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# (12) United States Patent Kaeppler et al.

#### (54) EXTENDING JUVENILITY IN GRASSES

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- (52) U.S. Cl.

CPC ..... C12N 15/8266 (2013.01); C12N 15/8261

(2013.01)

(58) Field of Classification Search

None

See application file for complete search history.

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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.

#### 45 Claims, 12 Drawing Sheets

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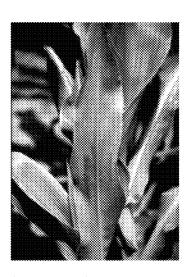
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FIG. 1.



### FIG. 2

AT5G55390.1 GRMZM26362718_P01	mtfvdddeeedfsvpcsasnytfedddrepvsfaplpiqusveekvbgs-glgfylpgrs mfddddgvdpQiedvwrytfedgeekfvcfsilpfqfgeddseavflokdvflcgfv :.***;; :***** ;;;**.*; **;*;. ::** *
AT5G55390.1 GRMZM2G362718_P01	DMGLLPLHKLVKAWRYDLSMFQPEISVLTKDNIWIKLEEPPKSYGELIPTVLVTLHSIQF - DKNLPVYKEVVAWKIRLDSEHPNIYVLSIEHKWIKLLKPPKCYGDIVPSTLITVQMLHF ; **;; * * **; *; *; * **; ; **** ; ***, **;;; *; *; ;; ;; *; *; *; *; *; *; *;
lT5055390.1 GENZN2G362718_P01	LPRNPQASEKALWEKLTESLRSYDVKPSONDLVDHIGLIAEAAKPDPNLANSKFILAFLT FGRGEQRSSNHLWDHLDEVFGKYNPKPVEDDLNKHHTLIKLFVEKDOTLNKSKILOPLIE : * * * .; **;; * . ; . * ; ** : ; **; * ; ; ; ; ; ; ; ; ; ; ;
AT5055390.1 GRMZMZG362718_P01	KKPTKPRLP
AT5055390.1 GRMZMZG362718_P01	ESVCAICONGGEILCCEGSCLESPHATEKDGEDSLCDSLGFNENQVEAIGEYFCPMCEHE DQICALCODGGELLSCOGPCESSPHPTEEDGPESECESLHYTSAEVERIGTYLCANCENE 1.:**;**(**,(*.*: * **** *****,(* *;** : (*: * .*); ***(.*
AT5055390.1 GPM2M2G362718_F01	IHQCFICKBLGSSDBSSGAAEVFQCVSATCGYFYHPHCVTBRLRLGNREESEALERQII- QHQCFRCGELEPSEGPNAKVFQCNQASCGYFYHPKCIAQLLDFBATDGELERRIBS **** * ! * *. *;**** .*;******;*;; * ***;*;
AT5055390.1 GPM2M2G362718_F01	AGEYTOPLHKOSVOENGEVKTDSNLQFAVORROPKSYHRKOLFREISFEDIEDEDILTRA GNSFPOPIHWOFKOGHMENKAQRALQLAVORROPRAYHREOLFPDLSFGTKDK-DGNQRA
AT5055390.1 GPM2M2G362718_F01	WDGLLHMRVLIYCQEHEIDEELLTPVRDHVKFFFTEEQKVFVKEQPRILEXHVGRBKARL WKLSDTIFIYCLDHEIDKDTGTTSRMHIKFFATPEYTKTKGLGNSKGRM *. *;;***;****;* *;*;****; * *;*;*** * *;
AT5G55390.1 GPMIM2C362718_F01	KVEDP
AT5G55390.1 GRMIM2G362718_FG1	
AT5G55390.1 GRMIM2G362718_F01	PSYTQEPNPVKPGRVIPVDSKHNKTDSIASKEPGSEIPTLDNDSQRRLLAVNKKATEEIT PGISFCVASTETEKRVTCLAQRGTC *.;;;;;*;;;;
AT5G55390.1 GRMZM2G362718_P01	BCTILERFRIQSTMSTMSTRNVVDRTITMGRVEGSVQAIRTALKKLEEGGBIEDARAVCE LGTQYDGPSTKGNYDCSVQDTPNDDBVELDNVAC1 ;** , **; * ; *** ; ; * ; *

## FIG. 2 (continued)

AT5655390.1 GPM2M2G362718_P01	PEVLSQILKUKDKLKVYLAPFLHGARYTSFGRHFTNPEKLQQIVDRLHNYADDGDNIVDF IAVDKYVNGRGKTQEDYTRKEAAQRK-DSSE ; ;;,* *;*. * *; *
AT5655390.1 GRM2M26362718_P01	CCGSMDFSCLMMARLEETGRECLYEMYDLFPARMNFNFEREDURTVSEDELEFGSEL NGGQMDALELDNLRM
ATSG55390.1 GRM2M2G362718_F01	INGLNPPFGVNASLANKFITKALEFRPKILILIVPPETERLDKKKSSYVLISEDKT WQKNVYGLGZAZGQKETLSREE
ATSG55390.1 CRMIM2C362718_PO1	PLSGNSFYLPGSVNEEDRQLEDWNLVPPPLSLWSRSDFAAKHKKIAEKHCHLSRLVGSSKSKTIYYPKGGTEVDNVDD
ATEGESEPO.: GRMIM2G362718_PO:	LRIVEEEANASLHPLGASDGRCDDIPMERDELEVAECVNKILVSEKIDTVETVAEVHQSD-PYLDENKKRNLPEDGPVA-HYEDBRSEENTAADTSGVRAQSE-EKFVWINTETGSRE(;*;;*; ** ;* ;* ;* ;* ;* ;* ;* ;* ;* ;*
AT5G5S390.1 GRMIM2G362718_PQ1	HLSRPSQLKREGETHDYSGRKLGKSMDSNWVDWKSNDMEBDQCELSRAPESIKVRIPEHT HSLDRQRIECGDSYRGTYWNRQRHEWLHPHASGNSSRIGWTDR * *.;(; * * . ; ). (* )).
AT5G55390.1 GRMIM2G362718_P01	SDWQSPVRSSPDDIYAVCTSISTTTPQRSHEAVEASLPAITRTKSNLGKNIREHOCKVQG PQWSSSPSFFFSAEFGGDESCSFAHFFGSKYRTGGRHDHPQYLG (*.* * ; * *; *; *; *; * ; *; *; *; *; *; *
AT5955390.1 GRMZM26362718_P01	TGKPEVSRDRPSSVETSREDITTVRPSPENTGQKPFEAFEPSYGASLSHFDDG LGTPQHGTSRPHHTEGWDRDTFHDHQHGRRPPHHTMGWDRAPFRDHQHGEYDDS *.*;** . , *; ** . , * ; ** . , . ; **.
AT5G55390.1 GRMZM2G362718_PQ1	LAAKYGGFOGGYRMFDFFFLFDQFFLRNOPNEMFDFRGYSDLDRGIGQREYFQQYGGHLD RYGEYDATDNGPDSAHRPYTAAGVAGRSAPSYQL-AGGYGEGSRAWR .:*. * . *: . **. : **. **. :
AT5G55390.1 GRMZM2G362718_PO1	PMLAPPPPPNLMDNAFFLQQRYAFHFDQMNYQRMSSFFPQPPLQPSGHNLLNPHDFPLPF 
AT5G55390.1 GRMZM2G362718_P01	PPPSDFEMSPRGFAPGPMPMYPYMSR3GGWIND

# FIG. 3

1_SB03g503430.1 -MSDDBBGVDFEIEDVWGYFEDGEGEPVCFSILFFGFGENDMEADFSRAVFIAGFVDQ PO1 1 13108.m23057 procein -knssppdiedvenytytyddnbyfysfdulffgfallfgfydgfyrgfydg 1,.** ::*:: *: **:*:: * * **:*:* : * * *:*:**	1 Sb02g03430.1 F01 1   13108.m23057   protein GLGRVYROYGABINIDGBSPEIAVLSTEGSBIALLRPEPSYEETIRSVLITVENLHFYR	. SPO2GOO3450.1 PGEQRSSWRLUPBLDEVPSKYNPKPVEDDLMKHHTLIKLPVEKDQTLMKZKILQKLIENG 1113108.m23057 protein brptdskkDbbblkVkpvekpleddfanhonliklpaordinyrrikdk 113108.m23057 protein brptdskkDbbblkVkpvekpleddfanhonliklpaordinyrrikdk	1 RDDLGGG3430.1 FERTKKV	1 SROZGOGAGO.1 POI 1   13108.123057   provein Redgelfrygocalcomgoellocegpckrsphakirdgebsycallgytkaevkalknfy	1_SBOLGGOO3430.1 FOI 13108.m23057 protesin ckncdhrohocfofiersdoffmarvflownatcohfyhfrotaollapmatdgele	1_SBGZGGG3430.1 RRINGONSPPCPIENCTROCHMENKAQRALQLAVCRECPRATHECLFRELSLGARDNDG PD1 [131D8.m230S7[protein krinagfsftopvenchentokelbetarrcheristedintog	1_SB02g503430.1 -WPRAGKLSTIFFYCLDHEIDKDIRTLSDNHIKFPATFECTKTRELONKROPHT P01 1 13108.m23057 proteid litragelskriliycldheidkdigfppnhikffhyrkTrolonskormi
Sorghum SbOZgOO3420.1_Sk	Sorghumisbosgoogase. 1 St	Sorghum; xb02g003420.1_Sb02g003430.1	%orghum; ShO2gOO3426.1, %bO2gOO3430.1	Sacghum XbACgOO%420.1_ShOZgOO343A.1	Sorghum; SbO2gOO342C.1_3k	Sarghum: %b02g003420.1_Sh02g003430.1	Sorghum; Sp02g003420.1_Sp02g003430.1
Maise GRMIMZO362718_PO1	Matre GRNZRSG363718 PO.	Maize; CRMIM20362718_P01	Maine: SRMZMZG362718, FG1	Maize QPNINZG362718_Põi	Maire; ORNZN2G362716_FO1	kaire: QPK2K2G962718_P01	Maize; ORMIM20362718_P01
Rice LOC_OSO6924946.1 11	Rice LOC_OsOsg24946.1 13	Pice; LOC_OsO8g24946.1; 13108.m23057;	Rice: LOC_O®O8g24946.1; 13:08.m23OS7{	Pice LOC_OsOSG24946.1 13108.u23057 protein	Rice; LOC_C&O6g24946.1113	Rice: LOC_O&O&g24946.1   13108.m23057	Rice; LOC_0908g24946.1; 13108.m23057;

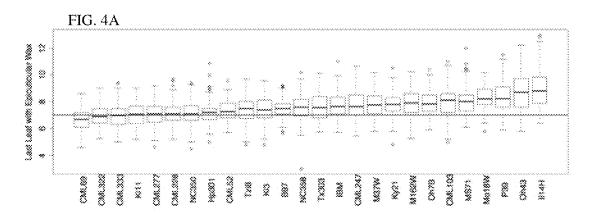
# FIG. 3 (continued)

Sorghum Sb02g003420.1_Sb02g003430.1 Maize GRMZM2G362718_P01 Rice LoC_os08g24946.1 13108.m23057 protein	GKRRKNTDQSTEPTELSNRLYGAESEQADNVGAKSTSPQIVVEPHCAAKVLK GKRRKNKRRKWTDQSTKPTDLPNRLCGAESEQADNVGAKSTLPQIVVEPHCAAKHLK DDSYVSEPLQKRAKLNEKFNAKGDKSKRAGVKSEFEEVLESEKKKTRSLK :: *: *: *: *: *: *: *: *: *: *: *: *: *
Sorghum Sb02g003420.1_Sb02g003430.1 Kaize GRNZNZG362716_P01 Rice LoC_os08g24946.1 13108.m23057 protein	GDPQIEGSIIGVAGSQWGAETHWGHEKOFGIS-CVARTETEKRVTY GDPQIAKQGVAARQWGAETHKGHENQFGISFCVASTETEKRVTC KRTQPEEPLVECAAAAANWANRPVKEREKELGTSSLDMGKIPLSSPPIVDSETEKRISA * : :****::
Sorghum Sb02g003420.1_Sb02g003430.1 Maize GRMZNZG362718_P01 Rice LOC_OSO8g24946.1 13108.m23057 protein	LAQKGKDFEL LAQRGTCLGTPYDGPSTKDMSDCSVQDTPVDKDFEL LAQRGDDVEL LVEKEVSSLTVADISRRCVIPSTYACSGRQIDKIVVRGKLERSIQAVKAALQKLENGGAV *.:: ::::::::::::::::::::::::::::::::::
Sorghum Sb02g003420.1_Sb02g003430.1 Kaize GRNZK2362718_P01 Rice LoC_os08g24946.1 13108.m23057 protein	DNVAYRIMEDKYANGREETQEDYTRKETAHRKDSSENQGON DNVACIIAVDKYVNGRGKTQEDYTRKEAAQRKDSSENQGON DDAKAVCESEVLRQLTRUHNKLRVYLAPFIHGMRYTSFGRHFTKKEK
Sorghum Sb02g003420.1_Sb02g003430.1 Maize GRMZMZG362718_P01 Rice LOC_Os08g24946.1 13108.m23057 protein	DVLELDNLUVEIQADGSPLEPGNKRYKEENAYGLGSASGHEKETSSSRRENV DALELDNLRMENQADERPLEPGNKRDRKUQKNVYGLGSASGQKETLSRRENP -LIEIAEKLHUYVQPGDUKSNNVDPETRPRRVNMLRGFGALSQFNKEKLDKVGKRCNF :*: : : : :: : :: :: :: :: :: :: :: :: :
Sorghum Sb02g003420.1_Sb02g003430.1 Kaize GRMZM2G362718_P01 Rice LoC_os08g24946.1 13108.m23057 protein	OSDRGHVPHNDSKTIDYRKG-GTTLDNHVYDHSSEGSYPCQGECSHSKCN RSDRGHVHSNDSKTIYYRKG-GTEVDNVD-DHPLE
Sorghum Sb02g003420.1_Sb02g003430.1 Maize GRNZNZG362718_P01 Rice LoC_os08g24946.1 13108.m23057 protein	DGLVAIDODTSSDRLKKRSOPVEKASDGWKTDLDKWKKHNLKED KQDHQDTSSDGSKKRSRPVDNASGGWRPYLDENKKRNLRED FKPKLIILIVPKEAERLDFKQQPYDLVUEDDQRLSGKSFYLPGSLDVSDKQIDQUNKSPP
Sorghum Sb02g003420.1_Sb02g003430.1 Maize GRMZM2G362718_P01 Rice LoC_OS08g24946.1 13108.m23057 protein	GR-DAHYEDRRTERNTAADTSRYKCRDKIQLDRREPELVGRNTRARSSEHSPERQRMERD GR-YAHYEDURSERNTAADTSGYKAOSEEKPVWTNTRTGSREHSLDRQRIEGG PLYLUSRPDUTQKHKRIARQHGHTKANVFSHNEEDLVYLFEDRATQNHDVMNKNYTSG

# FIG. 3 (continued)

Sorghum   SMC2g003420.1 SbC2g003430.1 Maire   ORMIM20362718 F01 Rice   LOC	
Sorghum   SD02g003420.1	eefoodhsh-l-y-prosh-ingrebhfqyloLoypqhgyzeph sernshkkysnsspdrykydgredhfqyloLoypqhgyzeph srrnshkkysnsspdrykydhladsdrilesdsrnylhksgrilepissp
Sorghum Sb02g003420.1_Sb02g003430.1 Naire GRWIM2G362716_P0.1 Rice LOC_CeUSg24946.1 13108.m23057 protein	RINGEBRUTTHUNGHGREPFHTNGBBAFFRDRORGEYDBSRYGEYDATUNGPDSAHRP 2GYTLERLRYHDNHFDHLVGERSSSSSSSSSTLOMPIPEBSYFRSVNE
Sorghum Sp02gD03420.1_Sp02gD03450.1 Maire GRMIM2G362718_P01 Rice LOC_OmUSG24946.1 13108.m23057 protein	THARSAACHSANCTRLAGGYGEGSRASERPYTDWTAPHLD
Sorghum  Spd2gdd1428.1_Spd2gdd2430.1 Maizæ GRMZM2G162718_PG1 Rice LOC_OwOBg24946.1 11108.m23057 pratein	NLEDTINDSSESAOMPYPGRBVQETARTITYGHURDEVPQTAINTPSKDIRTHIRNYGRH
%orgmum %pD%gU0%420.1_SbC2gU0%430.1 Maizæ GRMZM%%62718_PG1 Rice{LOC_O&O8g24946.1 13108.m23057 protein	IRIDHTQITIMPPANDIRAQIPAYGQHITSDHQHASRISSSSPUARFEQQPSFTSYGMPS
%orqhum %b02g003420.1_%b02g003430.1 Raize GPNINIO362718_P01 Rice LOC_Os08g24946.1 13108.m25057 protein	-LOASRPUTDRYAPGLELTHYPFPSQSDL
Sorghum ShOZgoO3420.1 ShOZgOO3430.1 Maire (PRMIM20362718 PU1 Rice (100 OsoBo24946.1   13108.m23057   protein	

FIG. 4



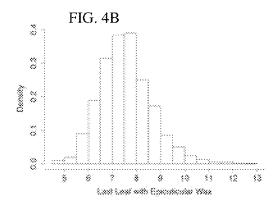


FIG. 4 (continued)

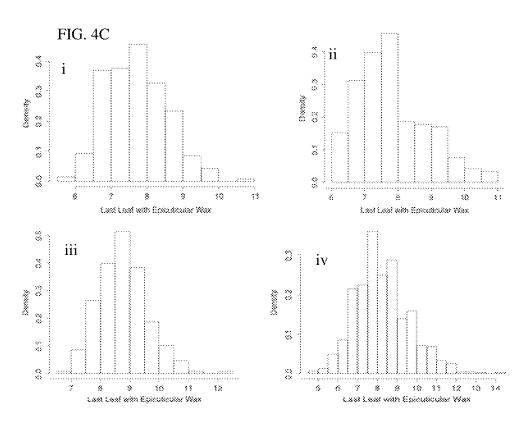


FIG. 5

No. of lines	3	875	3	02	243	277	573	509
Year <sup>reps</sup>	2008	20091	2008 <sup>7</sup> 2030 <sup>7</sup>	2009 <sup>)</sup> 2011	2010 <sup>,</sup> 2011 <sup>,</sup>	2010, 5011,	2009 2010	2016,
Location	ww	ARL	WW	ARE	WW	WW	ARL	WM
			ARL	ww				
Plot size/ Plants	3	/42	2)	42	3/15	1/15	2/92	1/15
Traits Measured	Constituti DAR CAS	Francisco Node # Place Height	Transf Notes Part		Transition	Transition	Transition Node # Plansitioght GCO	Transition

FIG. 6

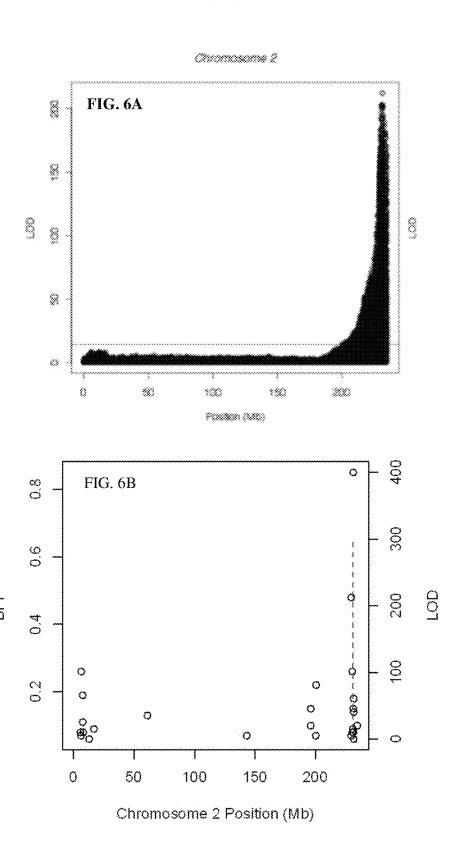


FIG. 7

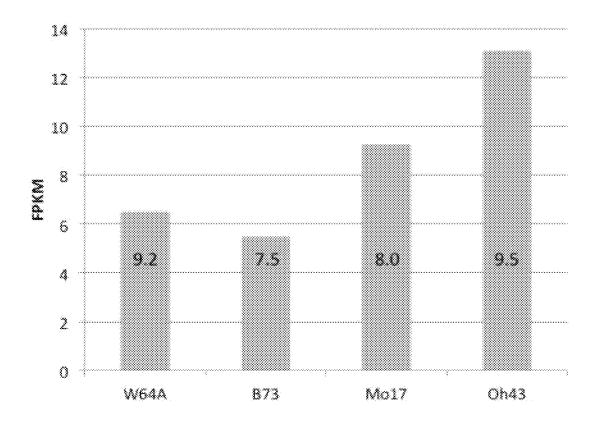
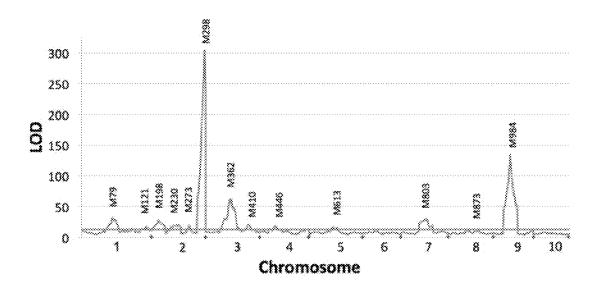


FIG. 8



#### **EXTENDING JUVENILITY IN GRASSES**

This application claims the priority of U.S. Provisional Appl. Ser. No. 61/651,540 filed May 24, 2012, the entire disclosure of which is incorporated herein by reference.

### STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with Government support under <sup>10</sup> 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 updated ST25.txt", which is 122,753 bytes (measured in MS-WINDOWS) and was created on Sep. 18, 2013, is filed herewith by electronic submission and incorporated herein by reference.

#### 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 dramatically in anatomy, biochemical composition, and in 30 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 generally less recalcitrant when used for processing for biofuels, 35 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. However, the genetic triggers controlling the transition 40 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 agronomic traits.

#### SUMMARY OF THE INVENTION

In one aspect the invention provides a polynucleotide molecule comprising a sequence selected from the group 50 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 55 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 compris- 60 ing 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), further wherein the 65 polynucleotide molecule is operably linked to a heterologous promoter functional in plants. In a particular embodi2

ment 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 promoter, a selectable marker, a leader sequence and a terminator. The additional sequence may be a heterologous sequence. In some embodiments the promoter is a tissue-specific promoter. In a particular embodiment the promoter directs expression in leaf tissue. In certain embodiments the 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 45 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 juvenile 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 recombinant 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 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 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.

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 embodithe 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 disease resistance, enhanced insect resistance, improved forage 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(SEQ ID NO:3) with *Arabidopsis* homolog AT5G55390.1(SEQ ID NO:10).

FIG. **3** shows a CLUSTAL protein alignment of GRMZM2G362718(SEQ ID NO:3) with homologs from sorghum(SEQ ID NO:19) and rice(SEQ ID NO:11).

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×Mo17; 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 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 5 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 sequence from Z. mays Oh43.

SEQ ID NO:8 GRMZM2G362718 nucleotide coding sequence from Z. mays W64A.

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 Os08g24946.1 from Oryza sativa.

SEQ ID NO:12 Predicted protein sequence of 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 25 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 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 40 the adult phase of growth. By modulate is meant to either 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, 45 and resistance to a plant pest or plant disease, and is also an 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 50 enhanced. Such traits may include one or more of: improved 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 digest- 55 ibility of an animal feed ingredient such as plant vegetative tissue; and improved nutritional content of plant vegetative tissue.

GRMZM2G362718 is a gene of previously unknown function in corn (maize) which was identified through 60 chromosomal mapping of juvenile plant tissue, and apparently 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 65 Arabidopsis, rice, Brachypodium, and sorghum (displaying approximately 52.9, 56.7, 42.9, 63.2, percent similarity,

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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 10 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 SEQ ID NO:9 GRMZM2G362718 predicted protein 15 transcript presence/absence as the dependent variable, identified 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 20 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 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 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 **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 expression of GRMZM2G362718 and/or GRMZM2G096016, or homologs thereof. Thus, for expression Glossy15 (Gl15; instance. the of

GRMZM2G160730), or Cg1, or a homolog thereof, may be 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 10 coding sequence, or a GRMZM2G096016 coding sequence. 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 15 NO:9, and SEQ ID NO:17, respectively. Constructs may 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 20 addition to a GRMZM2G362718 coding sequence, 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 25 for instance, such constructs, in addition to comprising all or 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 30 ID NO:14, encoding the polypeptide of SEQ ID NO:15, and 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 35 sequence displaying at least 85%, 90%, 95%, or 99% 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 40 for the polypeptide or a fragment thereof in a reading frame 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, non- 45 coding 5' and 3' sequences, such as the transcribed, untranslated 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 50 included to facilitate the purification of a fused polypeptide. 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 55 between two or more polypeptide sequences or two or more 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 60 such sequences. Methods to determine "identity" are 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 65 used to determine "identity" between two sequences these programs include but are not limited to, GCG; suite of

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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., 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 the following: Algorithm: Needleman and Wunsch (1970); 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 GRMZM2G362718 GRMZM2G096016 or 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 5 represent one way of altering agronomic characteristics in 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 10 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 15 alter agronomic characteristics (e.g., leaf morphology or 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 25 virtually any method by which DNA can be introduced into 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 30 cells, the next steps generally concern identifying the transformed 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 35 invention. In this case, one would then generally assay the 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 40 cells that have been scored positive in a screening assay, 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 45 and 2,4-D. However, other growth regulators may be 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 50 growth regulators until sufficient tissue is available to begin 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 55 needed on this medium. Shoot development will signal the 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, and hardened, e.g., in an environmentally controlled chamber, for example, at about 85% relative humidity, 600 ppm CO<sub>2</sub>, and 25-250 microeinsteins m-2 s-1 of light. Plants may be matured in a growth chamber or greenhouse. Plants can 65 be regenerated from about 6 wk to 10 months after a transformant is identified, depending on the initial tissue.

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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 PCR<sup>TM</sup>; "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 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 embryo rescue due to cessation of seed development and 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 10-5 M 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 cross

One embodiment of the present invention provides for a 10 plant that contains a genetic marker linked to one or more locus allowing for extended juvenile growth. By "extended juvenile growth locus" is meant a locus that contributes to such extended or enhanced juvenile growth either alone or in combination 15 with one more other locus.

#### IV. Definitions

As used herein, a "desirable trait" or "desirable traits" 20 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 preparation of, for instance, biofuel, among others.

As used herein, "polymorphism" means the presence of 25 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 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 of meiosis, such as unequal crossing over, genome duplication and chromosome breaks and fusions. The variation can 35 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 important phenotypic variation. Useful polymorphisms may include single nucleotide polymorphisms (SNPs), insertions 40 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 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 polymorphisms. In addition, the presence, absence, or variation in copy number of the preceding may comprise a 50 polymorphism.

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

As used herein, linkage of two nucleic acid sequences, including a nucleic acid marker sequence and a nucleic acid 60 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 enhanced juvenile growth trait are genetically linked, and exhibit a LOD score of greater than 2.0, as judged by 65 interval mapping for the trait based on maximum likelihood methods described by Lander and Botstein, 1989 (*Genetics*,

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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 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 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

Appropriate stringency conditions which promote DNA hybridization are known in the art, for example 6.0× sodium chloride/sodium citrate (SSC) at about 45° C., followed by a wash of 2.0×SSC 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, 5.0×SSC, 1% SDS and incubation at 42° C. for 14 hours, followed by a wash using 0.2×SSC, 1% SDS and

incubation at  $65^{\circ}$  C. Alternative wash conditions, such as of 0.15 M NaCl and  $70^{\circ}$  C. for 10 minutes may also be used.

The specificity of hybridization can be affected by posthybridization washes. For example, the salt concentration in 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 stringency conditions at room temperature, about 22° C., to 10 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 other variable is changed. In some aspects, the wash step can 15 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, temperature, and time. In another aspect, the wash step is 20

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 profile and phenotype is crossed with a second inbred of 25 known genetic marker profile and phenotype it is possible to predict the phenotype of the F<sub>1</sub> hybrid based on the combined genetic marker profiles of the parent inbreds. Methods for prediction of hybrid performance from genetic marker data are disclosed in U.S. Pat. No. 5,492,547, the disclosure 30 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.

Additional markers, such as SSRs, AFLP markers, RFLP 35 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, 1993); Burow and Blake, *Molecular Dissection of Complex* 40 *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 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 described herein can be established by a gene mapping 50 model such as, without limitation, the flanking marker model, and the interval mapping, based on maximum likelihood methods described by Lander and Botstein, 1989 (*Genetics*, 121:185-199), and implemented in the software 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 of a marker is calculated, together with an MLE assuming no 60 trait effect, to avoid false positives. A log<sub>10</sub> of an odds ratio (LOD) is then calculated as: LOD=log<sub>10</sub> (MLE for the presence of a trait (MLE given no linked trait)).

The LOD score essentially indicates how much more likely the data are to have arisen assuming the presence of 65 a resistance allele rather than in its absence. The LOD threshold value for avoiding a false positive with a given

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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 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 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., F<sub>3</sub> or back cross self families) to identify heterozygous individuals. The information gathered can be equivalent to that obtained in a completely classified F<sub>2</sub> population. This procedure is, however, often prohibitive because of the cost and time involved in progeny testing. Progeny testing of F<sub>2</sub> 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<sub>3</sub> 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 (F2, F3), where linkage has not been completely disassociated by recombination events (i.e., maximum disequilibrium).

Recombinant inbred lines (RILs) (genetically related lines; usually >F<sub>5</sub>) 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 popu-

lations 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., loci become more independent), the information in RIL 5 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 F<sub>1</sub> to one of its parents. Typically, backcross 10 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, which is often referred to as the donor. A series of back- 15 crosses 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 a mosaic of genomic regions from the donor parent. Back- 20 cross 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; 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, gametes are sampled per plant. Backcross populations, however, 30 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 tight linkages, but may be undesirable in the construction of 35 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 under interrogation can be used as a mapping population. In 40 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 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 drawn from a segregating population originating from a 50 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 the target trait will not differ between the bulked samples of 55 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 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.

For highly heritable traits, a choice of superior individual 65 plants evaluated at a single location will be effective, whereas for traits with low heritability, selection should be

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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 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 com- 10 monly 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 plants produced by a method of the present invention. Parts of 15 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 of a fruit. In one embodiment of the present invention, the plant 20 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 greater than 60%, or a greater than 80% reduction in foliar 25 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 displaying enhanced juvenile growth may comprise increased 30 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 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 discovered by the inventor to function well in the practice of 45 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 which are disclosed and still obtain a like or similar result 50 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 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.

The molecular network controlling the process of devel- 65 analysis of the NAMs: opmental transition has been poorly understood. The present study utilizes the dramatic variation in the timing of juvenile

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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 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

where Y is the last leaf with epicuticular wax of the ith genotype (G) in the kth year (Y) and  $\mu$  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 the Wisconsin Diversity Panel:  $_{15}$ 

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

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  $\mu$  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:

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

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, <sup>35</sup> 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.nesu.edu/qtlcart/WQTCart.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-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.

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

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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 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, 56 total QTL 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 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), 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

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 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 <sup>10</sup> 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, and OWRI results are from composite interval mapping <sup>15</sup> (LOD threshold 2.5).

TABLE 1

Summary of QTL detected acros	s all RIL mapping populations.
Mapping Population	LOD score of QTL on chromosome 2
NAM IBM NyH	21.3, 20.1, 18.2, 303.9 18.7 3.3

Numbers indicate LOD score. Overlapping QTL based on physical position of QTL support intervals are italicized.

Two common QTL were detected across multiple mapping populations. The QTL on the long arm of chromosome 300 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 35 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 juvenile-adult transition.

#### Example 4

#### Candidate Gene GRMZM2G362718

The gene model nearest the most significant SNP on chromosome two is GRMZM2G362718 whose predicted 50 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 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 QTL. Mutations in EDM2 show a delay in flowering and elevated transcripts of the flowering suppressor FLC 60 (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 22

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 (Peragine et al *Genes Devel.* 18:2368-2379, 2004). This suggests EDM2's role in vegetative phase change may be 20 independent of the siRNA pathway, and GRMZM2G362718 may act similarly.

Sekhon et al (*Plant J.* 66:553-563, 2011) developed a 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 levels 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 ("G115" (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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bp after clipping using both Illumina 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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<213> ORGANISM: Zea mays

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Gly	Thr	Thr 435	Ser	Arg	Asn	His	Ile 440	Lys	Phe	Pro	Ala	Thr 445	Pro	Glu	Tyr
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Lys 625	Glu	Ala	Ala	Gln	Arg 630	Lys	Asp	Ser	Ser	Glu 635	Asn	Gln	Gly	Gln	Asn 640
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Arg	Pro	Leu	Glu 660	Pro	Gly	Asn	Lys	Arg 665	Asp	Arg	Lys	Trp	Gln 670	Lys	Asn
Val	Tyr	Gly 675	Leu	Gly	Ser	Ala	Ser 680	Gly	Gln	ГÀз	Glu	Thr 685	Leu	Ser	Arg
Arg	Glu 690	Asn	Pro	Arg	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	Asp	His	Gln 730	Asp	Thr	Ser	Ser	Asp 735	Gly
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Tyr	Ala 770	His	Tyr	Glu	Asp	Trp 775	Arg	Ser	Glu	Arg	Asn 780	Thr	Ala	Ala	Asp
Thr 785	Ser	Gly	Tyr	Lys	Ala 790	Gln	Ser	Glu	Glu	Lys 795	Pro	Val	Trp	Thr	Asn 800
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<211> LENGTH: 3021

<212> TYPE: DNA

<213 > ORGANISM: Zea mays

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<210> SEQ ID NO 5

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<sup>&</sup>lt;211> LENGTH: 1005

<sup>&</sup>lt;212> TYPE: PRT

<sup>&</sup>lt;213> ORGANISM: Zea mays

<sup>&</sup>lt;400> SEQUENCE: 5

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Arg	Lys 50	Asp	Val	Phe	Leu	Cys 55	Gly	Phe	Val	Asp	Lys 60	Asn	Leu	Pro	Val
Tyr 65	Lys	Glu	Val	Val	Ala 70	Trp	Lys	Ile	Arg	Leu 75	Asp	Ser	Glu	His	Pro 80
Asn	Ile	Tyr	Val	Leu 85	Ser	Ile	Glu	His	Lys	Trp	Ile	Lys	Leu	Leu 95	Lys
Pro	Arg	Lys	Cys 100	Tyr	Gly	Asp	Ile	Val 105	Arg	Ser	Thr	Leu	Ile 110	Thr	Val
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	Tyr 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
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Ser	Ile	Val 195	Ser	Asp	Gly	Trp	Arg 200	Ala	Arg	Lys	Asn	Asp 205	Asp	Asn	Asn
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CAa	Ala	Leu	Cys	Asp 245	Asp	Gly	Gly	His	Leu 250	Leu	Ser	CAa	Asp	Gly 255	Pro
CAa	ГÀЗ	Arg	Ser 260	Phe	His	Pro	Thr	Lys 265	ГÀв	Asp	Gly	Arg	Glu 270	Ser	Lys
Cys	Glu	Ser 275	Leu	His	Tyr	Thr	Ser 280	Ala	Glu	Val	Lys	Arg 285	Ile	Gly	Thr
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Leu	Leu	Asp	Pro 340	Asn	Ala	Thr	Asp	Gly 345	Glu	Leu	Glu	Arg	Arg 350	Ile	Met
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His	Met 370	Glu	Asn	ràs	Ala	Gln 375	Arg	Ala	Leu	Gln	Leu 380	Ala	Val	Cys	Arg
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<211> LENGTH: 3021

<212> TYPE: DNA

<213> ORGANISM: Zea mays

<400> SEQUENCE: 6

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<210> SEQ ID NO 7
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<sup>&</sup>lt;211> LENGTH: 1005

<sup>&</sup>lt;212> TYPE: PRT

<sup>&</sup>lt;213 > ORGANISM: Zea mays

<sup>&</sup>lt;400> SEOUENCE: 7

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Pro	Arg	Lys	Cys 100	Tyr	Gly	Asp	Ile	Val 105	Arg	Ser	Thr	Leu	Ile 110	Thr	Val
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
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Pro Ser Tyr Gln Leu Ala Gly Gly Tyr Gly Glu Gly Ser Arg Ala Trp 980 985 990

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<210> SEQ ID NO 8

<211> LENGTH: 3021

<212> TYPE: DNA

<213> ORGANISM: Zea mays

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Glu	Phe	Cys	Gly	Asp	His	Ser	His	Leu	Tyr	Pro	Arg	Asp	Ser	Thr	Ile

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His Ile Trp Asp Cys Gly Lys Gln Val Tyr Leu Gly Gly Phe Asp Thr 130 135 140

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Val Thr Gln His Lys Cys Gly Lys Trp Glu Ala Arg Ile Gly Gln Leu 210 215 220
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Pro Arg Ala Trp Lys Leu Ser Lys Thr Ile Phe Phe Tyr Cys Leu Asp

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Phe Pro Ala Thr Pro Glu Cys Thr Lys Thr Lys Glu Leu Gly Asn Arg 165 \$170\$

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CÀa	Gln	Ser	Ser	Phe	Pro	Leu	Pro	Glu	Phe	Cys	Gly	Asp	His	Ser	His

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Pro 705	Pro	Arg	Ser	Gln	Ser 710	Asp	Leu	Gln	Tyr	Cys 715	Thr	Thr	Thr	Ile	

What is claimed is:

- 1. A polynucleotide molecule comprising a sequence selected from the group consisting of:
  - (a) a sequence encoding a polypeptide at least 95% identical to SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9; 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, or SEQ ID NO:8;
  - (c) a sequence comprising at least 95% sequence identity over its full length to the full length of SEQ ID NO:2, wherein the sequence encodes a protein that regulates 35 juvenile to adult phase change in grass plant leaves; and (d) a sequence complementary to (a), (b), or (c),
  - further wherein the polynucleotide molecule is operably linked to a heterologous promoter functional in plants.
- 2. The polynucleotide molecule of claim 1, comprising 40 the coding sequence of SEQ ID NO:2.
- 3. A recombinant vector comprising the polynucleotide molecule of claim 1.
- **4**. The recombinant vector of claim **3**, further comprising at least one additional sequence chosen from the group 45 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 pro- 50 moter is a tissue-specific promoter.
- 7. The recombinant vector of claim 3, Wherein the promoter directs expression in leaf tissue.
- **8**. An expression cassette comprising the polynucleotide molecule of claim **1**.
- 9. A recombinant vector comprising a polynucleotide encoding a siRNA, wherein the polynucleotide comprises a first sequence comprising all or a part of the sequence of claim 1, and a second sequence comprising the reverse complement of the first sequence, wherein the expression of 60 the polynucleotide in a plant down regulates the expression of a polypeptide encoded by the polynucleotide molecule of claim 1 in the plant.
- 10. A transgenic plant comprising the recombinant vector of claim 3.
- 11. A transgenic plant comprising the recombinant vector of claim 9.

- 12. The transgenic plant of claim 10 or 11, wherein the plant is a monocotyledonous plant.
- 13. The transgenic plant of claim 10 or 11, wherein the plant is a member of the Poaceae.
- **14**. The transgenic plant of claim **10** or **11**, wherein the plant is a member of the Panicoideae or the Pooideae.
- 15. The transgenic plant of claim 10 or 11, wherein the plant is a maize, a rice, a sorghum, or a switchgrass plant.
- 16. A seed of the transgenic plant of claim 10 or 11, wherein the seed comprises recombinant vector.
- 17. The plant of claim 10 or 11, 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.
- 18. A cell transformed with the recombinant vector of claim 3 or 9.
- 19. 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, expressing the construct of claim 9 in the plant, or mutagenizing the polynucleotide molecule of claim 1.
- 20. The method of claim 19, comprising expressing the construct of claim 3 in the plant.
- 21. The method of claim 19, comprising expressing the construct of claim 9 in the plant.
- 22. The method of claim 19, comprising mutagenizing the polynucleotide sequence of SEQ ID NO:2.
- 23. The method of claim 19, wherein the timing of juvenile to adult phase in the plant is extended relative to a wild type plant.
- 24. The method of claim 23, wherein the timing of juvenile to adult phase change is calculated by a method comprising counting the last leaf displaying epicuticular wax.
  - 25. The method of claim 19, 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 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 relative to a wild type plant.

- 26. The method of claim 19, 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 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.
- 27. A method of producing plant biomass, the method  $^{10}$  comprising:
  - (a) obtaining a plant according to claim 10 or 11; and
  - (b) preparing biomass from said plant or a part thereof.
- 28. The method of claim 27, further comprising producing biofuel from the biomass.
- 29. The method of claim 27, comprising producing food or feed from the biomass.
- **30**. The recombinant vector of claim **3**, further comprising an additional polynucleotide sequence which, after being transcribed, regulates the timing of the juvenile to adult <sup>20</sup> phase change in a plant.
- **31**. The recombinant vector of claim **30** wherein the additional polynucleotide sequence 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.
- 32. 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.
- 33. A transgenic plant comprising the recombinant vector of claim 30.
- **34.** A transgenic plant comprising the recombinant vector of claim **32.**

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- 35. A seed of the transgenic plant of claim 33 or claim 34.
- 36. The method of claim 19, 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.
- 37. The method of claim 36, wherein the second gene is selected from the group consisting of: Glossy15 and Cg1.
- 38. The method of claim 36, comprising expressing the recombinant vector of claim 30 or 32 in the plant.
- **39**. The method of claim **36**, wherein the timing of juvenile to adult phase in the plant is extended relative to a wild type plant.
- **40**. A method of producing plant biomass, the method comprising:
  - (a) obtaining a plant according to claim 33 or 34; and
  - (b) preparing biomass from said plant or a part thereof.
- 41. The method of claim 40, further comprising producing biofuel from the biomass.
- **42**. The method of claim **40**, comprising producing food or feed from the biomass.
- **43**. The polynucleotide molecule of claim **1**, wherein the sequence encodes a polypeptide that is at least 95% identical to SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or SEQ ID NO:9; wherein the polypeptide regulates juvenile to adult phase change in grass plant leaves.
- **44**. The polynucleotide molecule of claim **1**, wherein the sequence comprises SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, or SEQ ID NO:8.
- **45**. The polynucleotide molecule of claim **1**, wherein the sequence comprises at least 95% sequence identity over its full length to the full length of SEQ ID NO:2, wherein the sequence encodes a protein that regulates juvenile to adult phase change in grass plant leaves.

\* \* \* \* \*