

# Cyanogenesis in the *Sorghum* genus: from genotype to phenotype

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**Supplementary Figure S1** CLUSTAL O (1.2.4) multiple sequence alignment of the amino acid sequences of CYP79A1 obtained by PCR from wild sorghum species.

**Table S1:** Accession and provenance details of the sorghum crop wild relatives.

**Table S2:** Genes selected for variant analysis.

**Table S3:** Sequence of the primers used to amplify CYP79A1 from the wild sorghum species.

## Supplementary References

## Supplementary Figure S1

CLUSTAL O (1.2.4) multiple sequence alignment of the amino acid sequences of CYP79A1 obtained by PCR from wild sorghum species.

Highlighted in blue are amino acids identified as important for function as determined using EMS mutant plants showing reduced HCnp. P414L and C493Y results in acyanogenic plants [1,2].

Yellow Highlighted regions are motifs shown to be important by modelling and experimental analysis [3,4]. E408, R411 and R460 form the E-R-R triad which is key to docking of the substrate tyrosine.

Transmembrane region		
brachypodum	MATKEVEAAAATVLAAPLLSTSTILKLLLFVVTL	SYLARALSRPRKTTTKCSSS--A--- 55
plumosum	MATNEVEAAAATVLAAPLLSTSTILKLLLSVVAL	SYLARALSRPRKTTTKCSS-TTS--- 56
interjectum	MATMEVEAAAATVLAAPLLSTSTILKLLLFVVTL	SYLARALSRPRKTTTKCGS-TTS--- 56
amplum	MATMEVEAAAATVLDAPLLSTSTILKLLLFVGT	SYLARALSRPRKTTTKCSS-TTS--- 56
bulbosum	MATMEVEAAAATVLGAPLLSTSTILKLLLFVVTL	SYLARALSRPRKTTTKCSSSTTS--- 57
purpureosericeum	MATKEVEAAAATVLAAPLLSTSAILKLLLFVVTL	SYLARALRRPRKTTTN-----KCS-- 53
versicolor	MATMEVEAAAATVLAAPLLSTSAILKLLLFVVTL	SYLARALSRPRKTTTKCSSTTSSG-- 58
bicolor	MATMEVEAAAATVLAAPLLSSSAILKLLLFVVTL	SYLARALRRPRKSTTKCSSTTCASPP 60
laxiflorum	MATMEVDAAAATVLAAPLLSSSAILKLLLFVVTL	SYLARALSRPRKTSTKCSSTTCASP- 59
angustum	MATMEVDAAAGATVLAAPLLSSSAILKLLLVVAT	SYLSRALIRPRKTTTKCSSTT----- 55
ecarinatum	MATMEVEAAAATVLAAPLLSSSAILKLLLVVAT	SYLSRALIRPRKTTTKCSSTT----- 55
intrans	MATMEVEAAAATVLAAPLLSSSAILKLLLFVAT	SYLSRALIRPRKTTTKCGSTT----- 55
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brachypodum	GVGSNPALPPGPVWPWPIVGNLPEMLMNKPAFRWIHQMMREMGT	DIACVKLGGIHVVSITC 115
plumosum	GVGSNPALPPGPVWPWPIVGNLPEMVINKPAFRWIHQMMREMGT	DIACVKLGGIHVVSITC 116
interjectum	CVGSNPPLPPGPVWPWPIVGNLPEMLINKPAFRWIHQMMREMGT	DIACVKLGGIHVVSITC 116
amplum	CVGSNPALPPGPVWPWPIVGNLPEMLINKPAFRWIHQMMREMGT	DIACVKLGGIHVVSITC 116
bulbosum	CVGSNPALPPGPVWPWPIVGNLPEMLINKPAFRWIHQMMREMGT	DIACVKLGGIHVVSITC 117
purpureosericeum	-VGSNPPLPPGPVWPWPIVGNLPEMLLNKPAFRWIHQMMRDMGT	DIACVKLGGIHVVSITC 112
versicolor	-VGSNPPLPPGPVWPWPVGNLPEMLLNKPAFRWIHQIMRDMGT	DIACVKLGVIHVVSITC 117
bicolor	AGVGNPPLPPGPVWPWPVGNLPEMLLNKPAFRWIHQMMREMGT	DIACVKLGVIHVVSITC 120
laxiflorum	-GVGNPPLPPGPVWPWPVGNLPEMLLNKPAFRWIHQMMHEMGT	DIACVKLGVIHVVSITC 118
angustum	-GVGNLPLPPGPAPWPWPVGNLPEMLLNKPAFRWIHQMMSEMGT	GIACVKLGDVHVVCVTC 114
ecarinatum	-GVGNLPLPPGPAPWPWPVGNLPEMLLNKPAFRWIHQMMSEMGT	MACVKLGDVHVVCVTC 114
intrans	-GVGNLPLPPGPAPWPWPVGNLPEMLLNKPAFRWIHQMMSEMGT	DIACVKLGAVHVVCVTC 114
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E145 R152 Heme binding		
brachypodum	PEMAREVLRKQDANFISRPLTFASETFSGGYRNAVLSPYGDQWKKMRRVLTSEIICPSRH	175
plumosum	PEIAREVLRKQDANFISRPLTFASETFSGGYRNAVLSPYGDQWKKMRRVLTSEIICPSRH	176
interjectum	PEMAREVLRKQDANFVSRPLTFASETFSGGYRNAVLSPYGDQWKKMRRVLTSEIICPSRH	176
amplum	PEIAREVLRKQDANFTSRPLTFASETFSGGYRNAVLSPYGDQWKKMRRVLTSEIICPSRH	176
bulbosum	PEIAREVLRKQDANFISRPLTFASETFSGGYRNAVLSPYGDQWKKMRRVLTSEIICPSRH	177

purpureosericeum	PEIAREVLRKQDANFISRPLTFASETFSGGYRNAVLSPYGDQWKKMRRVLTSEIICPSRH	172
versicolor	PEIAREVLRKQDANFISRPLTFASETFSGGYRNAVLSPYGDQWKKMRRVLTSEIICPSRH	177
bicolor	PEIAREVLRKQDANFISRPLTFASETFSGGYRNAVLSPYGDQWKKMRRVLTSEIICPSRH	180
laxiflorum	PEIAREVLRKQDANFISRPLTFASEMFSGGYRNAVLSPYGDQWKKMRRVLTSEIICPSRH	178
angustum	PEIAREVLRKQDANFISRPLTFASETFSGGYRNAVLSPYGAQWKKMRRVLTSEIICPPRH	174
ecarinatum	PEIAREVLRKQDANFISRPLTFASETFSGGYRNAVLSPYGAQWKKMRRVLTSEIICPSRH	174
intrans	PEIAREVLRKQDANFISRPLTFASETFSGGYRNAVLSPYGAQWKKMRRVLTSEIICPSRH	174
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brachypodium	AWLHDKRADEADNLTRYVYNLATKAA-AGDDAVVDVRHVARHYCGNVIRRLMFNRRYFGE	234
plumosum	AWLHDKRADEADNLTRYVYNLATKAAAAGDDVVDVRHVARHYCGNVIRRLMFNRRYFGE	236
interjectum	AWLHDKRADEADNLTRYVYNLATKAA-AGDDVVVDVRHVARHYCGNVIRRLMFNRRYLGE	235
amplum	AWLHDKRADEADNLTRYVYNLATKAA-AAAGDVVDVRHAARHYCGNVIRRLMFNRRYFGE	235
bulbosum	AWLHDKRADEADNLTRYVYNLATKAA-AAGDDVVDVRHVARHYCGNVIRRLMFNRRYFGE	236
purpureosericeum	AWLHDKRTDEADNLTRYVYNLATTKA-TGRDDVVDVRHVARHYCGNVIRRLMFNRRYFGE	231
versicolor	AWLHDKRTDEADNLTRYVYNLATTKA-TGG-DVVDVRHVARHYCGNVIRRLMFNKRYFGE	235
bicolor	AWLHDKRTDEADNLTRYVYNLATKAA-T-GDVAVDVRHVARHYCGNVIRRLMFNRRYFGE	238
laxiflorum	AWLHDKRTDEADNLTRYIYNLATKSA-G---DVVDVRHVARHYCGNVIRRLMFNRRYFGE	234
angustum	VWLHDKRSDEADNLTRYVYNLAT-GA--AGGNVVDVRHIARHYCGNVIRRLMFNKRYFGE	231
ecarinatum	VWLHDKRADEADNLTRYVYNLATTRA--AGGNVVDVRHIARHYCGNVIRRLMFNKRYFGE	232
intrans	AWLHDKRADEADNLTRYVYNLATTRA--AGGNVVDVRHIARHYCGNVIRRLMFNKRYFGE	232
	.*****:*****:***** * .***** *****:***:**	
brachypodium	PQPDGGPGPMEELHMDAVFTSLGLLYAFCVSDYLPWLRGLDLDGHEKIVKEANQTVNRLH	294
plumosum	PQPDGGPGPMEELHMDAVFTSLGLLYAFCVSDYLPWLRGLDLDGHEKIVKQANETVNRLH	296
interjectum	PQPDGGPGPMEELHMDAVFTSLGLLYAFCVSDYLPWLRGLDLDGHEKIVKEANETVNRLH	295
amplum	PQPDGGPGPMEELHMDAVFTSLGLLYAFCVSDYLPWLRGLDLDGHEKIVKEANETVNRLH	295
bulbosum	PQPDGGPGPTEELHMDAVFTSLGLLYAFCVSDYLPWLRGLDLDGHERIVKEANETVNRLH	296
purpureosericeum	PQPDGGPGPMEELHMDAVFTSLGLLYAFCVSDYLPWLRGLDLDGHEKIVKEANETVNRLH	291
versicolor	PQPDGGPGPMEVLHMDAVFTSLGLLYAFCVSDYLPWLRGLDLDGHEKIVKEANAAVNRLH	295
bicolor	PQADGGPGPMEVLHMDAVFTSLGLLYAFCVSDYLPWLRGLDLDGHEKIVKEANVAVNRLH	298
laxiflorum	PQPDGGPGPMEVLHMDAVFTSLGLLYAFCVSDYLPWLRGLDLDGHEKIVKEANEAVNRLH	294
angustum	PQPDGGPGPMEVLHMDAVFTSLGLLYAFCISDYLPWLRGLDLDGHERMVKEANETVNRLH	291
ecarinatum	PQPDGGPGPMEVLHMDAVFTSLGLLYAFCISDYLPWLRGLDLDGHEKIVKEANETVNRLH	292
intrans	PQPDGGPGPMEVLHMDAVFTSLGFLYAFCISDYLPWLRGLDLDGHEKIVKEANETVNRLH	292
	** ***** * *****:*****:*****:***:** :*****	
	D347	D354 N355
brachypodium	DTVIDGRWRQWKSGERKEMEDFLDVLITLKDAQGNPLLTIEEVKAQSQDITFAAVDNPSN	354
plumosum	DAVIDDRWRQWKSGERKEMEDFLDVLITLKDAQGNPLLTIEEVKAQSQDITFAAVDNPSN	356
interjectum	DAVIDDRWRQWKSGERKEMEDFLDVLITLKMAQGNPLLTIEEVKAQSQDITFAAVDNPSN	355
amplum	DAVIDDRWRQWKSGERKEMEDFLDVLITLKDAQGNPLLTIEEVKAQSQDITFAAVDNPSN	355
bulbosum	DTVIDDRWRQWKSGERKEMEDFLDVLITLKDAQGNPLLTIEEVKAQSQDITFAAVDNPSN	356

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**E408 R411 P414**

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### PERF domain - R460

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## C493

brachypodium	NDLRFISFSTGRRG <b>C</b> IAASLGTAMNIMLFGRLLQGFTWSKPAGVEAVDLSESKSDTFMAT	533
plumosum	NDLRFISFSTGRRG <b>C</b> IAASLGTAMSIMLFGRLQLQGFTWSKPAGVEAVDLSESKSDTFMAT	535
interjectum	NDLRFISFSTGRRG <b>C</b> IAASLGTAMSIMLFGRLQLQGFTWSKPAGVEAVDLSESKSDTFMAT	534
amplum	NDLRFISFSTGRRG <b>C</b> IAASLGTAMSIMLFSRLLQGFTWSKPAGVEAVDLSESKSDTFMAT	534
bulbosum	NDLRFISFSTGRRG <b>C</b> IAASLGTAMSIMLFGRLQLQGFTWSKPAGVEAVDLSESKSDTFMAT	535
purpureosericeum	NDLRFISFSTGRRG <b>C</b> IAASLGTAMSIMLFGRLQLGFAWSKPAGVEAVDLSESKSDTFMAT	529
versicolor	NDLRFISFSTGRRG <b>C</b> IAASLGTAMSIMLFGRLQLQGFTWSKPAGVEAVDLSESKSDTFMAT	533
bicolor	NDLRFISFSTGRRG <b>C</b> IAASLGTAMSVMLFGRLQLQGFTWSKPAGVEAVDLSESKSDTFMAT	538
laxiflorum	NDLRFISFSTGRRG <b>C</b> IAASLGTAMSIMLFGRLQLQGFTWSKPAGVEAVDLSESKSDTFMAT	533
angustum	KDLRFISFSTGRRG <b>C</b> IAASLGTMTSIMLFGRLQLQGFTWSKPAGVEAVDLSECKNTTFMAT	530
ecarinatum	NDLRFISFSTGRRG <b>C</b> IAASLGTAMSIMLFGRLQLQGFTWSKPAGVEAVDLSESKNDTFMAT	531
intrans	NDLRFISFSTGRRG <b>C</b> IAASLGTMTSIMLFGRLQLQGFTWSKPAGVEAVDLSESKNGTFMAT	531

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brachypodium	PLVLHAEPRLPAPHLIPTISV---	553
plumosum	PLALHAEPRLPAPHLIPSISI---	555
interjectum	PLVLHAQPRLPAPHLIPSISI---	554
amplum	PLVLHAEPRLPAPHLIPSFSI---	554
bulbosum	PLVLHAEPRLPAPHLIPSISII--	556
purpureosericeum	PLVLRAEPRLPAPHLIPSFSI---	549
versicolor	PLVLRAEPRLPAPHLIPSISI---	553
bicolor	PLVLHAEPRLPAPHLIPSISI---	558
laxiflorum	PLVLNAEPRLPAPHLIPAISI---	553
angustum	PLALNAVPRLPVHLIPSISI---	550
ecarinatum	PLALNAEPRLLVHLIPSFSI---	551
intrans	PLALNAEPRLPVHLIPAISPSPSD	554

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**Table S1:** Accession and provenance details of the sorghum crop wild relatives examined in the current study. Seeds were obtained from the Australian Grains Genebank (AGG), Horsham, Victoria (Coordinator: Dr Sally Norton).\*denotes species of the *Eusorghum* subgenus.

Species	Accession number	Provenance	Latitude	Longitude
<b>HCN analysis</b>				
<i>S. amplum</i>	302623	Kimberley, WA	-14.5982	125.7928
<i>S. angustum</i>	302596	Central Highlands, QLD	-13.4583	142.9613
<i>S. brachypodium</i>	302670	Kakadu, NT	-12.5667	-132.8833
<i>S. bulbosum</i>	302645	Kimberley, WA	-16.0965	128.39
<i>S. ecarinatum</i>	302656	Kimberley, WA	-17.1838	124.9158
<i>S. exstans</i>	302577	Melville Island, NT	-11.6403	130.6317
<i>S. interjectum</i>	302428	Kimberley, WA	-15.7695	128.6462
<i>S. intrans</i>	302394	Katherine, NT	-14.4503	132.2432
<i>S. laxiflorum</i>	302525	Katherine, NT	-16.6477	135.8495
<i>S. leiocladum</i>	300170	New England, NSW	-28.9263	152.3453
<i>S. macrospermum</i>	302367	Katherine, NT	-14.4097	132.1977
<i>S. matarankense</i>	302521	Katherine, NT	-16.0797	136.3077
<i>S. plumosum</i>	302415	Katherine, NT	-14.7922	131.9427
<i>S. propinquum</i> *	302546	NA	NA	NA
<i>S. purpureosericeum</i>	318068	NA	NA	NA
<i>S. stipoidum</i>	302442	Kimberley, WA	-18.0412	127.8037
<i>S. timorensis</i>	302660	Kimberley, WA	-17.3657	124.2937
<i>S. versicolor</i>	321128	Usagara, Tanzania	NA	NA
<i>S. halepense</i> *	300167	Western Downs, QLD	-28	152
<i>S. × alnum</i> *	316842	Central Highlands, QLD	-24.1187	148.0901
<b>Illumina sequencing</b>				
<i>S. brachypodium</i>	302481	Arnhem Land, NT	-12.6767	132.8435
<i>S. laxiflorum</i>	302525	NT	-16.6477	135.8495
<i>S. leiocladum</i>	300148	Maidenwell, QLD	-26	151
<i>S. macrospermum</i>	302367	Katherine, NT	-14.4097	132.1977
<i>S. matarankense</i>	302521	Booraloola, NT	-16.0797	136.3077
<i>S. purpureosericeum</i>	318068	NA	NA	NA
<b>PCR</b>				
<i>S. amplum</i>	302455	WA	-15.977	128.9632
<i>S. angustum</i>	302588	QLD	-15.308	144.8133
<i>S. brachypodium</i>	302480	Arnhem Land, NT	-12.7145	132.4167
<i>S. bulbosum</i>	302417	Katherine, NT	-15.1425	131.6812
<i>S. ecarinatum</i>	302581	Katherine, NT	-14.4393	132.2755
<i>S. exstans</i>	302401	Katherine, NT	-14.553	133.0117
<i>S. interjectum</i>	302569	Nhulunbuy, NT	-12.7758	136.23
<i>S. intrans</i>	302389	NT	-13.5838	131.4568
<i>S. laxiflorum</i>	302503	QLD	-17.8917	139.2853
<i>S. leiocladum</i>	300148	Maidenwell, QLD	-26	151
<i>S. macrospermum</i>	322277	Katherine, NT	NA	NA
<i>S. matarankense</i>	302521	Booraloola, NT	-16.0797	136.3077
<i>S. plumosum</i>	322440	QLD	-20.8777	144.51245
<i>S. purpureosericeum</i>	321133	NA	NA	NA
<i>S. stipoidum</i>	302449	Kimberley, WA	-18.0043	125.2893
<i>S. timorensis</i>	302634	Cloncurry, QLD	-20.6457	141.0265
<i>S. versicolor</i>	321127	NA	NA	NA

**Table S2:** Genes selected for variant analysis. Enzyme family, identification, function, and reference information for the genes examined in this study involved in dhurrin biosynthesis, bioactivation, recycling and transport. In addition, two genes were selected that are putatively involved in ethylene synthesis and tyrosine biosynthesis.

Enzyme	Gene	Gene ID	Function	Reference
Cytochrome P450	<i>CYP79A1</i>	Sobic.001G012300	Biosynthesis	[5]
Cytochrome P450	<i>CYP71E1</i>	Sobic.001G012200		[5]
UDP-glucosyltransferase	<i>UGT85B1</i>	Sobic.001G012400		[5]
Cytochrome P450 reductase	<i>POR</i>	Sobic.002G295100		[5]
MATE transporter	<i>MATE</i>	Sobic.001G012600	Transport	[6]
$\alpha$ -hydroxynitrile lyase	<i>HNL</i>	Sobic.004G335500	Bioactivation	[5]
$\beta$ -glucosidase	<i>DHR1</i>	Sobic.008G079800		[7]
$\beta$ -glucosidase	<i>DHR2</i>	Sobic.008G080400		[7]
$\beta$ -cyanoalanine synthase	<i>BCAS</i>	Sobic.006G016900	HCN detoxification	[5]
$\beta$ -cyanoalanine synthase	<i>CAS26</i>	Sobic.003G333700		[8]
Nitrilase	<i>NIT4A</i>	Sobic.004G225200	HCN detoxification, N recycling	[5]
Nitrilase	<i>NIT4B1</i>	Sobic.004G225000		[5]
Nitrilase	<i>NIT4B2</i>	Sobic.004G225100		[5]
Glutathione S-transferase	<i>GST1</i>	Sobic.001G012500	N recycling	[5,7,9]
Glutathione S-transferase	<i>GST1B</i>	Sobic.001G065800		[5]
Glutathione S-transferase	<i>GST3</i>	Sobic.003G416300		NA
ACC oxidase	<i>ACC</i>	Sobic.003G197200	Ethylene synthesis	
Chorismate mutase	<i>CM7</i>	Sobic.007G141500	Tyrosine synthesis	NA

**Table S3:** Details of the primers used to amplify and sequence *CYP79A1* from the wild sorghum species.

Primer name	Sequence	Tm (°C)	Application
CYP79A1 coding For	5' GGCGACAATGGAGGTAGAGG 3'	66	PCR and sequencing
CYP79A1 coding Rev	5' GATGGAGATGGACGGGTAGAG 3'	65	
CYP79A1 Int Rev 5' 1	5' ATTAATTACCTGTGACTGCGC 3'	61	sequencing
CYP79A1 Int Rev 5' 2	5' CGCATCTTCTTCCACTGGTC 3'	65	sequencing
CYP79A1 Int For 3' 1	5' CTCCTGGATGTGCTCATCAC 3'	65	sequencing
CYP79A1 Int For 3' 2	5' GCGAAGGCGATGGAGGAG 3'	68	sequencing

## Supplementary References

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