

Supplementary Table 1. List of *BdCYP711A29*-specific primers used for the construction of overexpressing lines and the selection of TILLING mutant lines.

Primer name	Use	Polarity	Nucleotide sequence	Amplicon size (bp)	Reference
Bd1g75310_BI5'ATG [†]	Cloning	Forward	5'-GAGGATCCATGGAGTCGCCATTGG-3'	2347 (gDNA) / 1621 (cDNA)	This study
Bd1g75310_EV3'TAA [†]		Reverse	5'-CCGATATCTTAGTGCTCTTCTCGATCG-3'		
CYP711A29-F1 [‡]	TILLING	Forward	5'-ttccctacacgacgctcttccgatctcGGCCACATCAATTTTCATT-3'	452 (gDNA)	This study
CYP711A29-R [‡]		Reverse	5'-agttcagcgtgtgtcttccgatctT TAGTGCTCTTCTCGATCGA-3'		

[†], underlined letters correspond to the added restriction sites (*Bam*HI for Bd1g75310_BI5'ATG and *Eco*RV for Bd1g75310_EV3'TAA, respectively).

[‡], small-case letters correspond to Illumina adapters sequences.

Supplementary Table 2. List of primers used in qPCR experiments.

Gene	Nucleotide sequence	Polarity	Putative function	Reference
<i>Bradi4g00660</i>	5'-ACCCTCTACGCTGGTGAGAC-3'	Forward	UBC18 (plant reference gene)	Pasquet et al. (2014)
	5'-TTGCTGTAAATGTGCGGATG-3'	Reverse		
<i>Bradi4g41850</i>	5'-CCTGAAGTCCTTTTCCAGCC-3'	Forward	ACT3-like (plant reference gene)	Pasquet et al. (2014)
	5'-AGGGCAGTGATCTCCTTGCT-3'	Reverse		
<i>BdCYP711A5</i>	5'- TTCGGCCCTCAATCTCAATCC-C-3'	Forward	Cytochrome P450 monooxygenase	This study
	5'-TGGTCCAGGTACGGGAATTTTCG-3'	Reverse		
<i>BdCYP711A6</i>	5'-TGCACATGTACCGGAGATTTCGTG-3'	Forward	Cytochrome P450 monooxygenase	This study
	5'-ATTGCGCGTCAGCCTCTTGATG-3'	Reverse		
<i>BdCYP711A29</i> (<i>Bradi1g75310</i>)	5'- GCTACGTCTTCAGGCACTCC-3'	Forward	Cytochrome P450 monooxygenase	This study
	5'- CGATCGATGACTTGGAGCTT-3'	Reverse		
<i>BdCYP711A30</i>	5'-TACATCTTCCGCCACTCTCC-3'	Forward	Cytochrome P450 monooxygenase	This study
	5'-TTGAGGGGATCGATGTCTGG-3'	Reverse		
<i>BdCYP711A31</i>	5'-CAACTTCAAGCACGGTGTCA-3'	Forward	Cytochrome P450 monooxygenase	This study
	5'-TATTGTACGCCACTTGTTCGC-3'	Reverse		
<i>BdMAX3</i>	5'- ATGTCGCCAATGGGTCAACG-3'	Forward	CCD7-like	This study
	5'- TTTGTCTTCGGATACCGCATACTC-3'	Reverse		
<i>BdMAX4</i>	5'-TCGTCTCTGCTTCTTGCATACTGG-3'	Forward	CCD8-like	This study
	5'-GCTGCACAAACATCCACGTACAG-3'	Reverse		
<i>Bradi1g57590</i>	5'-CAGGTGGTGTGGAGGAAGTC -3'	Forward	Pathogenesis-related protein 1-5	Kouzai et al. (2016)
	5'-CTCTCCCCGTTGAAGTTCCC -3'	Reverse		
<i>Bradi1g39190</i>	5'-TCCGACCAGGCTCTCTAC-3'	Forward	Pathogenesis-related protein PR9	Pasquet et al. (2014)
	5'-GGTATGTTCCCCATCTTGAC-3'	Reverse		
<i>Bradi3g47110</i>	5'-CCAAACAATTAAGGAGATCAATTAGAA-3'	Forward	Phenylalanine ammonia-lyase	Pasquet et al. (2014)
	5'- CCCGAATACTGGAAAGTAAGATACA-3'	Reverse		
18S <i>F. graminearum</i>	5'-GTCCGGCCGGGCCTTTCC-3'	Forward	Ribosomal region 18S (fungal reference gene)	Mudge et al. (2006)
	5'-AAGTCCTGTTTCCCCGCCACGC-3'	Reverse		

Supplementary Table 3: List of characteristic parent and product ions detected during multiple reaction monitoring (MRM) in *B. distachyon* (Bd21-3) exudates.

Compounds (parent ion form) detected in MRM	<i>m/z</i> Parent ion	<i>m/z</i> Daughter ion	RT (min)
4-deoxyorobanchol [M + Na] ^a	353	241	4,24
5-deoxystrigol [M + H] ^{b, c, d, *} / Unknown 5 from Maize [M - OH] ^{b, *}	331	97	8,56
5-deoxystrigol [M + H] ^{b, c, d, *} / Unknown 5 from Maize [M - OH] ^{b, *}	331	97	9,52
Carlactonoic acid [M + H] ^{b, *}	333	97	8,91
DidehydroOrobanchol [M + Na] [*] / DidehydroStrigol [M + H] [*]	345	97	7,36
DidehydroOrobanchol [M + Na] [*] / DidehydroStrigol [M + H] [*]	345	248	9,64
DidehydroOrobanchol [M + Na] ^{e, *} / DidehydroStrigol [M + Na] ^{e, *}	367	270	6,03
Hydroxyorobanchol [M + Na] ^{f, *}	385	288	9,2
Methoxy-5-Deoxystrigol [M + H] ^{g, *} / Heliolactone [M + H] ^{b, d, *} / Unknown 6 from Maize [M + H] ^{b, *}	361	97	10,67
Orobanchol [M - H ₂ O] [*]	329	97	9,2
Orobanchol [M - H ₂ O] [*] / Strigol [M - H ₂ O] ^{c, d, *} / Sorgomol [M - H ₂ O] [*]	329	97	7,41
Orobanchol [M + H] ^{c, d, h, i, m}	347	205	9,19
Orobanchol [M + H] ^{c, d, h, m, *}	347	97	9,2
Orobanchol [M + H] ^{d, h, i, j}	347	233	9,2
Orobanchyl acetate (Alectrol) [M + H] ^{h, m}	389	347	8,05
Orobanchyl acetate (Alectrol) [M + H] ^{c, d, h, k, m}	389	233	8,9
Solanacol [M + H] [*]	343	246	9,64
Solanacol [M + Na] ^{e, n, *}	365	268	7,02
Solanacyl acetate [M + H] ^{c, *}	325	97	4,15
Solanacyl acetate [M + H] ^c	325	279	11,51
Sorgomol [M + H] ^c / Strigol [M + H] ^c	347	215	7,49
Sorgomol [M + Na] ^{e, l, *} / Strigol [M + Na] ^{d, *}	369	272	5,45
Strigol [M - H ₂ O] ^c	329	215	5,89
Unknown 4 from Maize [M - OH] ^b	375	343	10,94
Zealactones [M + H] ^b / Unknown 2 from Maize [M + H] ^b	377	345	9,14

Each transition has been detected at least in 1 out of 3 independent experiments. The three orobanchol transitions in grey have been validated using a standard in a preliminary experiment.* SL specific transition: detection or release of a *m/z* 97 fragment. ^a Boari et al. (2016), ^b Charnikhova et al. (2017), ^c Xie et al. (2015), ^d Iseki et al. (2018), ^e Yoneyama et al. (2008), ^f Khetkam et al. (2014), ^g Cardoso et al. (2014), ^h Kohlen et al. (2011), ⁱ Boutet-Mercey et al. (2018), ^j Foo & Davies (2011), ^k Kohlen et al., (2013), ^l Yoneyama et al. (2010), ^m Pavan et al. (2016), ⁿ Xie et al. (2013).

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Supplementary Table 4. Protein identity matrix based on ClustalW alignment of CYP711A protein sequences from *B. distachyon*, *H. vulgare*, *O. sativa*, *A. thaliana* and *S. moellendorffii*. At: *A. thaliana* ; Bd: *B. distachyon* ; Hv: *Hordeum vulgare* ; Os: *O. sativa* ; Sm: *S. moellendorffii*. Protein sequences used in this analysis are available under the following accession numbers: AtCYP711A1, OAP07831.1; BdCYP711A5, XP_003571126.1; BdCYP711A6: XP_003560652.1; BdCYP711A29, XP_003562092.2; BdCYP711A30: XP_003575594.2; BdCYP711A31, XP_010237353.2; HvCYP711A5, BAJ97619.1; HvCYP711A6, KAE87888859.1; HVCYP711A29, BAJ98237.1; HvCYP711A30, KAE8810993.1; OsCYP711A2, XP_015633367.1; OsCYP711A3, XP_015644699.2; OsCYP711A4, XP_015642272.1; OsCYP711A5, XP_015626073.1; OsCYP711A6, XP_015644019.1; SmCYP711A1, XP_002972055.1. Values are indivated in percentages.

	HvCYP711A30	HvCYP711A6	HvCYP711A29_ HvMAX1	HvCYP711A5	AtCYP711A1_ AtMAX1	OsCYP711A2	OsCYP711A3	OsCYP711A4	OsCYP711A5	OsCYP711A6	BdCYP711A5	BdCYP711A6	BdCYP711A29	BdCYP711A30	BdCYP711A31	SmCYP711A1
HvCYP711A30	100.00															
HvCYP711A6	58.00	100.00														
HvCYP711A29_HvMAX1	58.93	56.55	100.00													
HvCYP711A5	51.34	56.86	48.42	100.00												
AtCYP711A1_AtMAX1	61.02	58.00	56.71	53.18	100.00											
OsCYP711A2	70.42	57.14	58.05	50.48	58.61	100.00										
OsCYP711A3	71.91	60.39	59.35	53.16	63.24	81.97	100.00									
OsCYP711A4	71.64	57.20	58.43	52.79	62.33	78.62	82.53	100.00								
OsCYP711A5	51.76	56.42	47.94	79.82	52.17	50.67	52.60	51.55	100.00							
OsCYP711A6	57.48	71.87	53.86	54.63	59.19	58.09	58.88	58.83	55.15	100.00						
BdCYP711A5	51.57	54.71	47.08	75.00	50.51	47.75	50.88	51.37	74.02	55.35	100.00					
BdCYP711A6	59.77	80.98	57.42	58.06	59.11	58.25	60.38	58.58	58.05	72.31	55.90	100.00				
BdCYP711A29	59.68	53.32	73.31	49.01	56.60	57.48	59.33	59.96	47.72	52.98	46.40	55.51	100.00			
BdCYP711A30	68.48	55.66	53.37	50.10	55.70	63.40	65.50	66.29	49.16	55.58	48.57	55.68	54.79	100.00		
BdCYP711A31	62.50	59.15	70.91	53.74	60.47	59.47	60.70	61.00	63.20	56.80	51.16	60.15	71.59	55.30	100.00	
SmCYP711A1	39.33	39.07	38.54	37.41	39.43	38.45	39.71	38.56	36.43	37.86	35.25	38.53	38.25	36.51	38.04	100.00

Supplementary Table 5. *BdCYP711A29* TILLING mutant families identified following screening of the Bd21-3 TILLING collection (Dalmais et al., 2013). The two mutant families selected for further analysis are indicated in bold letters.

Family name	Nucleic acid transition	Amino acid substitution	Type of Mutation	SIFT Score
7758	G1803A	G421G	Silent	1
8687	C1831T	P431S	Missense	0
7708	G1855A	E439K	Missense	0
5374	C1888T	R450*	Stop	0
7424	C1940A	P467H	Missense	0.02
8496	C1941T	P467P	Silent	0.06
7123	G1944A	G468G	Silent	1
6511	C1972T	L478F	Missense	0.08
7846	C2034T	P498P	Silent	1
8480	C2076T	G512G	Silent	1

Supplementary Table 6: Raw data and calculation of relative quantity of orobanchol in exudates of wild-type (Bd21-3), overexpressing (OE-CYP12.20) and mutant (M5374#135) lines after 7 d phosphorus starvation. AUC: Area under the curve. MRM transitions 321 > 224 and 347 > 97 were used to quantify GR24 and orobanchol signals, respectively.

Genotype	Bd21-3 (WT)			OE-CYP12.20			M5374#135		
Sample	Bd21-3 (WT)-A	Bd21-3 (WT)-B	Bd21-3 (WT)-C	OE-CYP12.20-A	OE-CYP12.20-B	OE-CYP12.20-C	M5374 #135-A	M5374 #135-B	M5374 #135-C
Root FW (g)	1.0467	2.32	1.014	1.88	1.27	1.431	1.451	1.4555	1.2059
Orobanchol 347>97 MH ⁺ relative to GR24 321>224 MH ⁺	0.091	0.130	0.122	1.854	1.629	1.566	0.058	0.123	0.06
AUC Orobanchol / AUC GR24 / g root FW	0.087	0.056	0.121	0.986	1.282	1.094	0.040	0.085	0.053
Mean AUC Orobanchol / AUC GR24 / g root FW	0.088			1.121			0.059		
Standard deviation	0.032			0.150			0.023		

FW: fresh weight