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### *A new genome allows the identification of genes associated with natural variation in aluminium tolerance in Brachiaria grasses*

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*Published in:*

Journal of Experimental Botany

*DOI:*

[10.1093/jxb/eraa469](https://doi.org/10.1093/jxb/eraa469)

[10.5281/zenodo.3941963](https://doi.org/10.5281/zenodo.3941963)

*Publication date:*

2021

*Citation for published version (APA):*

Worthington, M., Perez, J., Mussurova, S., Silva-Cordoba, A., Castiblanco, V., Arango, J., Jones, C., Fernandez Fuentes, N., Skot, L., Dyer, S., Tohme, J., Di Palma, F., Arango, J., Armstead, I., & De Vega, J. (2021). A new genome allows the identification of genes associated with natural variation in aluminium tolerance in Brachiaria grasses. *Journal of Experimental Botany*, 72(2), 302-319. <https://doi.org/10.1093/jxb/eraa469>, <https://doi.org/10.5281/zenodo.3941963>

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# A new genome allows the identification of genes associated with natural variation in aluminium tolerance in *Brachiaria* grasses

- **Supplementary table S1:** Root length, diameter and biomass in the *B. decumbens* CIAT 606 and *B. ruziziensis* BRX 44-02 (cv. Basilisk) progenitors after growing for 20 days in control and high 200  $\mu\text{M}$   $\text{AlCl}_3$  concentration hydroponic solutions.
- **Supplementary Table S2:** Statistics of the intermediate steps, alternative assemblies, final assembly and pseudo-molecules for the *B. ruziziensis* CIAT 26162 genome.
- **Supplementary Table S3:** Classification of the repeat content in the *Brachiaria* genome.
- **Supplementary Table S4:** Alignment of the transcripts and proteins from five sequenced species in the Panicoideae subfamily in the *Brachiaria ruziziensis* genome.
- **Supplementary Table S5:** EggNOG clusters in six sequenced species in the Panicoideae subfamily classified by number of proteins per cluster.
- **Supplementary Table S6:** Peak and interval positions for the identified QTLs, as well as corresponding *S. italica* chromosome.
- **Supplementary Table S7:** Enrichment analysis of the GO SLIM terms over-represented among DE genes in *B. ruziziensis* BRX 44-02 (Bruz), *B. decumbens* CIAT 606 (cv. Basilisk) (Bdec), or PRJNA314352 from Salgado *et al.* (2017).
- **Supplementary Figure S1:** 31mer frequency analysis comparing the short-reads assemblies produced with *Platanus assembler* or ABySS and SOAP2.
- **Supplementary Figure S2:** Divergence (Kimura) rates between the flanking tails in each *Gypsy* and *Copia* LTR duplication events in the *Brachiaria* genome.
- **Supplementary Figure S3:** Species of the top Blastp hit for each 35,982 of the coding transcripts which had a homologous protein in the NCBI non-redundant (nr) database.
- **Supplementary Figure S4:** Shared eggnoG clusters of proteins among *Brachiaria ruziziensis* (Bruz), foxtail millet, *S. viridis*, maize, *Panicum halli* and switchgrass.
- **Supplementary Figure S5:** Kimura rates between homologous gene pairs between *B. ruziziensis* and sequenced relatives including foxtail millet, *S. viridis*, maize, and *P. halli*.

- **Supplementary Figure S6:** Phylogenetic tree based on nucleotide divergence rate between sequences in the same eggnog cluster from *B. ruzizensis* and sequenced relatives.
- **Supplementary Figure S7:** The final genetic map for the *B. decumbens* CIAT 606 (cv. Basilisk) progenitor of the interspecific population included 4,427 markers placed at LOD 10 in 18 linkage groups.
- **Supplementary Figure S8:** RNA-seq from stem and root tissue samples extracted from the *B. decumbens* and *B. ruzizensis* progenitors. We also incorporated a reanalysis of public RNA-seq data (PRJNA314352) from *B. decumbens* var. Basilisks roots.
- **Supplementary Figure S9:** Enrichment analysis of the “Molecular function” GO terms overrepresented among differentially expressed upregulated (red) or downregulated (blue) genes in roots in *B. decumbens* CIAT 606 and *B. ruzizensis* BRX 44-02.
- **Supplementary Figure S10:** Enrichment analysis of the “Biological Process” GO terms overrepresented among differentially expressed upregulated (red) or downregulated (blue) genes in roots in *B. decumbens* CIAT 606 and *B. ruzizensis* BRX 44-02.
- **Supplementary Figure S11:** Correlation matrix plot among GO terms based on the DE genes included in each annotation.
- **Supplementary Figure S12:** Comparison the enriched GO Slim terms between *B. decumbens* cv. Basilisk exposed to 200  $\mu\text{M}$   $\text{AlCl}_3$  for 72 hours and 8 hours, the latter from the reanalysis of public raw data from Salgado *et al.* 2017.

**Supplementary table S1:** Root length, diameter and biomass in the *B. decumbens* CIAT 606 and *B. ruziziensis* BRX 44-02 (cv. Basilisk) progenitors after growing for 20 days in control and high 200  $\mu\text{M}$   $\text{AlCl}_3$  concentration hydroponic solutions

	Root Length (mm)			Root Tip Diameter (mm)			Root biomass (milligrams)		
	Control	Stress	Ratio	Control	Stress	Ratio	Control	Stress	Ratio
<i>B. decumbens</i> CIAT 606	428	261	0.61	0.29	0.31	0.109	54	34	0.63
<i>B. ruziziensis</i> BRX 44-02	177	72	0.41	0.39	0.46	0.118	30	17	0.55
Mean population	474	212	0.45	0.32	0.38	0.118	64	39	0.61

**Supplementary Table S2:** Statistics of the intermediate steps, alternative assemblies, final assembly and pseudo-molecules for the *B. ruzizensis* CIAT 26162 genome.

<b>Step</b>	<b>Total length (Mbp)</b>	<b>% Ns</b>	<b>Sequences</b>	<b>N50 (Kbp)</b>
WGS Platanus	712.4	17.45	196,321	17.4
ABySS+SOAP2 (Discarded)	815.4	12.74	268,486	5.5
Pacbio Gapfilling	796.8	11.39	191,540	23.3
Deposit WGS (GCA_003016355)	732.5	10.59	102,579	27.8
Unanchored reference (Sequences over 10Kb)	533.9	11.7	23,076	44.6
Anchored in 9 chrs	525.1	12.18	9	55.88*Mbp

**Supplementary Table S3:** Classification of the repeat content in the Brachiaria genome.

<b>Category</b>	<b>Superfamily</b>	<b>Coverage (bps)</b>	<b>Fraction genome*</b>
Class 1 Transposable elements (TEs)	Gypsy	156,824,480	23.9
	Copia	62,486,851	9.5
	Pao	55,071	0.0
	Other LTRs	972,934	0.1
	SINEs	2,939,316	0.4
	LINEs	11,929,645	1.8
		<b>(235,208,297)</b>	<b>(35.8)</b>
Class 2 (DNA) Transposable elements (TEs)	hAT	631,797	0.1
	hAT_Ac	2,534,322	0.4
	hAT_Tag1	1,082,474	0.2
	hAT_Tip100	306,963	0.0
	Harbinger/PIF	9,477,600	1.4
	MULE	7,895,375	1.2
	Stowaway	4,436,872	0.7
	CMC_EnSpm	27,724,058	4.2
	Helitron	1,339,398	0.2
		<b>(55,428,859)</b>	<b>(8.4)</b>
Non TEs	Unclassified TE	40,000,354	6.1
	Simple Repeats	482,035	0.1
	Satellites	2,999,591	0.5
		<b>(43,481,980)</b>	<b>(6.6)</b>
Unclassified TE	Other	335,155	0.1
<b>TOTAL</b>		<b>334,454,291</b>	<b>51.0</b>

\*656Mbp after excluding ambiguous nucleotides (Ns)

**Supplementary Table S4:** Alignment of the transcripts and proteins from five sequenced species in the Panicoideae subfamily [foxtail millet (*Setaria italica*), green foxtail (*Setaria viridis* (L.) Beauv.), *Panicum halli* Vasey, switchgrass (*Panicum virgatum* L.), and maize (*Zea mays* L.)], in the *Brachiaria ruziziensis* genome with a minimum identify of 70 %. Transcripts (longest one per gene) were aligned with GMAP and proteins were aligned with Exonerate. Sequences were obtained from Phytozome v.12 or Ensembl (v.284) in the case of maize.

	<b>Species</b>	<b>Total</b>	<b>PID&gt;70%</b>		<b>PID&gt;70% &amp; PCOV&gt;50%</b>	
<b>Transcripts</b>	<b><i>S. italica</i></b>	43,001	37,449	87.1	29,534	68.7
	<b><i>S. viridis</i></b>	47,205	36,372	77.1	23,110	49
	<b><i>P. halli</i></b>	49,852	40,818	81.9	31,599	63.4
	<b><i>P. virgatum</i></b>	91,838				
	<b><i>Z. mays</i></b>	88,760	58,312	65.7	36,642	41.3
<b>Proteins</b>	<b><i>S. italica</i></b>	43,001	34,749	80.8	29,975	69.7
	<b><i>S. viridis</i></b>	47,205	33,157	70.2	27,953	59.2
	<b><i>P. halli</i></b>	49,852	37,516	75.3	32,753	65.7
	<b><i>P. virgatum</i></b>	91,838				
	<b><i>Z. mays</i></b>	88,760	54,091	60.9	45,951	51.8

**Supplementary Table S5:** EggNOG clusters in six sequenced species in the Panicoideae subfamily, *B. ruziziensis*, foxtail millet (*Setaria italica*), green foxtail (*Setaria viridis* (L.) Beauv.), *Panicum halli* Vasey, switchgrass (*Panicum virgatum* L.), and maize (*Zea mays* L.), classified by number of proteins per cluster.

	<i>P. virgatum</i>		<i>S. italica</i>		<i>S. viridis</i>		<i>P. halli</i>		<i>B. ruziziensis</i>		<i>Z. mays</i>	
<b>GENES</b>	<b>Num</b>	<b>%</b>	<b>Num</b>	<b>%</b>	<b>Num</b>	<b>%</b>	<b>Num</b>	<b>%</b>	<b>Num</b>	<b>%</b>	<b>Num</b>	<b>%</b>
<b>1</b>	1897	8.4	18629	84.4	18508	83.4	18413	86.2	12572	66.7	14369	70.3
<b>2</b>	11955	53.1	2432	11.0	2534	11.4	2176	10.2	3908	20.7	4250	20.8
<b>3</b>	4602	20.5	559	2.5	630	2.8	464	2.2	1164	6.2	1040	5.1
<b>4</b>	1808	8.0	193	0.9	235	1.1	157	0.7	472	2.5	381	1.9
<b>&gt;4</b>	2237	9.9	263	1.2	285	1.3	163	0.8	733	3.9	411	2.0
<b>total</b>	22499		22076		22192		21373		18849		20451	



**Supplementary Table S6:** Peak and interval positions for the identified QTLs, as well as corresponding *S. italica* chromosome.

Trait*	LG	Peak marker	Peak Position (cM)	Position interval (cM)	Marker interval	LOD	R2	additive effect	Si*
RLA	1	scaf_7018_3_123	12.65	5.22 - 31.250	scaf_245_124360 - scaf_1729_44015	4.81	13.6	-22.31	8
RLC	1	scaf_2065_28271	26.027	5.22 - 28.893	scaf_1787_41196 - scaf_3809_36896	5.78	16.1	-45.05	8
RRL	3	scaf_1152_23964	96.802	88.62-98.851	scaf_2718_14282 - scaf_5425_26287	4.75	13.4	3.12	7
RBA	1	scaf_1948_47501	5.22	5.22 - 32.481	scaf_245_124360 - scaf_1218_39495	5.14	14.4	-0.003	8
RBC	1	scaf_1801_0_6509	25.797	17.162 - 28.893	scaf_7830_644 - scaf_3809_36896	5.25	14.7	-0.004	8
RRD	3	scaf_1423_8_7306	79.738	79.738 - 83.853	scaf_14238_7306 - scaf_298_25199	4.02	11.5	-2.36	7
RRD	4	scaf_1104_2_5202	50.423	49.12 - 62.127	scaf_1413_25183 - scaf_1181_29646	4.54	12.8	2.48	3

\*Si: *Setaria italica* chromosome.

RLA: Root length in Al<sup>3+</sup> stress; RLC: Root length in control; RRD: Relative root length ratio (stress/control); RB: Root biomass; RD: Root tip diameter.

**Supplementary Table S7:** Enrichment analysis of the GO SLIM terms over-represented among DE genes in *B. ruzi* BRX 44-02 (Bruz), *B. decumbens* CIAT 606 (cv. Basilisk) (Bdec), or PRJNA314352 from Salgado *et al.* (2017).

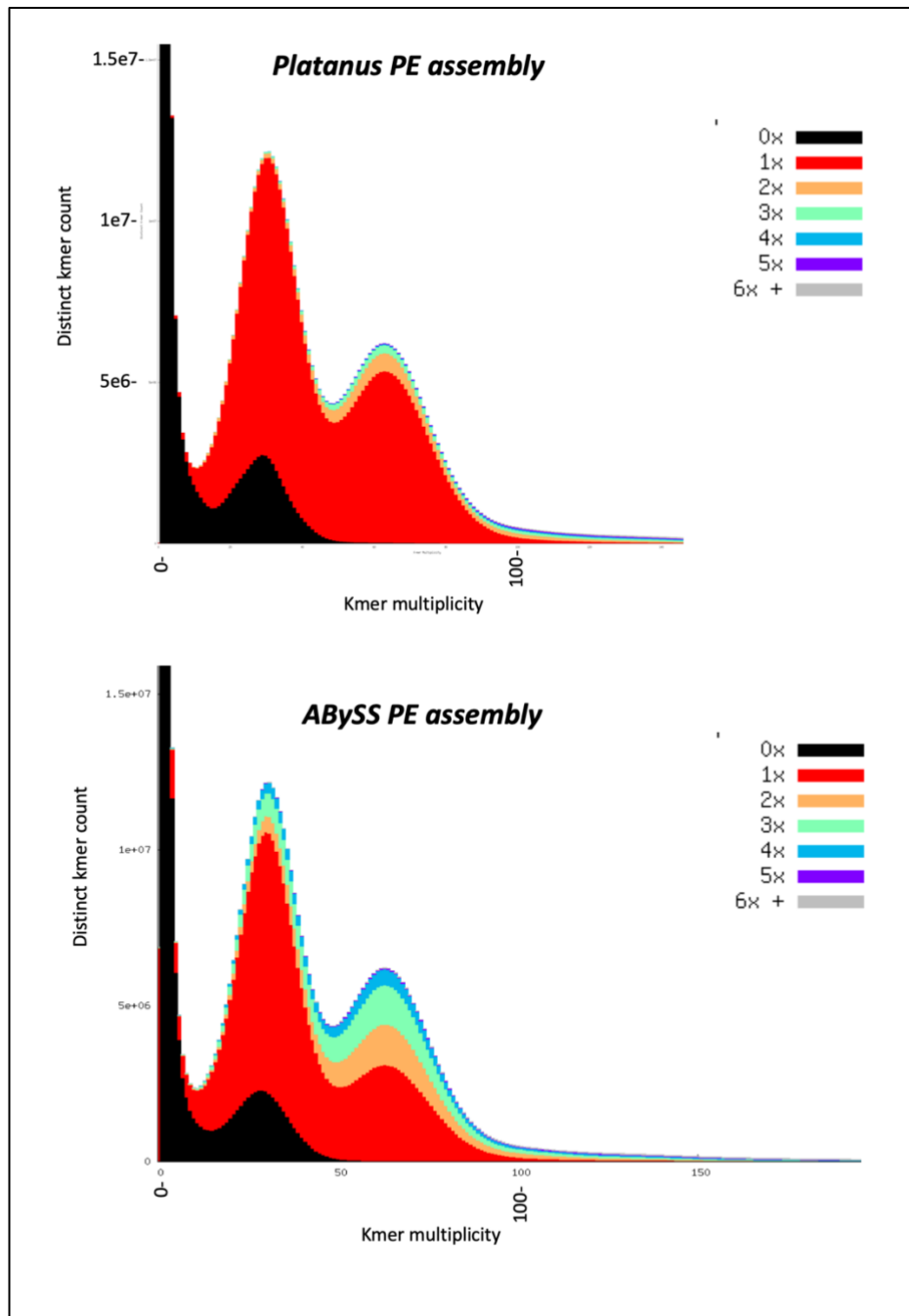
GO term	MOLEC. FUNC.	Bdec CIAT 606			Bruz BRX 44-02			Salgado <i>et al.</i> , 2017		
		Pval	REG	GENES	Pval	REG	GENES	Pval	REG	GENES
GO:0003723	RNA binding (3723)	0.05709	down	14	0.40064	down	40	0.988	down	3
GO:0003729	mRNA binding (3729)	0.13999	down	2	0.00358	down	8	0.419	down	1
GO:0003735	structural constituent of ribosome (3735)	5.5E-12	down	24	1E-30	down	112	0.93632	up	5
GO:0005198	structural molecule activity (5198)	0.19102	up	4	0.02756	down	120	0.583	down	2
GO:0008092	cytoskeletal protein binding (8092)	0.58493	down	1	0.25288	down	5	0.035	down	3
GO:0008134	transcription factor binding (8134)	0.0312	up	3	0.85829	down	1	1	0	0
GO:0008289	lipid binding (8289)	0.09253	up	5	0.21271	down	9	0.26684	up	5
GO:0008565	protein transporter activity (8565)	0.08093	up	3	0.14489	down	5	0.446	down	1
GO:0016491	oxidoreductase activity (16491)	0.00034	down	40	6.8E-09	down	143	0.00081	up	69
GO:0016757	glycosyl transferase (16757)	0.63379	up	7	0.0438	up	22	0.20773	up	14
GO:0016765	alkyl transferase (16765)	0.46513	down	3	0.03894	down	16	0.000019	up	17
GO:0016798	glycosyl hydrolase (16798)	0.00084	up	18	0.0002	down	39	0.00041	up	24
GO:0016829	lyase activity (16829)	0.01422	up	10	0.16411	down	16	2E-12	up	30
GO:0016853	isomerase activity (16853)	0.43965	down	4	0.00077	down	26	0.302	down	4
GO:0016874	ligase activity (16874)	0.11268	up	8	0.1587	up	13	0.0492	up	12
GO:0019843	rRNA binding (19843)	0.04791	down	3	0.0000021	down	14	0.81806	up	1
GO:0019899	enzyme binding (19899)	0.00567	down	8	0.90566	down	7	0.65	down	2
GO:0022857	transmembrane transporter activity (22857)	0.0000018	up	44	0.0000031	up	70	0.0000015	up	57
GO:0030234	enzyme regulator activity (30234)	0.58307	down	3	0.00354	down	22	0.09403	up	10
GO:0030674	protein binding bridging (30674)	1	0	0	0.06958	down	2	1	0	0
GO:0043167	ion binding (43167)	0.23666	up	44	0.0042	up	100	0.261	down	26
GO:0051082	unfolded protein binding (51082)	0.09328	down	2	0.32559	down	3	1	0	0

REG: Either up-regulated (up) or down-regulated (down)

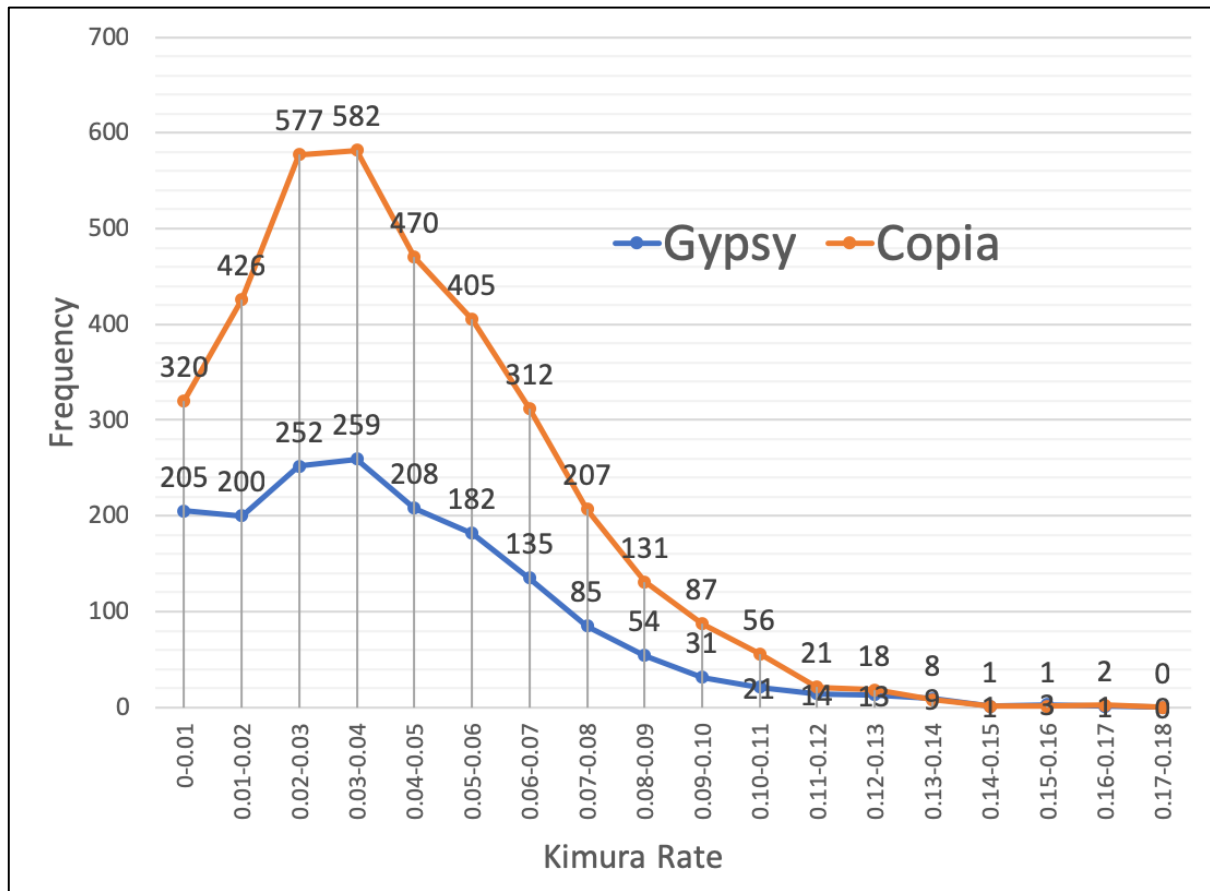
Supplementary Table S7 -Cont.-

GO term	BIOLOG. PROCESS.	Bdec CIAT 606			Bruz BRX 44-02			Salgado <i>et al</i> , 2017		
		Pval	RE G	GEN ES	Pval	RE G	GEN ES	Pval	RE G	GEN ES
GO:0005975	carbohydrate metabolic process (5975)	0.000087	1	21	0.00518	-1	42	0.00193	1	24
GO:0006091	generation of precursor metabolites (6091)	0.44019	-1	4	0.0175	1	15	0.000086	-1	13
GO:0006397	mRNA processing (6397)	0.06748	-1	5	0.6105	1	5	0.91865	1	2
GO:0006412	translation (6412)	0.0002	-1	18	1E-30	-1	103	0.90928	1	9
GO:0006457	protein folding (6457)	0.17529	-1	3	0.01588	-1	12	0.7229	-1	1
GO:0006464	cellular protein modification process (6464)	0.03977	-1	23	0.9702	1	28	0.3059	-1	16
GO:0006520	cellular amino acid metabolic process (6520)	0.4388	1	6	0.4677	1	11	0.03173	1	14
GO:0006629	lipid metabolic process (6629)	0.00645	-1	14	0.00613	-1	41	0.02375	1	20
GO:0006810	transport (6810)	0.0114	1	35	0.03057	-1	83	0.6354	-1	12
GO:0006913	nucleocytoplasmic transport (6913)	0.0052	1	7	0.232	1	6	0.7814	-1	1
GO:0006914	autophagy (6914)	1	-1	0	0.1436	1	3	0.0812	1	3
GO:0006950	response to stress (6950)	0.591	1	14	0.4765	1	29	0.0521	-1	16
GO:0007005	mitochondrion organization (7005)	0.08128	-1	3	0.02374	-1	9	0.57158	1	2
GO:0007010	cytoskeleton organization (7010)	0.76433	-1	1	0.13705	-1	9	0.0371	-1	4
GO:0007155	cell adhesion (7155)	1	-1	0	0.0699	1	1	1	-1	0
GO:0007165	signal transduction (7165)	0.1634	1	15	0.0845	1	29	0.09511	1	23
GO:0009056	catabolic process (9056)	0.13519	-1	17	0.20889	-1	58	1.2E-09	1	58
GO:0009058	biosynthetic process (9058)	0.09869	-1	52	0.0267	1	95	0.1459	-1	35
GO:0019748	secondary metabolic process (19748)	0.011	1	13	0.0066	1	22	0.00064	1	21
GO:0022618	ribonucleoprotein complex assembly (22618)	0.00041	-1	8	0.000012	-1	21	0.85132	1	2
GO:0030154	cell differentiation (30154)	0.5706	1	1	0.05932	-1	6	0.4517	-1	1
GO:0030198	extracellular matrix organization (30198)	1	-1	0	0.0699	1	1	0.05441	1	1
GO:0042592	homeostatic process (42592)	0.3365	1	7	0.0526	1	17	0.00568	1	17
GO:0044281	small molecule metabolic process (44281)	0.04068	-1	19	0.00017	-1	75	3.6E-09	1	60
GO:0048856	anatomical structure development (48856)	0.4292	1	13	0.0384	1	29	0.8382	-1	7
GO:0051186	cofactor metabolic process (51186)	0.1532	-1	6	0.01469	-1	24	0.000004	1	21
GO:0051301	cell division (51301)	1	-1	0	0.00458	-1	7	1	0	0
GO:0055085	transmembrane transport (55085)	0.14685	-1	3	0.30994	-1	7	0.01203	1	7
GO:0071554	cell wall organization or biogenesis (71554)	0.000041	1	17	2.8E-14	-1	51	0.01151	1	14

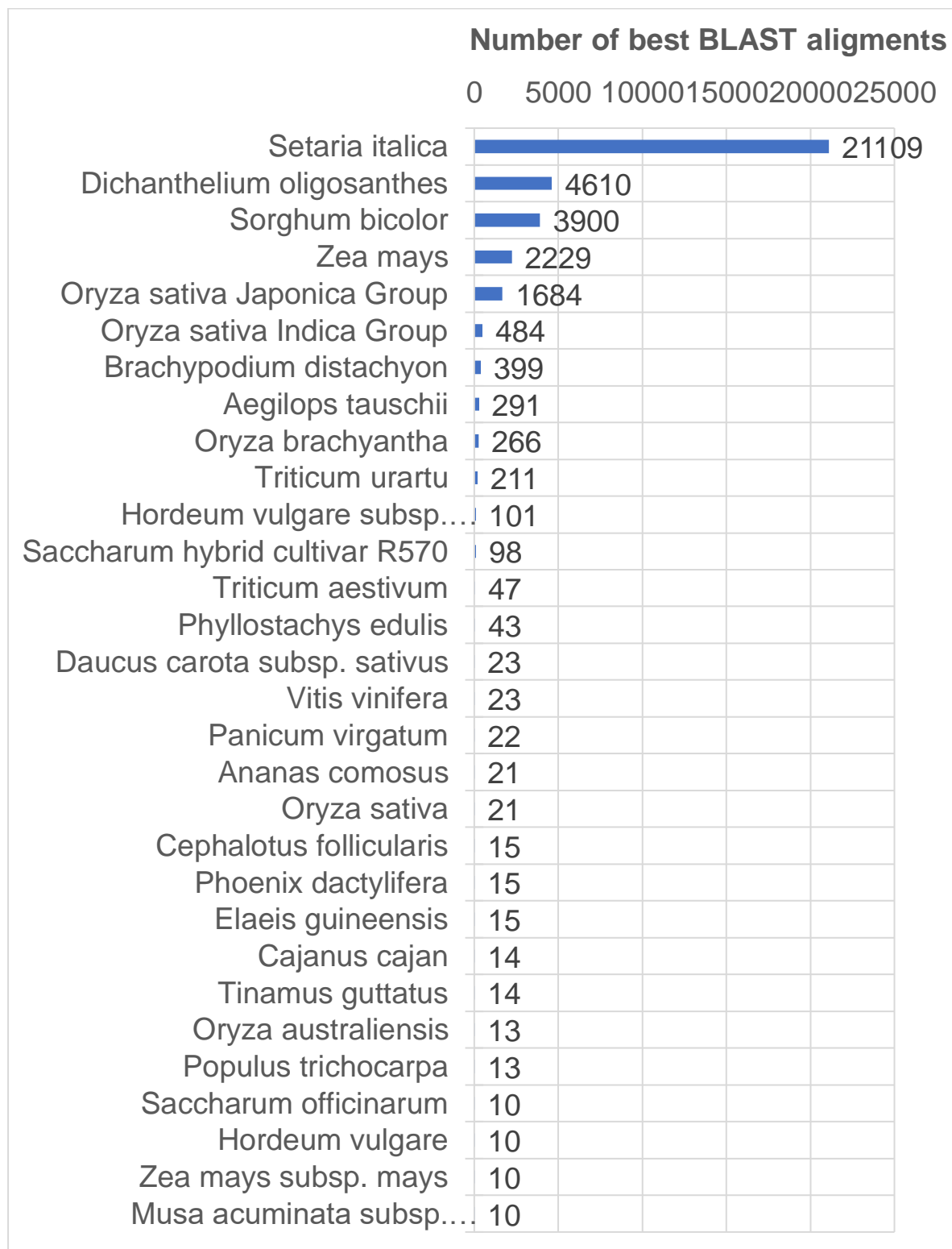
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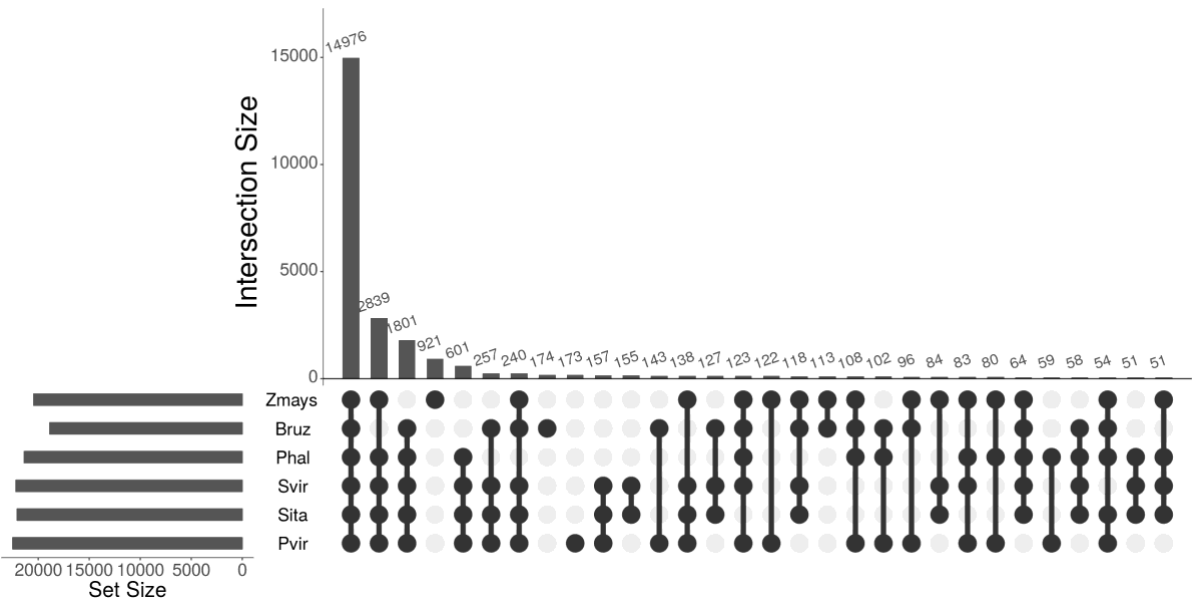
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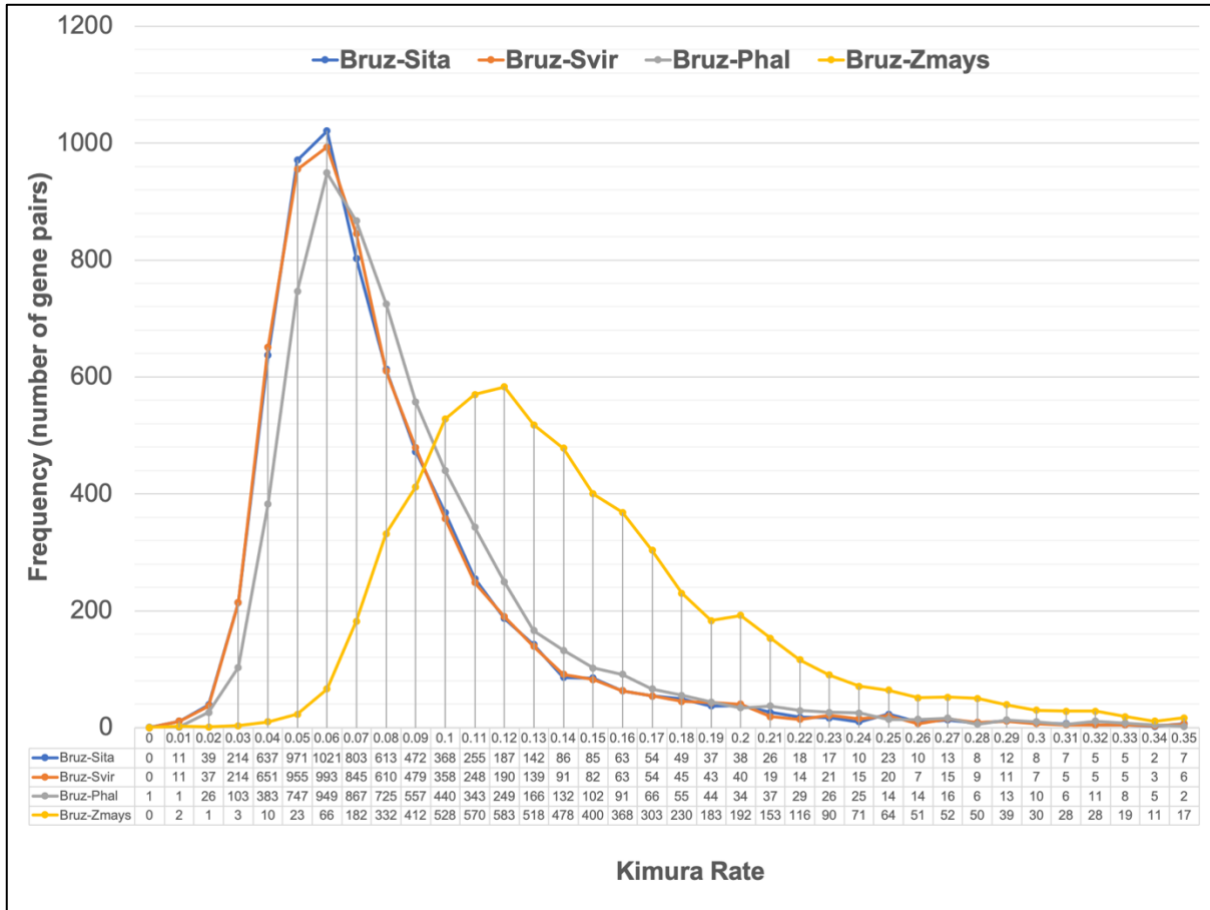
**Supplementary Figure S3:** Species of the top Blastp hit for each 35,982 of the coding transcripts which had a homologous protein in the NCBI non-redundant (nr) database.



**Supplementary Figure S4:** Shared egnog clusters of proteins among *Brachiaria ruziziensis* (Bruz), foxtail millet [*S. italica* (Sita)], *S. viridis* (Svir), maize [*Z. mays* (Zmays)], *Panicum halli* (Phal) and switchgrass [*P. virgatum* (Pvir)]. The “UpSet” plot format provides an efficient way to visualize the intersections (columns) of six species (Rows).

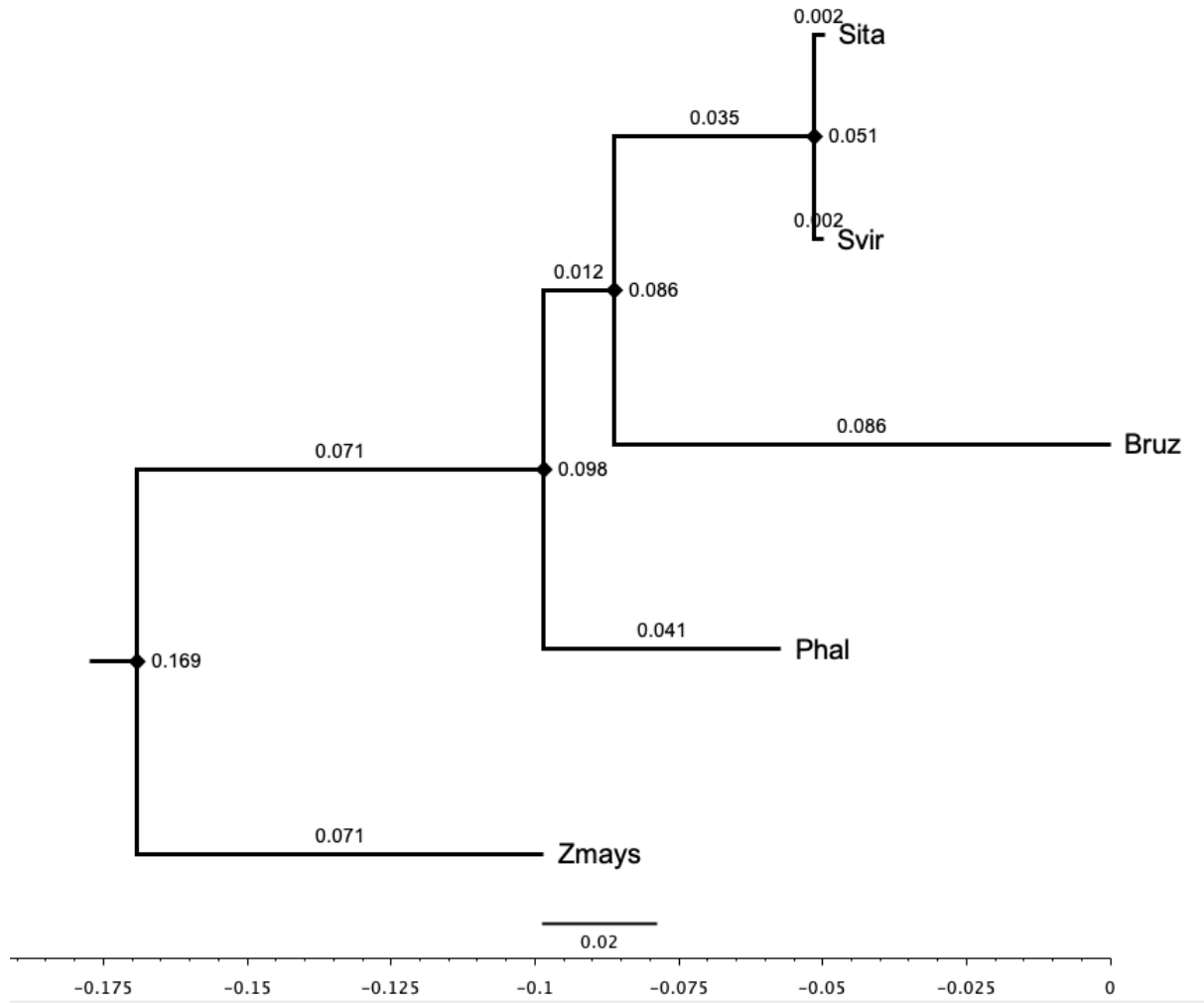


**Supplementary Figure S5:** Kimura rates between homologous gene pairs between *B. ruziziensis* and sequenced relatives including foxtail millet [*S. italica* (Sita)], *S. viridis* (Svir), maize [*Z. mays* (Zmays)], and *P. halli* (Phal)]. Gene pairs were build based on eggNOG clusters.

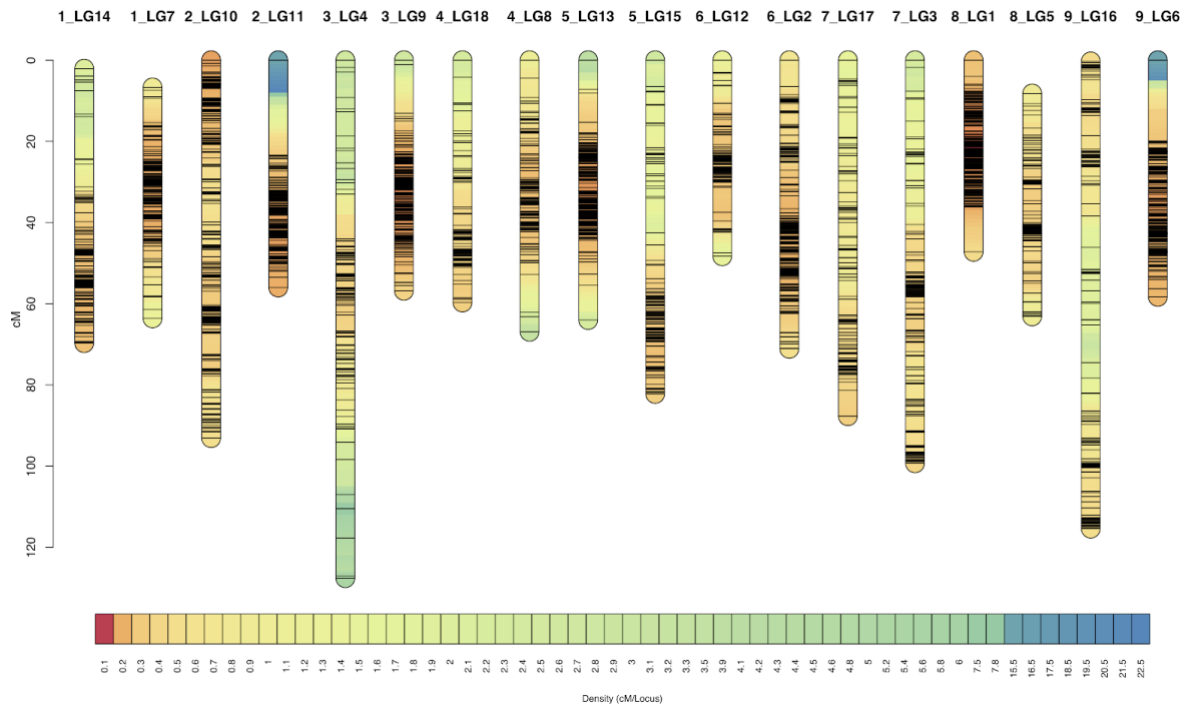




**Supplementary Figure S6:** Phylogenetic tree based on nucleotide divergence rate between sequences in the same eggnog cluster from *B. ruziziensis* and sequenced relatives including foxtail millet [*S. italica* (Sita)], *S. viridis* (Svir), maize [*Z. mays* (Zmays)], and *P. halli* (Phal)].

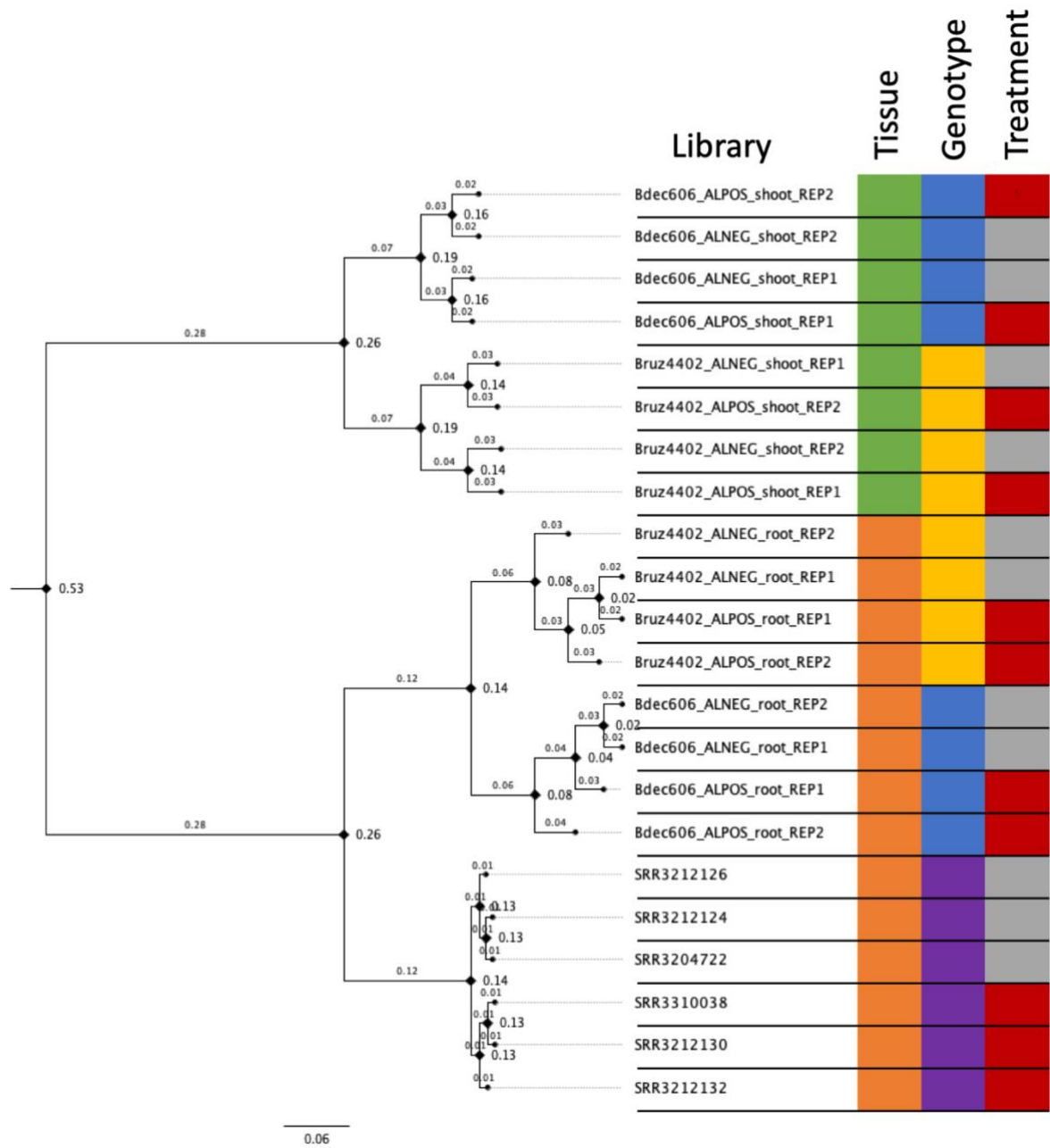


**Supplementary Figure S7:** The final genetic map for the *B. decumbens* CIAT 606 (cv. Basilisk) progenitor of the interspecific population included 4,427 markers placed at LOD 10 in 18 linkage groups

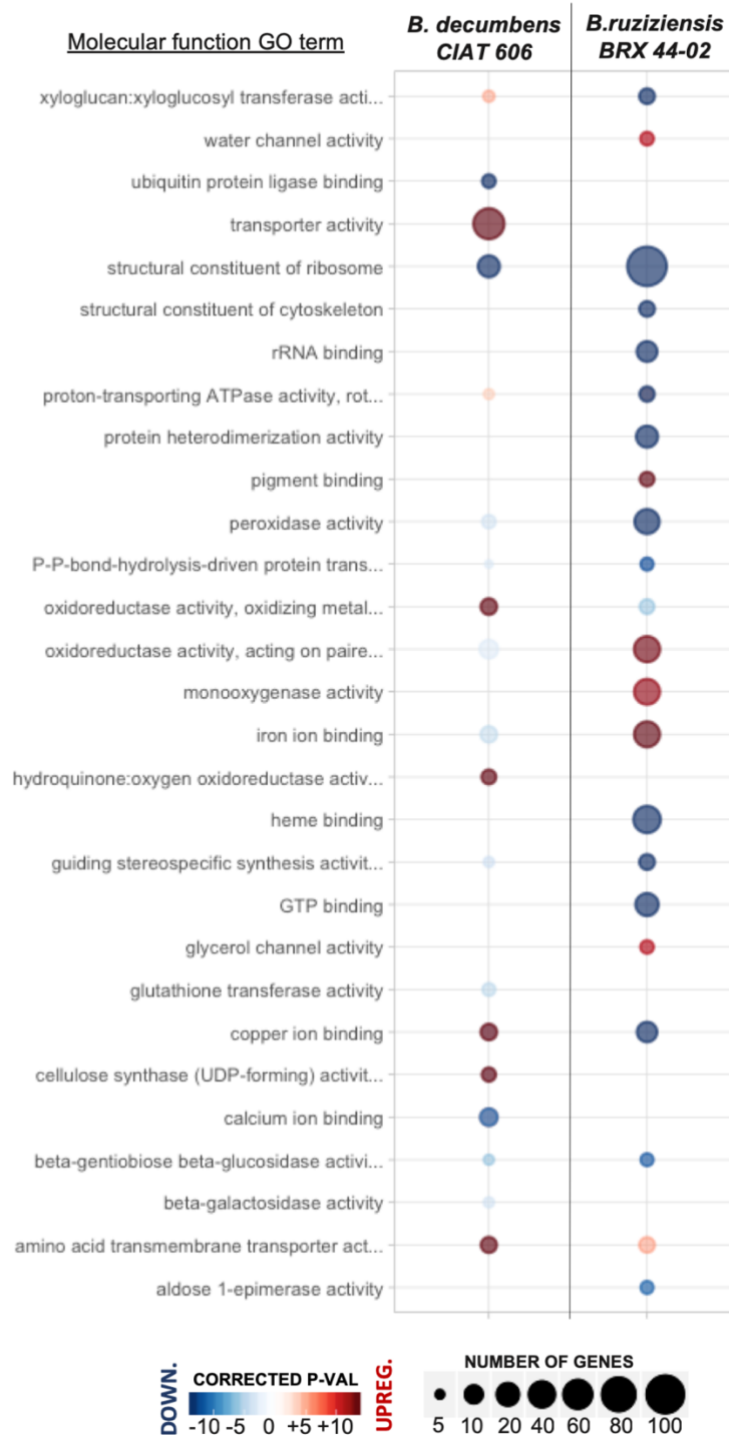


Rendered by LinkageMapView

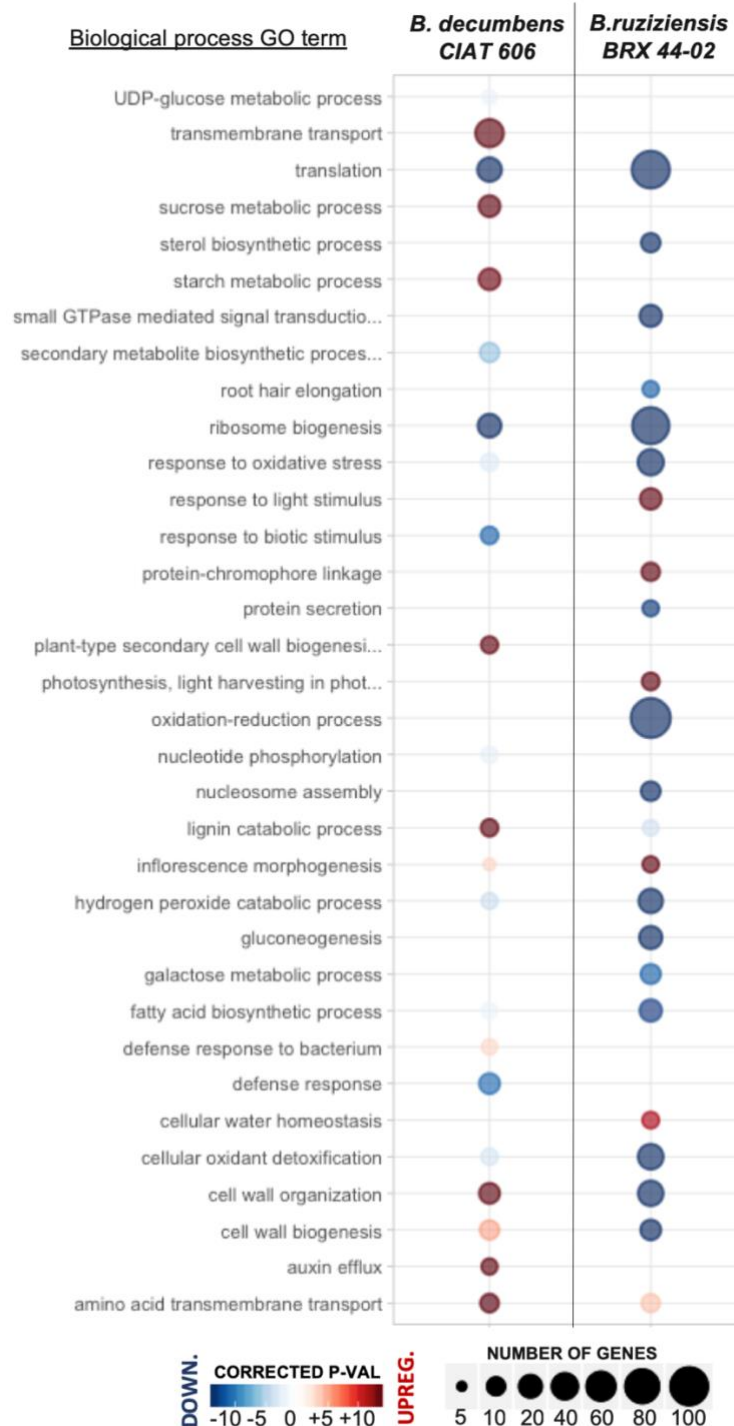
**Supplementary Figure S8:** RNA-seq from stem and root tissue samples extracted from the *B. decumbens* and *B. ruziziensis* progenitors. We also incorporated a reference-based reanalysis of public RNA-seq data (PRJNA314352) from *B. decumbens* var. Basilisks roots (Salgado et al., 2017, Plant Growth Regulation, 83,1:157-170). When the normalised counts for all the genes were used to cluster the samples, these clusters firstly grouped by tissue, secondly by genotype, and thirdly by treatment.



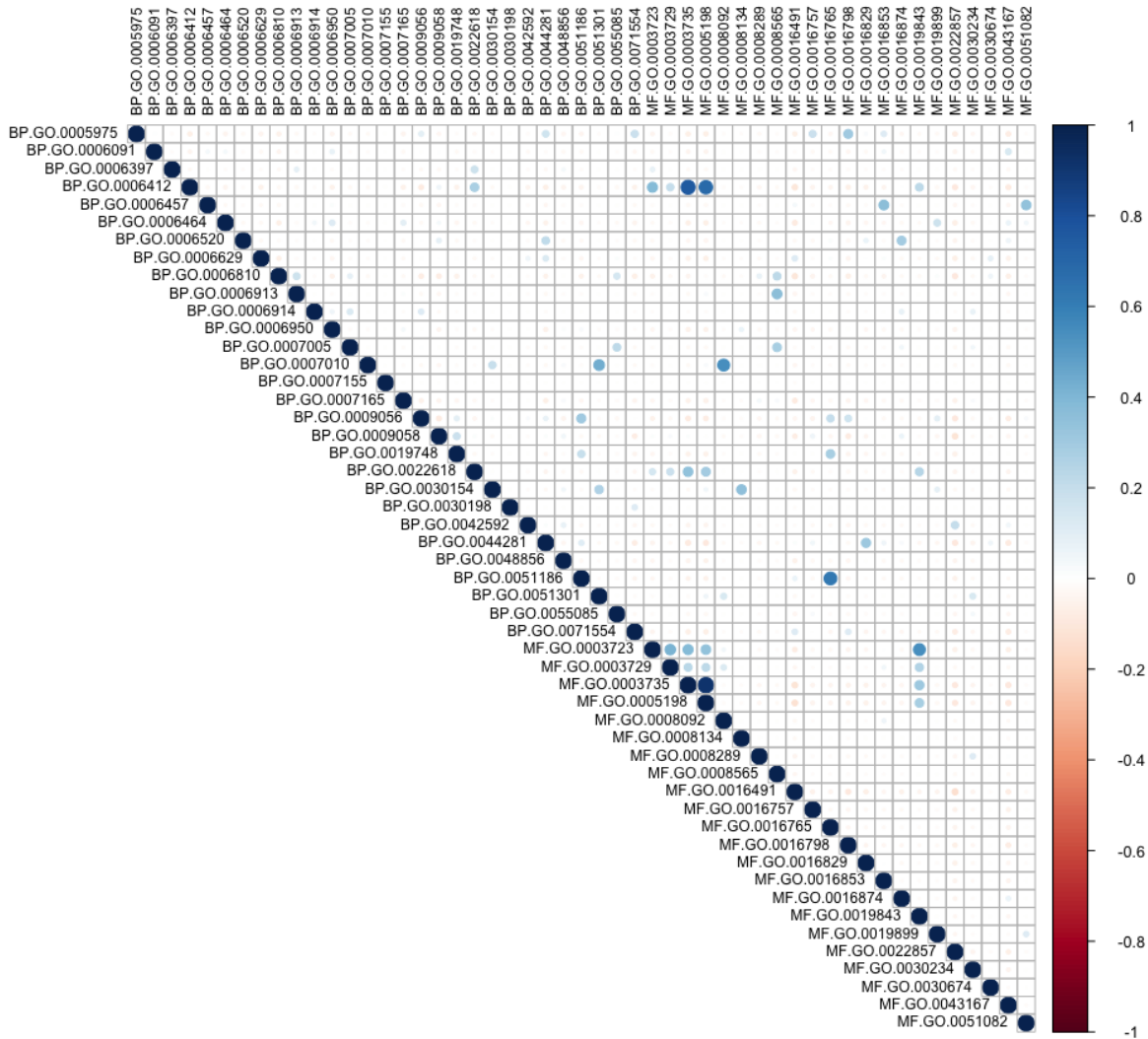
**Supplementary Figure S9:** Enrichment analysis of the “Molecular function” GO terms overrepresented among differentially expressed upregulated (red) or downregulated (blue) genes in roots in *B. decumbens* CIAT 606 and *B. ruziziensis* BRX 44-02.



**Supplementary Figure S10:** Enrichment analysis of the “Biological Process” GO terms overrepresented among differentially expressed upregulated (red) or downregulated (blue) genes in roots in *B. decumbens* CIAT 606 and *B. ruziziensis* BRX 44-02.



**Supplementary Figure S11:** Correlation matrix plot among GO terms based on the DE genes included in each annotation.



**Supplementary Figure S12:** Comparison the enriched GO Slim terms between *B. decumbens* cv. Basilisk exposed to 200  $\mu\text{M}$   $\text{AlCl}_3$  for 72 hours and 8 hours, the latter from the reanalysis of public raw data from Salgado *et al.* 2017.

