ı Ala	a Pro	Prc 121		ro	Pro	Pro	> As		eu 215	Met	Asp	Asn
Ala	Phe 1220) Leu	ı Glr	n Glr	1 Arg 122		yr.	Ala	Pro	> Hi		he 230	Asp	Gln	Met
Asn	Tyr 1235		n Arg	g Met	: Ser	: Ser 124		he	Pro	Pro	5 G1		ro 245	Pro	Leu	Gln
Pro	Ser 1250	-	/ His	s Ası	n Leu	1 Leu 125		sn	Pro	His	a As	-	he 260	Pro	Leu	Pro
Pro	Pro 1265		> Pro	Sei	a Ast	> Ph∈ 127		lu 1	Met	Ser	r Pr		rg 275	Gly	Phe	Ala
Pro	Gly 1280) Asr	n Pro) Asr	1 Tyr 128		ro	Tyr	Met	: Se		rg 290	Ser	Gly	Gly
Trp	Ile 1295		ı Asl	Ş												
<213	2> TY 3> OF 0> SE Met	GAN]	ISM: ICE:	11				Gl	u Pi	ro C	Jln	Leu	Lys	: Ala	a Val	l Glu
1				5	-	-			10	C			-		15	o Val
			20					25						30		
		35			-		40			-			45		-	s Lys
-	50	-		-	-	55		-	_	-	-	60		-		L Tyr
65					70					7	75					9 Glu 80
Ile	Ala	Val	Leu	Ser 85	Thr	Glu	Gly	Se	r Ti 9(Ile	Ala	Leu	. Lei	ι Lyε 95	s Pro
Arg	Pro	Ser	Tyr 100	Glu	Glu	Thr	Ile	Ar 10	-	er V	/al	Leu	Ile	Thi 110		l Glu
Met	Leu	His 115	Phe	Val	Arg	Arg	Arg 120		o Tł	nr A	/ab	Ser	Glu 125	_	a yab) Met
Trp	Asp 130	His	Leu	Tyr	Gly	Val 135	Phe	Gl	u Ai	rg I	Phe	Val 140	Val	. Arg	g Pro) Leu
Glu 145	Asp	Asp	Phe	Ala	Asn 150	His	Gln	As:	n Le		[le 155	Lys	Leu	l Phe	e Ala	a Gln 160
Arg	Asp	Pro	Asp	Leu 165	Ala	Asn	Ser	G1:		al I 70	Seu	Gln	Val	. Phe	e Ile 175	e Lys
Asp	Lys	Ile	Met 180	Glu	ГЛа	Thr	Asn	Gl 18		al C	Jly	Ser	Asr	Asr 190		ı Asp
Asn	Lys	Arg 195	Glu	Pro	Asp	Ile	Lys 200		n Gl	lu E	?ro	Asp	Ile 205	-	3 Glr	n Glu
Pro	Val 210	Ala	Ala	Gly	Asp	Glu 215	Met	Gl	u GI	lu 1	Ile	Val 220		ı Glu	ı Gly	/ Ile
												_ •				

Pro 225	Asp	Ala	Pro	Ser	Asn 230	Asp	Aap	Asp	Asp	Asp 235	Glu	Glu	Asp	Glu	Glu 240
	Gly	Asp	Leu			Ser	Val	Cys			Суз	Asp	Asn	-	
Glu	Leu	Leu	-	245 Cys	Glu	Gly	Pro		250 Met	Arg	Ser	Phe		255 Ala	Lys
Ile	Arg	_	260 Gly	Glu	Asp	Ser	-	265 Суз	Ala	Thr	Leu	-	270 Tyr	Thr	Lys
	~ 1	275				-	280	51				285	a		
Ala	Glu 290	Val	ГЛЗ	Ala	Leu	Lys 295	Asn	Phe	Val	Cys	100 200	Asn	Cys	Aab	His
Lуя 305	Gln	His	Gln	Суз	Phe 310	Val	Cys	Gly	Glu	Leu 315	Glu	Pro	Ser	Asp	Gly 320
Pro	Asn	Ala	ГЛа	Val 325	Phe	Leu	Суз	Asn	Asn 330	Ala	Thr	СЛа	Gly	His 335	Phe
Tyr	His	Pro	Arg 340	Cys	Val	Ala	Gln	Leu 345	Leu	His	Pro	Asn	Ser 350	Arg	Asn
Glu	Ala	Ser 355	Glu	Met	Glu	Lys	Lys 360	Ile	Met	Ala	Gly	Phe 365	Ser	Phe	Thr
Cya	Pro 370	Val	His	Trp	Cya	Phe 375	His	Cya	Lys	Gly	Leu 380	Glu	Aab	Arg	Thr
Gln 385	Glu	Pro	Leu	Gln	Phe 390	Ala	Val	Cys	Arg	Arg 395	Сув	Pro	Arg	Ser	Tyr 400
His	Arg	Lys	Cys	Leu 405	Pro	Arg	Glu	Ile	Ser 410	Phe	Glu	Asp	Ile	Asn 415	Thr
Gln	Gly	Ile	Ile 420	Thr	Arg	Ala	Trp	Glu 425	Leu	Ser	Lys	Arg	Ile 430	Leu	Ile
Tyr	Cys	Leu 435	Asp	His	Glu	Ile	Asp 440	Leu	Asp	Ile	Gly	Thr 445	Pro	Pro	Arg
Asp	His 450	Ile	Lys	Phe	Pro	His 455	Val	Glu	Lys	Ser	Ala 460	Tyr	Ser	Ala	Lys
Lys 465	Lys	Val	Lys	Glu	Leu 470	Ala	Glu	Lys	Lys	Arg 475	Arg	Ile	Суз	Asp	Asp 480
Ser	Tyr	Val	Ser	Glu 485	Pro	Leu	Gln	Lys	Arg 490	Ala	Lys	Leu	Asn	Glu 495	Lys
Phe	Asn	Ala	Lys 500	Gly	Asp	ГЛа	Ser	Lys 505	Lys	Ala	Gly	Val	Lys 510	Ser	Glu
Phe	Glu	Glu 515	Val	Leu	Glu	Ser	Glu 520	Lys	Lys	Lys	Thr	Arg 525	Ser	Leu	Lya
Lys	Arg 530	Thr	Gln	Pro	Glu	Glu 535	Pro	Leu	Val	Glu	Cys 540	Ala	Ala	Ala	Ala
Ala 545	Ala	Asn	Asn	Ala	Asn 550		Pro	Val	Lys	Glu 555	Arg	Glu	Lys	Glu	Leu 560
	Thr	Ser	Ser			Met	Gly	Lys			Leu	Ser	Ser		
T] -		7	Com	565	These	<i>a</i> 1	True	7	570	Com	71.0	T eu	170]	575	True
IIe	Val	Aab	580	GIU	Thr	GIU	гуа	Arg 585	шe	ser	AIa	Leu	vai 590	GIU	гуа
Glu	Val	Ser 595	Ser	Leu	Thr	Val	Ala 600	Asp	Ile	Ser	Arg	Arg 605	Cys	Val	Ile
Pro	Ser 610	Thr	Tyr	Ala	Суз	Ser 615	Gly	Arg	Gln	Ile	Asp 620	Lys	Ile	Val	Val
Arg 625	Gly	Lys	Leu	Glu	Arg 630	Ser	Ile	Gln	Ala	Val 635	Lys	Ala	Ala	Leu	Gln 640

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- 1	1

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Gly Val Ala Ser Val Glu	Asn Asn Ile Ala Leu	Ser Thr Asp Asn
1085	1090	1095
Val Gly Ala Gly Ser Arg	Met Tyr Ser Pro Asp	Pro Glu Leu Asn
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Gly Tyr Ala Val Asp Pro	Thr Val Asn Ala Tyr	Gly Ser Val Ser
1115	1120	1125
Gly Gly Thr Gly Gly Ser	Phe Tyr Arg Arg Gln	Asn Leu Glu Asp
1130	1135	1140
Tyr Thr Met Asp Ser Ser	Glu Ser Ala Gln Met	Asn Pro Val Pro
1145	1150	1155
Gly Arg Asp Val Gln Glu	Tyr Ala Arg Thr Tyr	Tyr Gly His Asn
1160	1165	1170
Arg Asp Glu Val Pro Gln	Thr Ala Ile Asn Thr	Pro Ser Met Asp
1175	1180	1185
Ile Arg Thr His Ile Arg	Met Tyr Gly Arg His	Ile Arg Asp Asp
1190	1195	1200
His Thr Gln Thr Thr Met	Asn Pro Pro Ala Asn	Asp Ile Arg Ala
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Gln Ile Arg Met Tyr Gly	Gln His Ala Thr Ser	Asp His Gln His
1220	1225	1230
Ala Ser Arg Tyr Ser Ser	Gly Ser Pro Asp Ala	Arg Phe Glu Gln
1235	1240	1245
Gln Pro Ser Phe Thr Ser	Tyr Gly Met Pro Ser	Leu Gly Ser Thr
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Gly Arg Ser Met Met Asp	Arg Tyr Ser Pro Ser	Ile Asp Glu Thr
1265	1270	1275
Ser Tyr Arg Thr Gly Gln	Arg Gly Pro Tyr Asn	Ala Ser Asp Phe
1280	1285	1290
Arg Arg Asp Arg His Pro	Asp Asp Met Asn Phe	Ala Leu His Asn
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Cys Pro Arg Ala Tyr His	Trp Glu Cys Leu Pro An	rg Glu Leu Ser Leu
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Gly Ala Lys Asp Lys Asp	Gly Asn Pro Arg Ala Ti	rp Lys Leu Ser Lys
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Thr Ile Phe Phe Tyr Cys 50	Leu Asp His Glu Ile As 55 60	
Thr Ala Ser Arg Asn His	Ile Lys Phe Pro Ala Th	nr Pro Glu Cys Thr
65 70	75	80
Lys Thr Lys Glu Leu Gly	Asn Arg Lys Gly Arg Me	et Thr Gly Lys Arg
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Leu	Tyr	Gly 115	Ala	Glu	Ser	Glu	Gln 120	Ala	Asp	Asn	Val	Gly 125	Ala	Lys	Ser
Thr	Ser 130	Pro	Gln	Ile	Val	Val 135	Glu	Pro	His	Суз	Ala 140	Ala	Lys	Val	Leu
Lys 145	Gly	Asp	Pro	Gln	Ile 150	Glu	Gln	Ser	Ile	Ile 155	Gly	Val	Ala	Gly	Ser 160
Gln	Asn	Gly	Ala	Glu 165	Thr	Met	Asn	Gly	His 170	Glu	ГЛа	Gln	Phe	Gly 175	Ile
Ser	Суз	Val	Ala 180	Arg	Thr	Glu	Thr	Glu 185	Lys	Arg	Val	Thr	Tyr 190	Leu	Ala
Gln	Lys	Gly 195	Thr	Суз	Leu	Gly	Thr 200	Pro	Tyr	Asp	Gly	Pro 205	Ser	Thr	Lya
Asp	Met 210	Ser	Asp	Суз	Ser	Val 215	Gln	Asp	Thr	Pro	Val 220	Asp	Lys	Asp	Phe
Glu 225	Leu	Asp	Asn	Val	Ala 230	Tyr	Arg	Ile	Met	Glu 235	Asp	Гла	Tyr	Ala	Asn 240
	Arg	Glu	Glu	Thr 245		Glu	Asp	Tyr	Thr 250		Гла	Glu	Thr	Ala 255	
Arg	Lys	Asp	Ser 260		Glu	Asn	Gln	Gly 265		Asn	Asp	Val	Leu 270	Glu	Leu
Asp	Asn	Leu 275		Val	Glu	Ile	Gln 280		Asp	Gly	Ser	Pro 285		Glu	Pro
Gly	Asn 290		Arg	Tyr	ГЛа	Glu 295		Asn	Ala	Tyr	Gly 300		Gly	Ser	Ala
Ser 305		His	Glu	Гла	Glu 310		Ser	Ser	Ser	Arg 315		Glu	Asn	Val	Gln 320
	Asp	Arg	Gly			Pro	Met	Asn			Lys	Thr	Ile	Asp	
Arg	Lys	Gly	-	325 Thr	Thr	Leu	Asp		330 Asn	Val	Tyr	Asp		335 Ser	Ser
Glu	Gly		340 Tyr	Pro	Суз	Gln	-	345 Glu	Суз	Ser	His		350 Lys	Суз	Asn
Asp		355 Leu	Val	Ala	Ile	-	360 Gln	Asp	Thr	Ser		365 Asp	Arg	Leu	Lys
-	370 Arg	Ser	Gln	Pro		375 Glu	Lys	Ala	Ser		380 Gly	Asn	Lys	Thr	_
385 Leu	Asp	Lys	Asn		390 Lys	His	Asn	Leu	-	395 Glu	Asp	Gly	Arg	Asp	400 Ala
His	Tyr	Glu	Asp	405 Arg	Arg	Thr	Glu	Arg	410 Asn	Thr	Ala	Ala	Asp	415 Thr	Ser
Arg	Tyr	Lys	420 Cys	Arg	Asp	Lys	Ile	425 Gln	Leu	Asp	Arg	Arg	430 Glu	Pro	Glu
-	-	435	-	-		-	440				-	445		Pro	
	450	-	-			455		-			460				
465		-			470	_	-		-	475	-		-	Asn	480
Arg	Arg	Tyr	Glu	Ser 485	Leu	His	Asn	Phe	Asn 490	Pro	Pro	Arg	Ser	Gly 495	Сув
Asp	Asp	Arg	Arg 500	Gln	Leu	Ser	Pro	Сув 505	Gln	Ser	Ser	Phe	Pro 510	Leu	Pro
Glu	Phe	Cys	Gly	Asp	His	Ser	His	Leu	Tyr	Pro	Arg	Asp	Ser	Thr	Ile

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Tyr 545	Met	Ala	Ala	Ser	Ala 550	Ala	Gly	His	Ser	Ala 555	Val	Суз	Tyr	Arg	Leu 560
Ala	Gly	Gly	Tyr	Gly 565	Glu	Gly	Ser	Arg	Ala 570	Ser	Arg	Pro	Val	Thr 575	Asp
Trp	Tyr	Ala	Pro 580	His	Leu	Asp	Arg	Thr 585	Asn	Суз	Gln	Pro	Arg 590	Ser	Gln
Ile	Asp	Leu 595	Gln	Leu	Gln	Ala	Ser 600	Arg	Pro	Val	Thr	Asp 605	Гла	Tyr	Ala
Pro	Gln 610	Leu	Glu	Leu	Thr	Asn 615	Tyr	Pro	Pro	Arg	Ser 620	Gln	Ser	Asp	Leu
Gln 625	Tyr	Сүз	Thr	Thr	Thr 630	Ile									
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Val	Lys	Asp	Tyr 20	Tyr	Phe	Val	Asp	Ala 25	Asp	Lys	Asn	Ala	Leu 30	Cys	Phe
Ser	Val	Leu 35	Pro	Ile	Trp	Phe	Lys 40	Glu	Asp	Ala	Val	Ala 45	Val	Pro	Glu
Суз	Lys 50	Thr	Gly	Val	Phe	Leu 55	Arg	Gly	Thr	Val	Asp 60	Pro	Gly	Ile	Pro
Val 65	Tyr	Lys	Gln	Val	Val 70	Ala	Trp	Lys	Leu	Gly 75	Leu	Asp	Ala	Arg	Gln 80
	Asp	Leu	Ala	Val 85		Ser	Lys	Glu	Gly 90		Trp	Ile	Asn	Leu 95	Ser
Lys	Pro	Lys	Asn 100		Tyr	Glu	Glu	Ser 105		Arg	Thr	Ile	Phe 110		Thr
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Asp	Leu 130		Ile	His	Leu	Arg 135		Val	Phe	Asp	Lys 140		Asp	Val	Arg
Pro 145	Ser	Lys	Asp	Asp	Phe 150		Asn	His	His	Thr 155		Met	Lys	Gln	Phe 160
	Glu	Lys	Asp			Leu	Ala	Asn			Ile	Leu	Lys		
Ile	Gly	Glu	-	165 Phe	Arg	Гла	Gln		170 Ser	Glu	Val	Asp		175 Gly	Asn
Phe	Glu	Val	180 Lys	Glu	Ser	Phe	Ile	185 Ala	Ala	Asp	Glu	Asp	190 Val	Glu	Asp
	Val	195					200					205			
	210					215					220				
Leu 225	Phe	Asp	ser	'I'hr	Суз 230	Ala	шe	СЛа	Asb	Asn 235	σтλ	сту	Asp	ьeu	Leu 240
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Glu	Ala	Met 275	Lys	Thr	Phe	Leu	Cys 280	Lys	Asn	Суз	Glu	Tyr 285	Lys	Gln	His
Gln	Cys 290	Phe	Ile	Сүз	Gly	Val 295	Leu	Glu	Pro	Ser	Asp 300	Gly	Pro	Thr	Ala
Lys 305	Val	Phe	Leu	Сүз	Asn 310	Asn	Ala	Thr	Сүз	Gly 315	Tyr	Phe	Tyr	His	Pro 320
Lys	Сүз	Val	Ala	Gln 325	Gln	Leu	His	Pro	Asn 330	Asn	Lys	Ile	Glu	Ala 335	Leu
Glu	Lys	Glu	Lys 340	Lys	Ile	Ala	Gly	Gly 345	Ser	Ser	Phe	Thr	Суз 350	Ser	Ile
His	Trp	Сув 355	Phe	Сув	Суа	Lya	Gly 360	Leu	Glu	Asp	Arg	Thr 365	Glu	Glu	His
Leu	Gln 370	Phe	Ala	Val	Сүз	Arg 375	Arg	Cys	Pro	Lys	Ser 380	Tyr	His	Arg	Lys
Суя 385	Leu	Pro	Ser	Glu	Ile 390	Pro	Phe	Glu	Asp	Ser 395	Asp	Glu	Asp	Ile	Val 400
Thr	Arg	Ala	Trp	Asp 405	Leu	Ser	Gln	Arg	Ile 410	Leu	Ile	Tyr	Суа	Met 415	Glu
His	Glu	Ile	Asp 420	Leu	Aap	Ile	Glu	Thr 425	Pro	Val	Arg	Asn	His 430	Ile	Lys
Phe	Pro	Gly 435	Leu	Pro	Ile	ГÀа	Pro 440	Thr	Glu	Tyr	Leu	Lys 445	Lys	Lys	Thr
Lys	Val 450	Leu	Ile	Lys	Lys	Lys 455	Lys	Arg	Thr	Phe	Asp 460	Glu	Ser	Phe	Leu
Asp 465	Glu	Pro	Ser	Ile	Lys 470	Pro	Ala	Lys	Phe	Pro 475	Gly	Lys	Val	Arg	Val 480
Gln	Glu	Asn	Glu	His 485	Ala	Arg	Lys	Ile	Ala 490	Val	Arg	Ser	Ser	Ser 495	Glu
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Ser 545	Thr	Thr	Met	Asn	Met 550	Pro	Gln	Ser	Ser	Phe 555	Pro	Ile	Val	Asp	Ser 560
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Tyr	Gln	Tyr	Phe 580	-	Met	Pro	Phe	Gly 585	His	Phe	Ser	Ala	Leu 590	Phe	Leu
Pro	Ala	Leu 595	Ala	Ile	Ser	Tyr	Thr 600	Ser	Gln	Thr	Leu	Ala 605	Gly	Gln	Phe
Phe	Asp 610	Lys	Val	Trp	Leu	Phe 615	Leu	Gly	Ser	Ser	Leu 620	Leu	Pro	Суз	Met
Cys 625	Lys	Val	Val	Ile	Ala 630	Leu	Val	Glu	Lys	Glu 635	Val	Ser	Ser	Leu	Thr 640
Leu	Asn	Asp	Ile	Ser 645	Arg	Lys	Сув	Leu	Met 650	Pro	Ser	Thr	His	Val 655	Tyr
Ser	Gly	Arg	Gln 660	Thr	Asp	ГЛа	Ile	Ile 665	Ala	Thr	Gly	ГÀа	Leu 670	Glu	Arg
Ser	Val	Gln		Val	Arg	Gln	Ala		His	Leu	Leu	Ala		Gly	Asp

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		675					680					685			
Val	Asn 690	Thr	Ala	Lys	Ala	Thr 695	Суз	Glu	Pro	Gln	Va] 700	L Leu)	Lys	Gln	Leu
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Gly	Ser	Arg	Tyr	Ser 725	Ser	Phe	Gly	Arg	His 730	Phe	Thi	r Lys	Val	Glu 735	-
Leu	Val	Glu	Ile 740	Val	Asp	Гла	Leu	His 745	Trp	Tyr	Val	L Glu	Pro 750	-	Asp
Met	Ile	Val 755	Asp	Phe	Суа	Суа	Gly 760	Ala	Asn	Asp	Phe	e Ser 765	Arg	Leu	Met
Lya	Glu 770	Гла	Leu	Asp	Leu	Val 775	Gln	Гла	Lys	Суз	Hi: 780	3 Phe	Гла	Asn	Tyr
Asp 785	Leu	Ile	Gln	Pro	Gln 790	Asn	Thr	Phe	Суз	Phe 795	Glu	ı Arg	Arg	Asp	Trp 800
Met	Thr	Val	Gln	Arg 805	Asn	Glu	Leu	Pro	Arg 810	Gly	Sei	r Arg	Leu	Val 815	Met
Gly	Leu	Asn	Pro 820	Pro	Phe	Gly	Val	Lys 825	Ala	Ala	Leu	ı Ala	Asn 830		Phe
Ile	Asp	Lys 835	Ala	Leu	Ser	Phe	Asn 840	Pro	Lys	Leu	Ile	e Ile 845	Leu	Ile	Val
Pro	Lys 850	Glu	Thr	Lys	Arg	Leu 855	Asp	Gln	Lys	Lys	Th1 860	r Pro	Tyr	Asp	Leu
Val 865	Trp	Glu	Asp	Gly	Asp 870	Сүз	Leu	Ala	Gly	Lys 875	Sei	r Phe	Tyr	Leu	Pro 880
Gly	Ser	Val	Asp	Val 885	Asn	Glu	Lys	Ile	Val 890	Gln	Glγ	/ Trp	Asn	Ala 895	Ser
Ala	Pro	Pro	Leu 900	Tyr	Leu	Trp	Ser	His 905	Pro	Asp	Trp	o Thr	Lys 910		His
Lys	Lys	Val 915	Ala	Glu	Glu	His	Asn 920	His	Thr	Ser	Leu	ı Ala 925	Lys	Ile	Ala
Суз	Arg 930	Ile	Glu	Glu	Gly	Asn 935	Leu	Ser	Asp	Asp	Va] 940	L Pro	Met	Lys	Lys
Glu 945	Ala	Glu	Ser	Ser	Asp 950	Val	His	Asn	Ser	Arg 955	Pro	> Arg	Lys	Glu	Asp 960
Glu	Asn	Thr	Gly	Arg 965		Ser	Суз	His	Leu 970	Glu	Glu	ı Ala	Ser	Leu 975	Ser
Asn	Val	Val	Pro 980		Gln	Arg	Gln	Ala 985	Glu	Pro	Lys	3 Ser	Lys 990		Asn
Ala	Arg	Ser 995	Gly	Lys	Ala	ГЛа	Trp 1000		r Ly:	s Glı	u Ai	rg Th 10		er C	Aa Yai
Val	Arg 1010	-	o Va	1 11	e Pro	o Se: 10:		∋p G	lu Tl	hr L		Ala L020	Lys	Lys (Gln
Asp	Arg 1025		r Gl	y Gl	u Asj	p Gl1 103		la Ly	γa G	lu P:		Asn L035	His	Leu '	Val
Gln	Lys 1040		n Se	r Ar	g Se:	r Gly 104		lu As	ab ri	ys A		јуя 1050	Glu	Pro .	Asn
Arg	Leu	Va:	l Ly	s Ly:	s Glı	n Ala	a Ai	rg Pl	ne G	ly G	lu (Jlu	Lys .	Asp	Lys
Glu		Ası	n Ar	g Le	u Vai		s Lj	∕s G	ln A	la A:	rg S	LO65 Ser	Gly	Glu .	Aap
Lys	1070 Tyr		r Asi	n Le	u Ala	107 a Gly		Ly Le	eu Se	er A		L080 Буз	Asn	Gln .	Ala
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Glu Gln Arg Asp Ala Phe Cys Glu Asn Leu Arg Asn Asp His Ile 1160 1165 1170	
Lys Glu Pro Ser Arg Gly Ser Ser Asp Met Asn Met Ser Ser Pro 1175 1180 1185	
Asp Thr Ser Asn Ala Pro Asn Arg Ser Thr Ser Tyr Ser Pro Tyr 1190 1195 1200	
Met Pro Thr Glu Gln Pro Ser Glu Phe Arg Pro Thr Ala Tyr Leu 1205 1210 1215	
Asp Gly Asn Met Ser Tyr Pro Val Lys Glu Pro His Val Ser Ala 1220 1225 1230	
Phe Ser Ser Ala Thr Tyr Gln Gly Ser Tyr Leu Ala Arg Ser Asp 1235 1240 1245	
Arg His Asn Asp Ala Leu Gly Val Lys Asn Asp Pro Met Leu Tyr 1250 1255 1260	
Thr His Ala Val Asp Gly Ser Lys Tyr Ser Pro Ser Phe Glu Glu 1265 1270 1275	
Leu Thr Met Arg Tyr Ala Ala Asn Pro Ala Gly Asp Gly Tyr Ser 1280 1285 1290	
Met Gln Ala Gln Gly Asp Asp Tyr Leu Pro Met Ser Arg His Ser 1295 1300 1305	
Leu Gly Ser Ser Gly Ala Arg Tyr Asp Gln Pro Ser Leu Arg Ser 1310 1315 1320	
Tyr Tyr Gly Leu Ser Gly Thr Thr Ala Pro Gln Ser Ser Ile Thr 1325 1330 1335	
Asp Lys Tyr Gly Pro Gly Leu Phe Gly Pro Ser Gly Ser Gly Ala 1340 1345 1350	
Ser Val Thr Asp Lys Tyr Ala Pro Gly Phe Leu Gly Pro Ser Ala 1355 1360 1365	
Pro Gly Ser Ser Val Ile Asp Asn Tyr Ala Ala Pro Leu Asn Gly 1370 1375 1380	
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Gln Phe Phe Pro Pro Thr Thr Thr Ala Ala Gln Gln Ala Thr Met Glu	
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Glu Gln Cys His Val Pro Ala Gly Ser Ala Ala Glu Gln Trp Val Arg 85 90 95	
Ser Ser Ala Ser Arg Lys Ser Arg Arg Gly Pro Arg Ser Arg Ser Ser 100 105 110	
Gln Tyr Arg Gly Val Thr Phe Tyr Arg Arg Thr Gly Arg Trp Glu Ser 115 120 125	
His Ile Trp Asp Cys Gly Lys Gln Val Tyr Leu Gly Gly Phe Asp Thr 130 135 140	
Ala Gln Ala Ala Ala Arg Ala Tyr Asp Gln Ala Ala Ile Lys Phe Arg 145 150 155 160	

Gly Leu Asn Ala Asp Ile Asn Phe Thr Leu Asp Asp Tyr Lys Asp Glu

Met Lys Lys Met Lys Asp Leu Ser Lys Glu Glu Phe Val Leu Val Leu

Arg Arg Gln Gly Ala Gly Phe Val Arg Gly Ser Ser Arg Phe Arg Gly

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Val Thr Gln His Lys Cys Gly Lys Trp Glu Ala Arg Ile Gly Gln Leu Met Gly Lys Lys Tyr Val Tyr Leu Gly Leu Tyr Asp Thr Glu Thr Glu Ala Ala Gln Ala Tyr Asp Lys Ala Ala Ile Lys Cys Tyr Gly Lys Glu Ala Val Thr Asn Phe Asp Ala Gln Ser Tyr Asp Lys Glu Leu Gln Ser Gln Pro Trp Asp Gly Glu Leu Asp Leu Glu Leu Ser Leu Gly Cys Ala Ser Ser Asp Pro Ser Thr Val Ala Val Glu Ala Phe Ser Pro Ala Thr Ser Ser Ser Arg Lys Gln Arg Thr Met Thr Leu Thr Leu Gly Leu Pro Glu Glu Glu Glu Thr Gly Ala Gly Tyr Pro His Pro Ala Ala Gly Met Phe Gly Arg Pro Ala Asp Gly His Val His Val Ala Pro Pro Pro His Arg Gln Trp Gln Gln Gln Gln Gln Gly Gln His Ala Ala Pro Asp Ala Ala Pro Glu Arg Arg Ala Ala Glu Pro Ala Asp Arg Gln Arg Trp Gly Arg Gly Ala Arg Trp Pro Ile Ala Ser Ala Ser Gly Ile Asn Trp Ala Trp Ala Pro Pro Tyr Ala Thr Ala Arg Ala Gly Thr Asp Asp Asp Asp Ala Ser Ser Ala Ala Ala Ala Ala Ser Ser Gly Phe Pro Leu Trp Gln Leu Gly Ala Ala Ser Ser Arg Ser Ser Trp Pro Ser Cys <210> SEQ ID NO 16 <211> LENGTH: 588 <212> TYPE: DNA <213> ORGANISM: Zea mays <400> SEQUENCE: 16 atggccgcgc acccggaget getgetgetg gacaggtace agtaceacea ceatggeege ttcgacctga ccgttgggca atccatggtg cttaacaacg acagcgccat tgctagccat cagatetatg gegeegetge gtactateee ttetaeggag eccaagetet geaegggagg gtgeteetge egeeggegat egeggeegae gageeggtet aegtgaaege eaageagtte aacggcatcc tccggcggcg cctggcgcgc gccaagcgcg cggccgccac ggaccgccgg gteteeggga geegeaagee gtaceteeae gagteaegge acetgeaege getgegeegg gegeggggea eeggeggeeg etteeteaae acceggagee gegaeggega eeeegaggee qqcaqcqcqq qqaaqqcqqc qqcqqcqqcq qcqaqqatqc aqqaqqaqqa ccqqcaqqcq

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Tyr Pro Phe Tyr Gly Ala Gln Ala Leu His Gly Arg Val Leu Leu Pro 50 55 60	
Pro Ala Ile Ala Asp Glu Pro Val Tyr Val Asn Ala Lys Gln Phe 65 70 75 80	
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Lys Ala Ala Ala Ala Ala Arg Met Gln Glu Glu Asp Arg Gln Ala 145 150 155 160	
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Lys Asn Val Phe Leu His Gly Phe Val Asp Gln Ser Pro His Val Tyr 50 55 60	
Lys Glu Val Val Ala Trp Lys Ile Leu Gln Arg Leu Ile Glu Asn Gly 65 70 75 80	
Phe Glu Arg Thr Lys Lys Val Cys Met Glu Asn Lys Thr Gln Arg Ala 85 90 95	
Leu Gln Leu Ala Val Cys Arg Arg Cys Pro Arg Ala Tyr His Trp Glu 100 105 110	
Cys Leu Pro Arg Glu Leu Ser Leu Gly Ala Lys Asp Lys Asp Gly Asn 115 120 125	
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His Glu Ile Asp Lys Asp Thr Arg Thr Ala Ser Arg Asn His Ile Lys 145 150 155 160	
Phe Pro Ala Thr Pro Glu Cys Thr Lys Thr Lys Glu Leu Gly Asn Arg	
165 170 175	

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Glu	Pro	Thr 195	Glu	Leu	Ser	Asn	Arg 200	Leu	Tyr	Gly	Ala	Glu 205	Ser	Glu	Gln	
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Pro 225		Суз	Ala	Ala	Lys 230	Val	Leu	Lys	Gly	Asp 235		Gln	Ile	Glu	Gln 240	
	Ile	Ile	Gly			Gly	Ser	Gln			Ala	Glu	Thr			
Gly	His	Glu	Lys	245 Gln	Phe	Gly	Ile	Ser	250 Cys	Val	Ala	Arg	Thr	255 Glu	Thr	
Glu	Lys	Arg	260 Val	Thr	Tyr	Leu	Ala	265 Gln	Lys	Gly	Thr	Cys	270 Leu	Gly	Thr	
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	290	-	-			295	-	-			300	-				
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	-		500	-	-			505	-			-	510			
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Ser	Tyr	Pro	Gly	Thr 565	Tyr	Asn	Arg	Arg	Arg 570	Tyr	Glu	Ser	Leu	His 575	Asn	
Phe	Asn	Pro			Ser	Gly	Суа	_		Arg	Arg	Gln			Pro	
Cys	Gln	Ser	580 Ser	Phe	Pro	Leu	Pro	585 Glu	Phe	Суз	Gly	Asp	590 His	Ser	His	

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		595					600					605			
Leu	Tyr 610	Pro	Arg	Asp	Ser	Thr 615	Ile	Gly	Arg	His	Asn 620	Pro	His	Arg	Tyr
Leu 625	Gly	Ile	Pro	Gln	Tyr 630	Gly	Pro	Tyr	Met	Ala 635	Ala	Ser	Ala	Ala	Gly 640
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Arg	Ala	Ser	Arg 660	Pro	Val	Thr	Asp	Trp 665	Tyr	Ala	Pro	His	Leu 670	Asp	Arg
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Arg	Pro 690	Val	Thr	Asp	Lys	Tyr 695	Ala	Pro	Gln	Leu	Glu 700	Leu	Thr	Asn	Tyr
Pro 705	Pro	Arg	Ser	Gln	Ser 710	Asp	Leu	Gln	Tyr	Cys 715	Thr	Thr	Thr	Ile	

50

What is claimed is:

1. A polynucleotide molecule comprising a nucleotide sequence selected from the group consisting of: 25

- (a) a nucleotide sequence encoding a polypeptide that comprises an amino acid sequence that is at least 96% identical over its full length to the amino acid sequence of SEQ ID NO: 17; wherein the polypeptide regulates 30 juvenile to adult phase change in grass plant leaves; (b) a nucleotide sequence comprising SEQ ID NO: 16;
- (c) a nucleotide sequence comprising at least 95% sequence identity over its full length to the full length of SEQ ID NO:16, wherein the nucleotide sequence encodes a protein that regulates juvenile to adult phase change in grass plant leaves; and
- (d) the nucleotide sequence complementary to (a), (b), or (c).
- wherein the polynucleotide molecule further comprises a $_{40}$ heterologous promoter functional in plants that is operably linked to the nucleotide sequence.

2. The polynucleotide molecule of claim 1, wherein the nucleotide sequence comprises SEQ ID NO:16.

3. A recombinant vector comprising the polynucleotide 45 molecule of claim 1.

4. The recombinant vector of claim 3, further comprising at least one additional sequence chosen from the group consisting of: a regulatory sequence, a selectable marker, a leader sequence and a terminator.

5. The recombinant vector of claim 4, wherein the additional sequence is a heterologous sequence.

6. The recombinant vector of claim 3, wherein the promoter is a tissue-specific promoter.

moter directs expression in leaf tissue.

8. The recombinant vector of claim 3, defined as an isolated expression cassette.

9. A recombinant vector construct comprising a polynucleotide sequence encoding a siRNA that targets a nucleo- 60 tide sequence, wherein expression of the siRNA in a plant down regulates expression of the nucleotide sequence, wherein the nucleotide sequence is selected from the group consisting of:

(a) a nucleotide sequence encoding a polypeptide that 65 claim 3. comprises an amino acid sequence that is at least 95% identical over its full length to the amino acid sequence

of SEQ ID NO: 17; wherein the polypeptide regulates juvenile to adult phase change in grass plant leaves;

- (b) a nucleotide sequence comprising SEQ ID NO: 16; (c) a nucleotide sequence comprising at least 95% sequence identity over its full length to the full length of SEQ ID NO:16, wherein the nucleotide sequence encodes a protein that regulates juvenile to adult phase change in grass plant leaves; and
- (d) the nucleotide sequence complementary to (a), (b), or (c).

10. A transgenic plant comprising a recombinant vector, the recombinant vector comprising a heterologous promoter functional in plants that is operably linked to a nucleotide sequence selected from the group consisting of:

- (a) a nucleotide sequence encoding a polypeptide that comprises an amino acid sequence that is at least 95% identical over its full length to the amino acid sequence of SEQ ID NO: 17; wherein the polypeptide regulates juvenile to adult phase change in grass plant leaves;
- (b) a nucleotide sequence comprising SEQ ID NO: 16;
- (c) a nucleotide sequence comprising at least 95% sequence identity over its full length to the full length of SEQ ID NO:16, wherein the nucleotide sequence encodes a protein that regulates juvenile to adult phase change in grass plant leaves; and
- (d) the nucleotide sequence complementary to (a), (b), or (c).

11. A transgenic plant comprising the recombinant vector construct of claim 9.

12. The transgenic plant of claim 10, further defined as a monocotyledonous plant.

13. The transgenic plant of claim 10, further defined as a 7. The recombinant vector of claim 3, wherein the pro- 55 member of the Poaceae, the Panicoideae or the Pooideae.

> 14. The transgenic plant of claim 10, further defined as maize, rice, sorghum, or switchgrass.

> 15. A seed of the transgenic plant of claim 10, wherein the seed comprises the recombinant vector.

> 16. The transgenic plant of claim 10, wherein the last leaf with epicuticular wax is produced later during plant development relative to that found in an otherwise isogenic plant lacking the recombinant vector.

> 17. A cell transformed with the recombinant vector of

18. A method of altering the timing of juvenile to adult phase change in a plant, the method comprising expressing

the construct of claim 3 in the plant or expressing the construct of claim 9 in the plant.

19. The method of claim **18**, wherein the timing of juvenile to adult phase change is calculated by a method comprising counting the last leaf displaying epicuticular 5 wax.

20. The method of claim **18**, wherein the plant exhibits an altered trait selected from the group consisting of: an increase of at least one in the numbering of the last leaf which displays epicuticular wax or which does not contain 10 abaxial trichomes; an altered proportion of juvenile, transitional, or adult leaves; enhanced yield of vegetative tissue; enhanced digestibility of vegetative tissue; enhanced resistance to a plant pest; and enhanced resistance to a plant disease, wherein the trait exhibited by the plant is altered 15 relative to a wild type plant.

21. The method of claim **18**, wherein the plant has altered development or morphology when compared to a wild type plant, further wherein the plant displays an altered trait selected from the group consisting of: enhanced disease 20 resistance, enhanced insect resistance, improved forage digestibility, enhanced abiotic stress tolerance, and improved utility for biofuel production, wherein the development, morphology, or trait is altered relative to a wild-type plant.

22. A method of producing plant biomass, the method comprising:

(a) obtaining a plant according to claim 10; and

(b) preparing biomass from said plant or a part thereof.

23. The method of claim **22**, further comprising producing biofuel, food or feed from the biomass.

24. The recombinant vector of claim **3**, further comprising an additional polynucleotide sequence that encodes all or part of a sequence of Glossy15 or Cg1.

25. The recombinant vector of claim **9**, further comprising an additional polynucleotide sequence which, after being transcribed, regulates the timing of the juvenile to adult phase change in a plant.

26. A transgenic plant comprising the recombinant vector of claim **3**.

27. A transgenic plant comprising the recombinant vector of claim 25.

28. A seed of the transgenic plant of claim **26** wherein the seed comprises the recombinant vector.

29. The method of claim **18**, further comprising modulating the expression of at least a second gene which regulates the timing of the juvenile to adult phase change in a plant.

30. The method of claim **29**, wherein the second gene is selected from the group consisting of: Glossy15 and Cg1.

31. The method of claim **29**, wherein the timing of juvenile to adult phase in the plant is extended relative to a wild type plant.

32. The polynucleotide molecule of claim **1**, wherein the nucleotide sequence encodes a polypeptide that comprises an amino acid sequence that is at least 99% identical over its full length to the amino acid sequence of SEQ ID NO: 17.

33. The polynucleotide molecule of claim **1**, wherein the nucleotide sequence comprises at least 95% sequence identity over its full length to the full length of SEQ ID NO:16.

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