

**Table S1** The DNA primers used in this study.

Primer name	Sequence (5'-3')
NPT2U	GCTATTCGGCTATGACTGG
NPT2R	ATAGAAGGCGATGCGCTG
PnPGK_LP	GTACTTGATGGGTCATGGTG
PnPGK_RP	AGGTCAACCTTCTCCAAGAG
PnFL1_47_C08_LP1	CTATGCTGCCATGGACTGC
PnFL1_47_C08_RP1	CCAGCGTTGAAGTAGAGAGGA
PnFL1-093_N05_LP1	CGGAGAACATTAACCTTGACAA
PnFL1-093_N05_RP1	TCCCACCATTTGTTGACCAC
PnFL1_55_M08_LP1	GGAGAACATTAACCTTGACAG
PnFL1_55_M08_RP1	TCCCACCATTTGTTGACCAC
PnFL2_32_B04_LP1	GGGTTAGCCAAGGGGCTAAA
PnFL2_32_B04_RP1	TGGATGAACAGGCTCAATCTC
PnFL2_46_L21_LP1	TTTGCTGAACAGGACTTCCTC
PnFL2_46_L21_RP1	CCAGCAGCACAATAGTGGAC
PnFL2-078_L13_LP1	AAAACATGGAGTCACACTCTG
PnFL2-078_L13_RP1	TGGCAATACTAGGCTCAAACA

**Table S2** Relationship of galactinol synthase (GolS) from *Populus nigra* with those from *P. trichocarpa*.

<i>P. nigra</i>		<i>P. trichocarpa</i>			
Gene name	Locus	Gene name		Predicted protein length	Identity (%)
		Philippe et al. (2010)	Zhou et al. (2014)	(Amino acids)	
<i>PnGolS1</i>	Potri.002G191600	<i>PtGolS6</i>	<i>PtrGolS8</i>	337	96
	Potri.014G116800	<i>PtGolS7</i>	<i>PtrGolS6</i>	336	92
<i>PnGolS2</i>	Potri.005G006800	<i>PtGolS5</i>	<i>PtrGolS3</i>	334	97
<i>PnGolS3.1</i>	Potri.013G005800	<i>PtGolS4</i>	<i>PtrGolS1</i>	334	97
<i>PnGolS3.2</i>	Potri.013G005800	<i>PtGolS4</i>	<i>PtrGolS1</i>	334	97
<i>PnGolS4.1</i>	Potri.013G005900	<i>PtGolS3</i>	<i>PtrGolS2</i>	337	100
<i>PnGolS4.2</i>	Potri.013G005900	<i>PtGolS3</i>	<i>PtrGolS2</i>	337	99

**Figure S1**

PnGolS1	1	MAPELVQS--ALKPAGF-TKLASLPS-----RAYVTFLAGDGDYVKGVVGLAKGLRKVKT	52
PnGolS2	1	MAPDITATL-ANNANSL-VKQASISS-----CAYVTFLAGDGDYWKGVVGLAKGLRKAKS	53
PnGolS3.1	1	MAPDITATL-ANNATTL-VKQASISS-----CAYVTFLAGDGDYWKGVVGLAKGLRKAKS	53
PnGolS3.2	1	MAPDITATL-ANNATTL-VKQASISS-----CAYVTFLAGDGDYWKGVVGLAKGLRKAKS	53
PnGolS4.1	1	MAPHITTAL-ANSTNSL-VKQASLSS-----CAYVTFLAGDGDYWKGVVGLAKGLRKAKS	53
PnGolS4.2	1	MAPHITTAL-ANSTNSL-VKQASLSS-----CAYVTFLAGDGDYWKGVVGLAKGLRKAKS	53
PtrGolS1	1	MAPDITATL-ANNATTL-VKQASISS-----CAYVTFLAGDGDYWKGVVGLAKGLRKAKS	53
PtrGolS2	1	MAPHITTAL-ANSTNSL-VKQASLSS-----CAYVTFLAGDGDYWKGVVGLAKGLRKAKS	53
PtrGolS3	1	MAPDITATL-ANNTNSL-VKQASISS-----CAYVTFLAGDGDYWKGVVGLAKGLRKAKC	53
PtrGolS4	1	MAPGV-----PMDVISC-TGKVSTASTGYSKRAFVTFLAGNGDYVKGVVGLAKGLRKVKS	54
PtrGolS5	1	MSPN-----IIEPTTD-LQR-----RAYVTFLAGNGDYVKGVVGLAKGLRKVKS	44
PtrGolS6	1	MAPELVQA--ALKPAGF-TKPASLPS-----RAYVTFLAGNGDYVKGVVGLAKGLRKVKT	52
PtrGolS7	1	MSPN-----IIEPTFN-SHK-----RAYVTFLAGNGDYVKGVVGLAKGLRKAKS	44
PtrGolS8	1	MAPELVRS--ALKPAGF-TKLANLPS-----RAYVTFLAGDGDYVKGVVGLAKGLRKVKT	52
PtrGolS9	1	MAPGV-----PIDGNILGTGKYSTVNTGYSKRAYVTFLAGNGDYVKGVVGLAKGLRKVKS	55
PaxgGolS1	1	MAPGV-----PMDVISC-TGKVSTASHGYSKRAFVTFLAGNGDYVKGVVGLAKGLRRVKS	54
PaxgGolS2	1	MAPHITTTTL-ANTNSL-VKQASLSS-----CAYVTFLAGDGDYWKGVVGLAKGLRKAKS	53
AtGolS1	1	MAPGLTQTADAMSTVTI-TKPSLPSVQDSRAYVTFLAGNGDYVKGVVGLAKGLRKVKS	58
AtGolS2	1	MAPEINTKL-TVPVHSA-TGGEK-----RAYVTFLAGTG DYVKGVVGLAKGLRKAKS	50
AtGolS3	1	MAPEM-----NNKLSYG-EKK-----RAYVTFLAGTG DYVKGVVGLAKGLRKTKS	44
AtGolS4	1	MAPEI-----SVNPMYL-SEKAHQAPPR--RAYVTFLAGNGDYVKGVVGLAKGLRKVKS	51
AtGolS5	1	MTMTVEK---RIEADVT-VSHEGVE-----RAYVTFLAGNKDYWMLVVGLAKGLRKVKS	50
AtGolS6	1	MAQMSMTVEKSIKADVT-VSHDRVK-----RAYVTFLAGNKDYWMGVVGLAKGLRKVKS	53
AtGolS7	1	MTPETHVDM-INASEKA-PKE-----RAYVTFLAGNGDYVKGVVGLAKGLRKVKS	48
PnGolS1	53	AYPLIVAVLPDVPEEHRQILLESQGCIVRETEPVYPPENQTFAMAYYVINYSKLRIWEFV	112
PnGolS2	54	NYPLVVAILPDVPEEHRKILASQGCIVRETEPVNPPENQTFAMAYYVINYSKLRIWEFV	113
PnGolS3.1	54	KYPLVVAILPDVPEEHRKILVSQGCIVRETEPVHPPENQTFAMAYYVINYSKLRIWEFV	113
PnGolS3.2	54	KYPLVVAILPDVPEEHRKILVSQGCIVRETEPVHPPENQTFAMAYYVINYSKLRIWEFV	113
PnGolS4.1	54	KYPLVVAILPDVPEEHRMILVSQGCIVRETEPVHPPENQTRFAMPYYVINYSKLRIWEFV	113
PnGolS4.2	54	KYPLVVAILPDVPEEHRMILVSQGCIVKEIEPVHPPENQTRFAMPYYVINYSKLRIWEFV	113
PtrGolS1	54	KYPLVVAILPDVPEEHRKILVSQGCIVRETEPVHPPENQTRFAMPYYVINYSKLRIWEFV	113
PtrGolS2	54	KYPLVVAILPDVPEEHRMILVSQGCIVRETEPVHPPENQTRFAMPYYVINYSKLRIWEFV	113
PtrGolS3	54	NYPLVVAILPDVPEEHRKILASQGCIVRETEPVNPPENQTFAMAYYVINYSKLRIWEFV	113
PtrGolS4	55	AYPLVVAMLPDVPEEHRDILRSQGCIVRETEPIYPPENQTFAMAYYVINYSKLRIWNEF	114
PtrGolS5	45	AYPLVVAILPDVPEEHRILESQGCIRETEPLYPPENQTFAMAYYVINYSKLRIWEFV	104
PtrGolS6	53	AYPLIVAVLPDVPEEHRILESQGCIVRETEPVYPPENQTFAMAYYVINYSKLRIWEFV	112
PtrGolS7	45	SYPLMVAILPDVPEEHRKILESQGCIVRETEPVYPPDNQTFAMAYYVINYSKLRIWEFV	104
PtrGolS8	53	AYPLIVAVLPDVPEEHRQILLESQGCIVRETEPVYPPENQTFAMAYYVINYSKLRIWEFV	112
PtrGolS9	56	AYPLVVAILPDVPEEHRDILRSQGCIVRETEPIYPPENQTFAMAYYVINYSKLRIWNEF	115
PaxgGolS1	55	AYPLVVAMLPDVPEEHRDILRSQGCIVRETEPIYPPENQTFAMAYYVINYSKLRIWNEF	114
PaxgGolS2	54	KYPLVVAILPDVPEEHRMILVSQGCIVRETEPVHPPENQTRFAMPYYVINYSKLRIWEFV	113
AtGolS1	59	AYPLVVAMLPDVPEEHRILVDQGCIVRETEPVYPPENQTFAMAYYVINYSKLRIWKFV	118
AtGolS2	51	KYPLVVAVLPDVPEDHRKQLVDQGCIVKEIEPVYPPENQTFAMAYYVINYSKLRIWEFV	110
AtGolS3	45	KYPLVVAVLPDVPADHRQLLDQGCIVKEIQPVYPPDNQTFAMAYYVINYSKLRIWKFV	104
AtGolS4	52	AYPLVVAMLPDVPEEHRILRSQGCIVRETEPVYPPDNQTFAMAYYVINYSKLRIWNEF	111
AtGolS5	51	AYPLVVAATLPDVPEEHRQILVDQGCIRDIEPVYPPENTTGYSMAYYVINYSKLRIWEFV	110
AtGolS6	54	AYPLVVAILPDVPEEHRQILLAQGCIRETEPVYPPENKTGYSMAYYVINYSKLRIWEFV	113
AtGolS7	49	AYPLVVAMLPDVPEEHRILERSQGCIVRETEPVHPPDSQDAYARAYYIINYSKLRIWNEF	108



Figure S1 (continued)

PnGolS1	113	EYSKMIYLDGDIQVYDNIDHLEFLDPDGRFYAVMDCFCEKTWSHTLQYKIGYCQQCPDKVN	172
PnGolS2	114	EYSKMIYLDGDIQVFDNIDHLEFDMFDGYFYAAMDCFCEKTWSNSPQYKIGYCQQCPDKVH	173
PnGolS3.1	114	EYSKMIYLDGDIQVFDNIDHLEFDMFDGYFYAVMDCFCEKTWSNSPQYKIGYCQQCPDKVQ	173
PnGolS3.2	114	EYSKMIYLDGDIQVFDNIDHLEFDMFDGYFYAVMDCFCEKTWSNSPQYKIGYCQQCPDKVQ	173
PnGolS4.1	114	EYSKMIYLDGDIQVFDNIDHLEFDMFDGYFYAVMDCFCEKTWSNSPQYKIGYCQQCPDKVQ	173
PnGolS4.2	114	EYSKMIYLDGDIQVFDNIDHLEFDMFDGYFYAVMDCFCEKTWSNSPQYKIGYCQQCPDKVQ	173
PtrGolS1	114	EYSKMIYLDGDIQVFDNIDHLEFDMFDGCFYAVMDCFCEKTWSNSPQYKIGYCQQCPDKVQ	173
PtrGolS2	114	EYSKMIYLDGDIQVFDNIDHLEFDMFDGYFYAVMDCFCEKTWSNSPQYKIGYCQQCPDKVQ	173
PtrGolS3	114	EYSKMIYLDGDIQVFDNIDHLEFDMFDGYFYAAMDCFCEKTWSNSPQYKIGYCQQCPDKVH	173
PtrGolS4	115	EYSKMIYLDADIQVFENIDHLEFDTQDGYFYAVMDCFCEKTWSHSPQHSIGYCQQCPDKVT	174
PtrGolS5	105	DYGKMIYLDGDIQVFDNIDHLEFKPTGTGYFYAVMDCFCEKTWSSTTPQYQIKYCQQCPDKVQ	164
PtrGolS6	113	EYSKMIYLDGDIQVYDNIDHLEFLDPDGHFYAVMDCFCEKTWSHTPQYKIGYCQQCPDKVN	172
PtrGolS7	105	DYEKMIYLDGDIQVFDNIDHLEFDPNGYFYAVMDCFCEKTWSSTTPQYQIKYCQQCPDKVR	164
PtrGolS8	113	EYSKMIYLDGDIQVYDNIDHLEFLDPDGRFYAVMDCFCEKTWSHTPQYKIGYCQQCPDKVN	172
PtrGolS9	116	EYSKMIYLDADIQVFENIDHLEFDTQDGYFYAVMDCFCEKTWSHSPQYSVGYCQQCPDKIT	175
PaxgGolS1	115	DYSKMIYLDADIQVFENIDHLEFDTQDGYFYAVMDCFCEKTWSHSPQYSIGYCQQCPDKVT	174
PaxgGolS2	114	EYSKMIYLDGDIQVFDNIDHLEFDMFDGYFYAVMDCFCEKTWSNSPQYKIGYCQQCPDKVQ	173
AtGolS1	119	EYSKMIYLDGDIQVYENIDHLEFLDPDGYLYAVMDCFCEKTWSHTPQYKIRYCQQCPDKVQ	178
AtGolS2	111	EYNKMIYLDGDIQVFDNIDHLEFLDPNGQFYAVMDCFCEKTWSHSPQYKIGYCQQCPDKVT	170
AtGolS3	105	EYSKLIYLDGDIQVFNENIDHLEFLDPDGNFYAVKDCFCEKTWSHTPQYKIGYCQQCPDKVT	164
AtGolS4	112	EYSKMIYLDADIQVFENIDHLEFLSDAYFYAVMDCFCEKTWSHSLQYSIGYCQQCPDKVT	171
AtGolS5	111	EYEKMIYLDGDIQVFNIDHLEFDTPRGYLYAVKDCFCEVSWSKTPQYKIGYCQQSPDKVT	170
AtGolS6	114	EYEKMIYLDGDIQVFSNIDHLEFDTPRGYLYAVKDCFCEISWSKTPQYKIGYCQQCPDKVT	173
AtGolS7	109	EYNKMIYLDADIQVFGNIDDLFDMDQDGYLHGVLSCFCEKIWSYTPLYSIGYCQYCPDKVV	168
PnGolS1	173	WP-AEMGQ-PPSPYFNAGMFVFEPSIATYHDLKTLKVTTPPTPFAEQDFLNMYFRDIYTP	230
PnGolS2	174	WP-AEMGP-KPPLYFNAGMFVYEPNLSTYHDLLETLKITTPPTLFAEQDFLNIFFRDVKYP	231
PnGolS3.1	174	WP-AEMGP-KPPLYFNAGMFVYEPNLSTYHDLLETLKITSETLFAEQDFLNMFRRDVKYP	231
PnGolS3.2	174	WP-AVMGP-KPPLYFNAGMFVYEPNLSTYHDLLETLKITSETLFAEQDFLNMFRRDVKYP	231
PnGolS4.1	174	WP-AEMGP-KPPLYFNAGMFVYEPNLSTYHDLLETVKVTSETLFAEQDFLNMFRRDVKYP	231
PnGolS4.2	174	WP-AEMGP-KPPLYFNAGMFVYEPNLSTYHDLLETVKVTSETLFAEQDFLNMFRRDVKYP	231
PtrGolS1	174	WP-AEMGP-KPPLYFNAGMFVYEPNLSTYHDLLETLKITSETLFAEQDFLNMFRRDVKYP	231
PtrGolS2	174	WP-AEMGP-KPPLYFNAGMFVYEPNLSTYHDLLETVKVTSETLFAEQDFLNMFRRDVKYP	231
PtrGolS3	174	WP-AEMGP-KPPLYFNAGMFVYEPNLSTYHDLLETLKVTTPPTLFAEQDFLNMFRRDVKYP	231
PtrGolS4	175	WP-AEMGS-PPPLYFNAGMFVFEPSRLTYESLLETLOITPPTPFAEQDFLNMFQKTYKP	232
PtrGolS5	165	WP-LEMGS-PPPLYFNAGMCLFEFFKLETYEDLLETLKVTTPPTSFAEQDFLNMFRRDVKYP	222
PtrGolS6	173	WP-AEMGQ-PPSLYFNAGMFVFEPSISTYHDLKTLKVTTPPTPFAEQDFLNMYFKDIYKP	230
PtrGolS7	165	WP-KEMGS-PPPLYFNAGMFVFEFKLLTYEDLLETLKVTTPPTSFAEQDFLNMFRRDVKYP	222
PtrGolS8	173	WP-AEMGQ-PPSPYFNAGMCFEPSIATYHDLKTLKVTTPPTPFAEQDFLNMYFKDIYTP	230
PtrGolS9	176	WP-AEMGS-PPPLYFNAGMFVFEPSRLTYESLLETLOITPPTPFAEQDFLNMFQKTYKP	233
PaxgGolS1	175	WP-AEMGS-PPPLYFNAGMFVFEPSRLTYESLLETLOITPPTPFAEQDFLNMFQKTYKP	232
PaxgGolS2	174	WP-AEMGP-KPPLYFNAGMFVYEPNLSTYHDLLETVKVTSETLFAEQDFLNMFRRDVKYP	231
AtGolS1	179	WPKAELGE-PPALYFNAGMFLYEPNLETYEDLLRTLKITTPPTPFAEQDFLNMYFKKIYKP	237
AtGolS2	171	WPEAKLGP-KPPLYFNAGMFVYEPNLSTYHNLLETVKIVPPTLFAEQDFLNMYFKDIYKP	229
AtGolS3	165	WPESELGP-KPPLYFNAGMFVYEPNLTYYNLLLETLKVPPTPFAEQDFLNMYFKDIYKP	223
AtGolS4	172	WPEDEMESPFPPLYFNAGMFVFEPSPLTYESLLQTLLETTPSPFAEQDFLNMFEEKVYKP	230
AtGolS5	171	WPVESLGA-PPPVYFNAGMLVGENLVTYEDLLRVVQITTPPTYFAEQDFLNIFYFRDIYKP	229
AtGolS6	174	WPVESLGS-PPPVYFNAGMLVFEENLLTYEDLLRVVQITTPPTYFAEQDFLNIFYFTDIYKP	232
AtGolS7	169	WP-AEMESAPPSPYFNAGMFVEEPNLTYESLLQTLQVTPPTPFAEQDFLNMFEGKVKKP	227



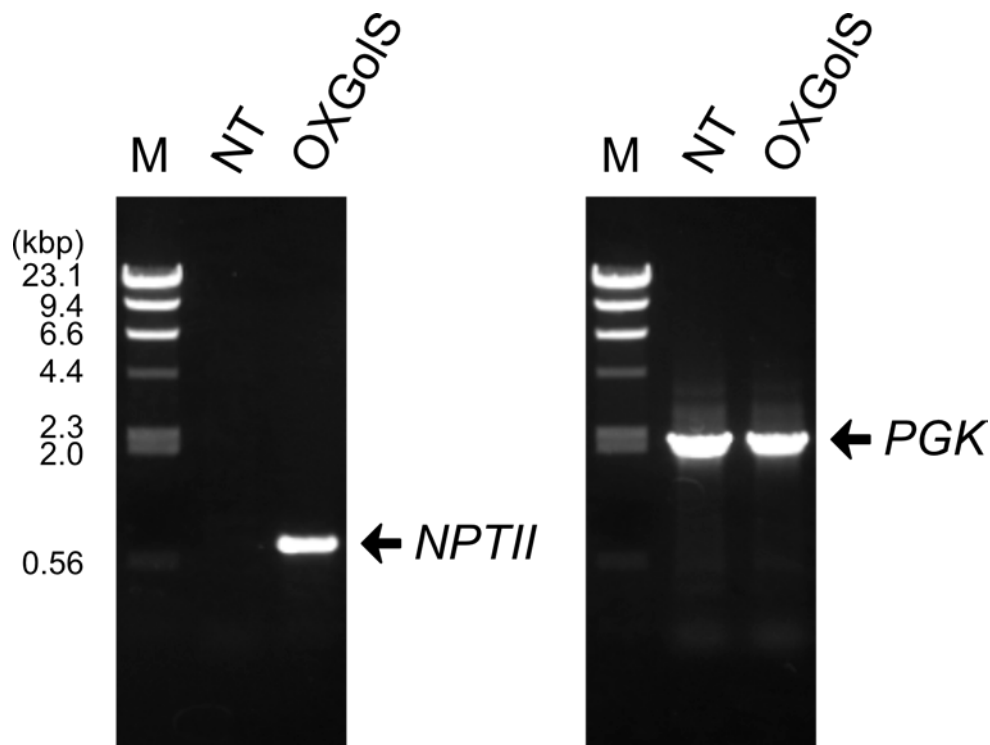
PnGolS1	231	IPLVYNLVLAMLRWRHFEENVELDKVKVVHYCAAGSKPWRYTGKEEKMQREDIEMLVKKWWD	290
PnGolS2	232	IPSDYNLVLALLWRHFEENINVDKVKVVHYCAAGSKPWRYTGKEDNMDREDIKMLVNKWWD	291
PnGolS3.1	232	IPSDYNLVLAMLRWRHFEENINLDKVKVVHYCAAGSKPWRYTGKEENMDREDIKMLVQKWWD	291
PnGolS3.2	232	IPSDYNLVLAMLRWRHFEENINLDKVKVVHYCAAGSKPWRFTGKEENMDREDIKMLVQKWWD	291
PnGolS4.1	232	IPSDYNLVLAMLRWRHFEENINLDKVKVVHYCAAGSKPWRFTGKEENMDREDIKMVVNKWWD	291
PnGolS4.2	232	IPSDYNLVLAMLRWRHFEENINLDKVKVVHYCAAGSKPWRFTGKEENMDREDIKMVVNKWWD	291
PtrGolS1	232	IPSDYNLVLAMLRWRHFEENINLDKVKVVHYCAAGSKPWRYTGKEENMDREDIKMLVQKWWD	291
PtrGolS2	232	IPSDYNLVLAMLRWRHFEENINLDKVKVVHYCAAGSKPWRFTGKEENMDREDIKMVVNKWWD	291
PtrGolS3	232	IPSDYNLVLALLWRHFEENINLDKVKVVHYCAAGSKPWRYTGKEDNMDREDIKMLVNKWWD	291
PtrGolS4	233	IPLMYNLVLAMLRWRHFEENVEVEKVKVVHYCAAGSKPWRYTGKEANMDREDIKMLVARWWD	292
PtrGolS5	231	IPPVYNLVSAMLWRHFDPKFDLDKVKVVHYCAAGAKPWRYTGKEENMDREDIKVLVKKWWE	282
PtrGolS6	231	IPLVYNLVLAMLRWRHFNVELDKVKVVHYCAAGSKPWRYTGKEENMDREDIKMLVEKWVG	290
PtrGolS7	223	IPAVYNLVSAMLWRHFENFELDVKVKVVHYCAAGAKPWRYTGKEENMDREDIQVLVKKWWE	282
PtrGolS8	231	IPLVYNLVLAMLRWRHFEENVELDRVKVVHYCAAGSKPWRYTGKEENMQREDIKMLVKKWWD	290
PtrGolS9	234	IPLLNYNLVLAMLRWRHFEENVEVEKVKVVHYCAAGSKPWRYTGEEANMDREDIKMLVAKWWD	293
PaxgGolS1	233	IPLMYNLVLAMLRWRHFEENVEVEKVKVVHYCAAGSKPWRYTGKEANMDREDIKMLVARWWD	292
PaxgGolS2	232	IPSDYNLVLAMLRWRHFEENINLDKVKVVHYCAAGSKPWRFTGKEENMDREDIKMVVNKWWD	291
AtGolS1	238	IPLVYNLVLAMLRWRHFEENVELGKVKKVVHYCAAGSKPWRYTGKEANMEREDIKMLVKKWWD	297
AtGolS2	230	IPPVYNLVLAMLRWRHFEENTIELDQVKVVHYCAAGAKPWRFTGEEENMDREDIKMLVKKWWD	289
AtGolS3	224	IPPVYNLVLAMLRWRHFEENTIELNEAKVVHYCAAGAKPWRFTGQEGNMEREDIKMLVEKWWD	283
AtGolS4	231	IPLVYNLVLAMLRWRHFEENVLEKVKVVHYCAAGSKPWRYTGEENMDREDIKMLVDKWWD	290
AtGolS5	230	IPTSYNLVMAMLRWRHFEHIDLDQISVHHYCAAGSKPWRFDEAEEHMDREDIKMLVKKWWE	289
AtGolS6	233	IPSTYNLVMAMLRWRHFEHIDLDQISVIHYCANGSKPWRFDETEEHMDREDIKMLVKKWWD	292
AtGolS7	228	VSPVYNLTLSVLWRHPGKVDLESVKVVHYCPPGSKPWRYTGEEPNDREDDVKMLIKKWWD	287

Comparison of the predicted amino acid sequence of galactinol synthase (GolS) from *Populus nigra*

with those of other GolSs from *P. trichocarpa*, *P. alba*  $\times$  *grandidentata*, and *Arabidopsis thaliana*. A

putative serine phosphorylation site is indicated by an asterisk and the characteristic hydrophobic pentapeptide (APSAA) at the C-terminus is shown by a black bar.

**Figure S2**



**Fig. S2**

PCR confirmation of transgene integration. The neomycin phosphotransferase gene (*NPTII*) was amplified from only the genomic DNA of a *PnGolS2*-overexpressed poplar (OXGolS), whereas a non-transformant (NT) did not have the *NPTII* gene. A phosphoglycerate kinase gene (*PGK*, accession number AB018410) was used as a positive control for PCR. M, Lambda DNA/HindIII marker.

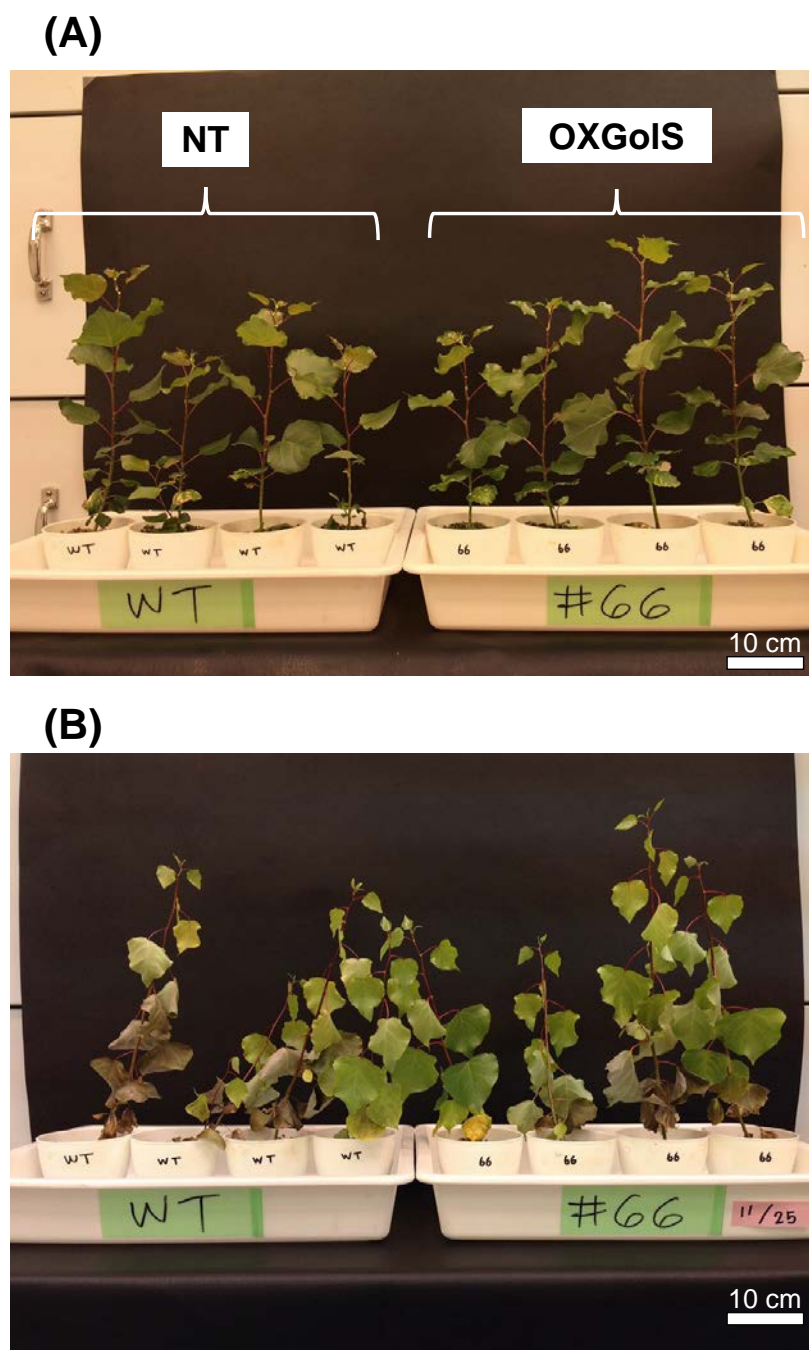


**Fig. S3**

Photographs of (A) non-transformants and (B) transformants overexpressing *PnGolS2* in *Populus nigra*. The photographs were taken 8 weeks after transfer to the Wagner-pots. The scale bar on the right bottom corner equals 10 cm in length.



**Figure S4**



**Fig. S4**

Photographs of the transformants overexpressing *PnGolS2* in *Populus nigra* (OXGoIS) and the

non-transformants (NT) (A) before and (B) on 14th day after withholding water supply. Aseptically cultivated clones were transplanted in a 1/10000 Wagner pot filled with 40 g vermiculite (in dry weight per pot). The plants were grown during about two months under an environmentally-controlled growth chamber before withholding water supply. The photosynthetically active photon flux density over the plants was  $300\text{--}500\ \mu\text{mol m}^{-2}\text{ s}^{-1}$ . The scale bar on the right bottom corner equals 10 cm in length.