

The genome of stress tolerant crop wild relative *Paspalum vaginatum* leads to increased biomass productivity in the crop *Zea mays*

Guangchao Sun^{1,2,3}, Nishikant Wase^{2,4,†}, Shengqiang Shu⁵, Jerry Jenkins⁶, Bangjun Zhou^{2,7}, Cindy Chen⁵, Laura Sandor⁵, Chris Plott⁶, Yuko Yoshinga⁵, Christopher Daum⁵, Peng Qi^{8,9,10}, Kerrie Barry⁵, Anna Lipzen⁵, Luke Berry^{2,4}, Thomas Gottilla⁸, Ashley Foltz^{1,2,3,‡}, Huihui Yu^{2,7}, Ronan O'Malley⁵, Chi Zhang^{2,7}, Katrien M. Devos^{8,9,10}, Brandi Sigmon¹¹, Bin Yu^{2,7}, Toshihiro Obata^{2,4}, Jeremy Schmutz^{5,6,*}, and James C. Schnable^{1,2,3,*}

¹Quantitative Life Sciences Initiative, University of Nebraska-Lincoln, Lincoln, NE, 68588 USA

²Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE, 68588 USA

³Department of Agronomy and Horticulture, University of Nebraska-Lincoln, Lincoln, NE, 68588 USA

⁴Department of Biochemistry, University of Nebraska-Lincoln, Lincoln, NE, 68588 USA

⁵Department of Energy Joint Genome Institute, Lawrence Berkeley National Laboratory, CA 94598, USA.

⁶HudsonAlpha Institute for Biotechnology, Huntsville, AL 35806, USA

⁷School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE, 68588 USA

⁸Institute of Plant Breeding, Genetics and Genomics, Department of Crop and Soil Sciences, University of Georgia, Athens, GA 30602, USA

⁹Department of Crop and Soil Sciences, University of Georgia, Athens, GA 30602, USA

¹⁰Department of Plant Biology, University of Georgia, Athens, GA 30602, USA

¹¹Department of Plant Pathology, University of Nebraska-Lincoln, Lincoln, NE, 68588 USA

[†]Present Affiliation: Biomolecular Analysis Facility. School of Medicine, University of Virginia, Charlottesville, VA 22903 USA

[‡]Present Affiliation: School of Biological Sciences, University of Nebraska-Lincoln, Lincoln, NE 68588 USA

*jschmutz@hudsonalpha.org & schnable@unl.edu

ABSTRACT

A number of crop wild relatives can tolerate extreme stressed to a degree outside the range observed in their domesticated relatives. However, it is unclear whether or how the molecular mechanisms employed by these species can be translated to domesticated crops. *Paspalum vaginatum* is a self-incompatible and multiply stress-tolerant wild relative of maize and sorghum. Here we describe the sequencing and pseudomolecule level assembly of a vegetatively propagated accession of *P. vaginatum*. Phylogenetic analysis based on 6,151 single-copy syntenic orthologous conserved in 6 related grass species placed paspalum as an outgroup of the maize-sorghum clade demonstrating paspalum as their closest sequenced wild relative. In parallel metabolic experiments, paspalum, but neither maize nor sorghum, exhibited significant increases in trehalose when grown under nutrient-deficit conditions. Inducing trehalose accumulation in maize, imitating the metabolic phenotype of paspalum, resulting in autophagy dependent increases in biomass accumulation.

1 **Running title: Paspalum shows role for trehalose in resilience**

2 Introduction

3 Domesticated crops from the grass family provide, directly or indirectly, the majority of total calories
4 consumed by humans around the globe. Among the domesticated grasses the yields of three crops
5 dramatically increased as part of the green revolution: rice, wheat and maize. These yield increases
6 resulted from both breeding and greater availability and application of fertilizer. From 1960 to 2014, the
7 amount of nitrogen (N) and phosphorus (P) fertilizer applied worldwide increased nine-fold and five-fold
8 respectively¹⁻³. Today these three crops account for approximately one half of total harvested staple
9 crop area and total global calorie production as well as greater than one half of total global fertilizer
10 consumption. Manufacturing N fertilizer is an energy intensive process⁴ and the production of P from
11 mineral sources may peak as early as 2030⁵. Fertilizer costs are often the second largest variable input
12 after seed in rain fed agricultural systems. In the United States Corn Belt alone, 5.6 million tons of N
13 and 2.0 million tons of P have been applied annually to maize (*Zea mays*) fields since 2010⁶. In the 2015
14 growing season, these fertilizers accounted for an estimated \$5 billion in input costs^{7,8}. Fertilizer runoff
15 resulting from inefficient uptake or over application can result in damage to both aquatic ecosystems and
16 drinking water quality⁹⁻¹².

17 Improving the productivity of crop plants per unit of fertilizer applied would increase the profitability
18 of agriculture while decreasing its environmental impact¹³⁻¹⁵. A significant portion of the overall increase
19 in maize yields appears to be explained by selection for increased stress tolerance and yield stability in
20 the decades since the 1930s^{16,17}. The observations from maize suggest it may be possible to increase
21 the stress tolerance and resource-use efficiency of crops in a manner that is either neutral or beneficial to
22 overall yield potential. Some crop wild relatives exhibit degrees of stress tolerance well outside the range
23 observed in their domesticated relatives, and therefore may employ mechanisms not present in the primary
24 germplasm of crops¹⁴.

25 *Paspalum vaginatum* (seashore paspalum – or simply paspalum) is a relative of maize (*Zea mays*)
26 and sorghum (*Sorghum bicolor*). It is currently found on saltwater beaches and in other regions of high
27 salinity around the globe^{18,19}. Reports suggest that paspalum is tolerant of drought²⁰⁻²³, cold stress²⁴⁻²⁶,
28 low light²⁷, and crude oil contamination²⁸. Paspalum grows primarily in the wild, but breeding efforts
29 have led to the development of turfgrass cultivars for use in areas with high soil salinity, limited access
30 to freshwater, or where turf is irrigated with wastewater^{27,29}. Paspalum requires less N to maintain

31 visible health than other grasses employed as turfgrasses in environments where paspalum thrives²⁹.
32 Historically few genetic resources have been available for this species, although a set of genetic maps
33 were recently published³⁰. The paucity of genetic and genomic investigations may in part result from the
34 challenging reproductive biology of this species; paspalum is self-incompatible and is primarily propagated
35 as heterozygous vegetative clones^{29,31}.

36 Here, we generate a pseudomolecule level genome assembly for a reference genotype of paspalum (PI
37 509022), enabling comparative transcriptomic and genomics analysis. Phylogenetic analyses employing
38 syntenic gene copies confirmed paspalum's placement as a close outgroup to maize and sorghum and
39 the paspalum genome exhibits a high degree of conserved collinearity with that of sorghum. The genes
40 involved in telomere maintenance and DNA repair have experience significant copy number expansion
41 in the paspalum lineage as do several gene families which transcriptionally respond to nitrogen or
42 phosphorous deficit stress. Changes in trehalose accumulation and the expression of genes involved
43 in trehalose metabolism were observed in response to multiple nutrient-deficit stresses were observed
44 in paspalum, but not in paired datasets collected from sorghum and maize under identical conditions.
45 Replicating the pattern of trehalose metabolism observed in wild-type paspalum by inhibiting the enzyme
46 responsible for degrading trehalose increased trehalose accumulation, biomass accumulation and shoot-
47 to-root ratios in maize under nutrient-optimal and -deficient conditions. The induced accumulation of
48 trehalose in maize was associated with lipidation of AUTOPHAGY-RELATED8 (ATG8) a marker for
49 autophagy activity. Treatment with a chemical inhibitor for autophagy abolished the increased biomass
50 accumulation observed in maize plants accumulating additional trehalose, suggesting that increased
51 autophagy as a potential mechanism for the observed increased productivity observed in maize plants
52 accumulating additional trehalose.

53 Results

54 Characteristics of the paspalum genome

55 We generated 5,021,142 PacBio reads with a median length of 9,523 bp from genomic DNA isolated from
56 dark-treated tissue of the heterozygous paspalum clone PI 509022. The reads were assembled into 1,903
57 main genome scaffolds with an N50 of 44.5 Mbp and a total length of 651.0 Mbp (Table S1). This is
58 modestly larger than the estimated haploid gene size of the paspalum genome of 593 Mbp (See Methods
59 and Supplementary Note 1)³². Flow cytometry carried out within this study confirmed that the genome
60 size of the paspalum clone employed in this study was approximately 590 Mbp (Figure S1A & B). This
61 modest over-assembly may represent haplotype-specific sequences which is supported by the binomial
62 distribution of read coverage mapped to the current genome assembly (Figure S1C). We used published
63 sequence data from markers genetically mapped in an F1 population generated from a cross between two
64 heterozygous paspalum individuals³⁰ to integrate a set of 347 scaffolds into ten pseudomolecules spanning
65 >82% of the estimated total haploid paspalum genome (Supplementary Note 2). Scaffolds that were not
66 anchored in a chromosome were classified into bins depending on sequence content. Contamination was
67 identified using BLASTN against the NCBI nucleotide collection (NR/NT) and BLASTX using a set of
68 known microbial proteins. Additional scaffolds were classified as repetitive (>95% masked with 24 mers
69 that occur more than 4 times in the genome) (197 scaffolds, 12.4 Mb), alternative haplotypes (unanchored
70 sequence with >95% identity and >95% coverage within a chromosome) (3,276 scaffolds, 187.9 Mb),
71 and low quality (>50% unpolished bases, post polishing) (9 scaffolds, 204.5 Kb) (Table S1). A set of
72 45,843 gene models were identified and annotated using a combination of approaches (See Methods).
73 A total of 22,148 syntenic orthologous gene pairs were identified between the paspalum and sorghum
74 genomes (Figure S2A & B). The large inversions observed on chromosome 4 and chromosome 7 were

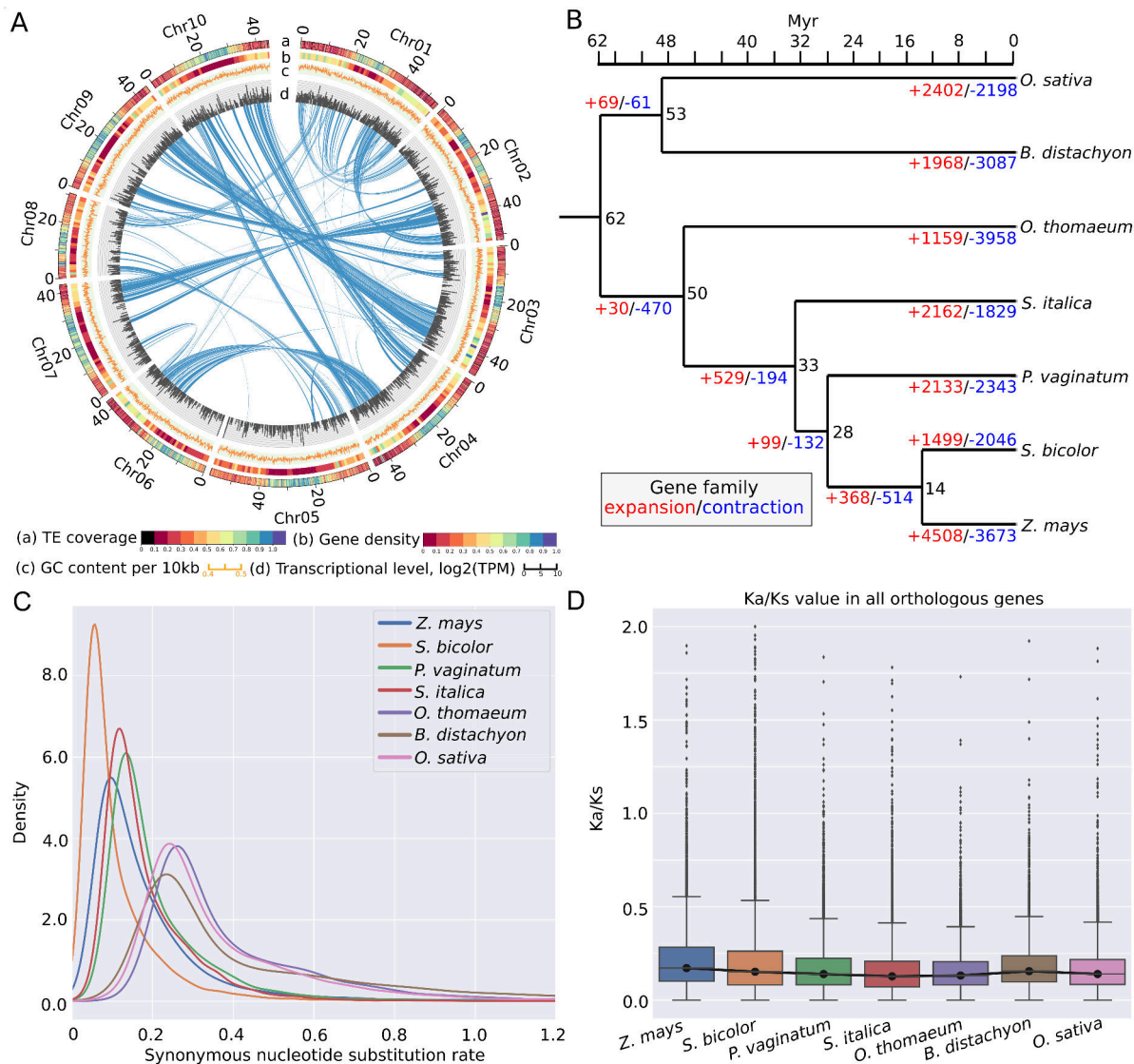


Figure 1. Paspalum (*Paspalum vaginatum*) genome and evolution. (A) Circos plot for the paspalum genome. a: TE coverage per 100 Kb, b:GC content per 10 kb region, c: gene density (generic region coverage) per 1 Mb, d: transcription represented by log₂(TPM) per 100 kb and e: inter- and intra- chromosomal synteny. (B) Phylogeny and estimated divergence times among maize (*Zea mays*), sorghum, paspalum, foxtail millet (*Setaria italica*), *Oropetium* (*Oropetium thomaeum*), *Brachypodium* (*Brachypodium distachyon*), and rice (*Oryza sativa*). Numbers in black indicate the estimated divergence time (in millions of years before present) for each node. Numbers in blue and red indicate the number of gene families predicted to have experienced copy number expansion or contraction along each branch of the phylogeny, respectively. (C) Distribution of the estimated lineage-specific synonymous substitution rates for syntenically conserved genes in each of the seven species shown in panel A (see Methods) (D) Distribution of the estimated lineage-specific ratios of nonsynonymous substitution rates to synonymous substitution rates for syntenically conserved genes among each of the seven species shown in panel A.

75 previously validated by a study a genetic map was constructed using GBS genotyping technology³⁰. Small
 76 translocations were also observed between chromosome 8 and chromosome 4 (Figure S2 A& B). The
 77 predicted protein sequences of annotated paspalum genes tended to cover the full length of the most
 78 closely related protein in sorghum, and vice versa, indicating most annotated gene models in the paspalum
 79 genome assembly are likely full length (Figure S2C). On a macro level, the paspalum genome displays

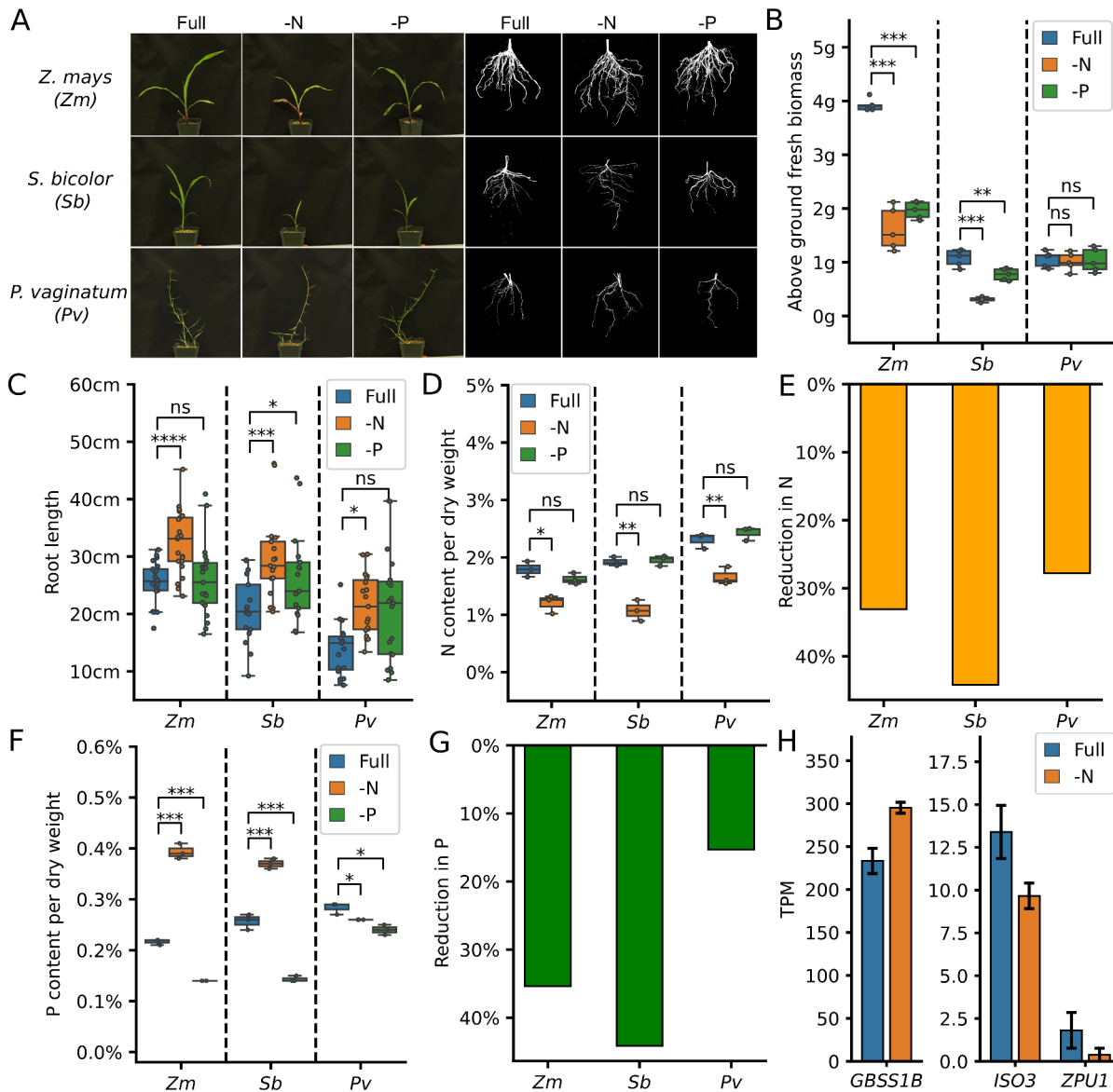
80 many features common to other grass genomes: a higher gene density in the distal chromosome regions
81 than pericentromeric regions and, conversely, a higher frequency of transposable elements and other
82 repetitive sequences in pericentromeric regions than distal chromosome regions, and syntenic evidence of
83 the pre-grass (rho) whole genome duplication (Figure 1 A).

84 **Comparative genomics analysis of paspalum and its relatives**

85 Paspalum belongs to the grass tribe Paspaleae, a group which, together with the Andropogoneae (which
86 includes maize and sorghum) – and the Arundinelleae, forms a clade sister to the Paniceae – which
87 includes foxtail millet (*Setaria italica*). Paspaleae, Andropogoneae and Paniceae are all members of the
88 grass subfamily Panicoideae, while *Oropetium* belongs to the grass clade Cynodonteae³³. We constructed
89 phylogenetic trees using DNA alignments for 6,151 single-copy syntenic orthologous genes present in
90 *Zea mays*, *Sorghum bicolor*, *Setaria italica*, *Oropetium thomaeum*, *Brachypodium distachyon*, *Oryza*
91 *sativa*, and *Paspalum vaginatum*. A total of 5,859 trees representing 49 unique topologies and placing *B.*
92 *distachyon* and *O. sativa* in a monophyletic outgroup survived quality filtering (see Methods for filtering
93 criteria). The most common topology among these trees – represented by 4,265 individual gene trees
94 (73%) – was consistent with the previous consensus placement of paspalum (Figure S3A & Figure 1B).
95 The second most common topology, represented by 762 individual gene trees (13%), placed paspalum
96 sister to foxtail millet (Figure S3A).

97 Dating placed the split of the Chloridoideae (represented by *Oropetium thomaeum*) from the Pani-
98 coideae at 50 million years before present and indicated that, within the Panicoideae, the Paniceae shared
99 a common ancestor with the Andropogoneae/Paspaleae clade (represented by paspalum, sorghum, and
100 maize) at 33 million years (Myr) before present, a date modestly earlier than previous estimates (26
101 Myr ago)^{34,35} (Figure 1B). The divergence of the lineage leading to paspalum from that leading to maize
102 and sorghum – (the split between the Andropogoneae and Paspaleae) – was estimated to have occurred
103 approximately 28 million years before present. We calculated branch specific synonymous (Ks) and
104 nonsynonymous (Ka) nucleotide substitution rates for syntenic orthologous gene groups based on known
105 species relationships (Figure 1C; Supplementary note 3). Consistent with previous reports, maize exhibited
106 greater modal synonymous substitution rates than sorghum, even though these are sister lineages in the
107 phylogeny³⁶ (Figure 1C). The modal synonymous substitution rates in paspalum were modestly higher
108 than those observed in foxtail millet (Figure 1D).

109 Annotated protein sequences for sorghum, foxtail millet, *Oropetium*, *Brachypodium* (*Brachypodium*
110 *distachyon*), and paspalum were grouped into 25,675 gene families. Of these families 16,038 were
111 represented by at least one gene copy in each of the five species, with the remainder being present in 1-4
112 species (Figure S3B). A set of 721 gene families were unique to paspalum. This number was modestly
113 less than the number of species-specific gene families identified in brachypodium and modestly more
114 than the number of species-specific gene families observed in sorghum and foxtail millet (Figure S3B).
115 Of the 21,091 gene families present in paspalum, 75% (15,769) were represented by only a single copy
116 in the paspalum genome and 17 % (3,524) were represented by two copies. These values are similar to
117 those observed in sorghum and foxtail millet which shared the same most recent common pre-grass (rho)
118 whole genome duplication (Figure S3C). A set of 149 gene families were identified as undergoing copy
119 number expansion with a significantly different evolution rate (lambda) in the paspalum lineage. These
120 included families of genes annotated with gene ontology (GO) terms related to homeostatic processes
121 such as telomere maintenance and DNA repair, protein modification, stress response, nutrient reservoir
122 activity and oxidation-reduction process (Supplementary note 4).



123 General and paspalum-specific physiological responses to nutrient-deficit stress

124 Paspalum requires little fertilizer in order to remain visibly healthy, however it was unclear whether
 125 paspalum is actually more efficient at producing biomass under nitrogen limited conditions. A comparison
 126 was conducted of growth of paspalum, sorghum, and maize plants under ideal, nitrogen limited and

127 phosphorous limited conditions. Visible effects were apparent in maize and sorghum seedlings under
128 N- or P-deficient conditions but not in clonally propagated paspalum plants (ramets) three weeks after
129 planting (Figure 2A). Both maize and sorghum exhibited significant decreases in above ground fresh
130 biomass accumulation when grown under N- or P- deficient conditions whereas paspalum did not (Figure
131 2B). This result should be interpreted with the caveat that paspalum accumulated the least biomass per
132 plant of the three species under nutrient-optimal conditions. In both maize and sorghum, N-deficit was
133 associated with significant increases in root length. However, a statistically significant increase in root
134 length in response to P-deficit stress was only observed for sorghum (Figure 2C). Paspalum ramets grown
135 under N-deficient conditions showed a modest but statistically significant increase in root length compared
136 to optimal nutrient conditions, while P-deficit stress did not produce any statistically significant increase
137 in root length in this species (Figure 2C).

138 One potential explanation for the relative lack of plasticity observed in paspalum in response to
139 N-deficit or P-deficit stress is that paspalum has limited potential to utilize N under optimal conditions
140 and hence did not experience substantial internal declines in N availability in response to N-deficient
141 conditions. We therefore measured the contents of N and P in plants of all three species included in this
142 study. Substantial decreases in N as a proportion of total biomass were observed in all three species
143 grown under N-deficient conditions relative to the full nutrient controls (Figure 2 D & E). P-deficient
144 treatments produced significant declines in P as a proportion of dry biomass for all three species (Figure 2
145 F), although the decline in P abundance for paspalum was notably smaller in magnitude than the declines
146 in maize and sorghum, with sorghum exhibiting the greatest reduction in P content (43%), followed by
147 maize (36%) and paspalum (15%; Figure 2 G). N-deficient treatment produced significant increases in P as
148 a proportion of dry biomass in maize and sorghum (Figure 2 F), which is consistent with previous reports
149 of enhanced P uptake in plants grown under N-deficient conditions³⁷. N deficit stress is also associated
150 with increased starch accumulation in maize^{38,39}. In shoot tissues of paspalum seedlings grown under
151 N-deficit conditions, the expression of the syntenically conserved gene *GBSS1B* (encoding granule-bound
152 starch synthase 1)⁴⁰ increased and the expression of *ISO3* and *ZPUI*(encoding starch debranching enzyme
153 involved in starch degradation) decreased⁴¹ relative to nutrient optimal conditions (Figure 2 H). Taken as a
154 whole, these results indicate that the external N-deficient treatment protocol employed here was sufficient
155 to produce declines in internal N levels and N-deficit stress in paspalum.

156 **Comparisons of primary metabolic responses to nutrient-deficit stress**

157 Numerous metabolic changes were observed between plants grown under nutrient-replete and nutrient-
158 deficient conditions, with more metabolites exhibiting significant changes in abundance in response to N-
159 or P-deficit stress in maize and sorghum than in paspalum (Figure 3A & B, Supplementary note 5). Twelve
160 metabolites showed significant decreases in abundance in response to N-deficit stress in both sorghum and
161 maize, and four metabolites showed significantly increased abundance in response to N-deficit stress (18
162 of the 32 metabolic responses were shared between the two species) (Figure 3A). A smaller number of
163 statistically significant metabolic changes were observed in response to P-deficit stress, which is consistent
164 with the less severe phenotype observed for P deficiency in the experimental design employed (Figure
165 3 B; Figure 2 A). A number of metabolic changes were again shared between maize and sorghum, with
166 the levels of five tested metabolites decreasing in both species in response to P-deficit stress and one
167 increasing (6 of the 16 metabolic responses were shared) (Figure 3B). All metabolic changes associated
168 with N-deficit stress in paspalum were either species specific or shared with both maize and sorghum
169 while all metabolic changes associated with P-deficit stress in paspalum were species specific (Figure 3 A
170 & B). Metabolic changes associated with N-deficit stress shared by maize and sorghum but not paspalum
171 included decreases in the abundance of many amino acids, including L-asparagin, L-glutamine, L-alanine

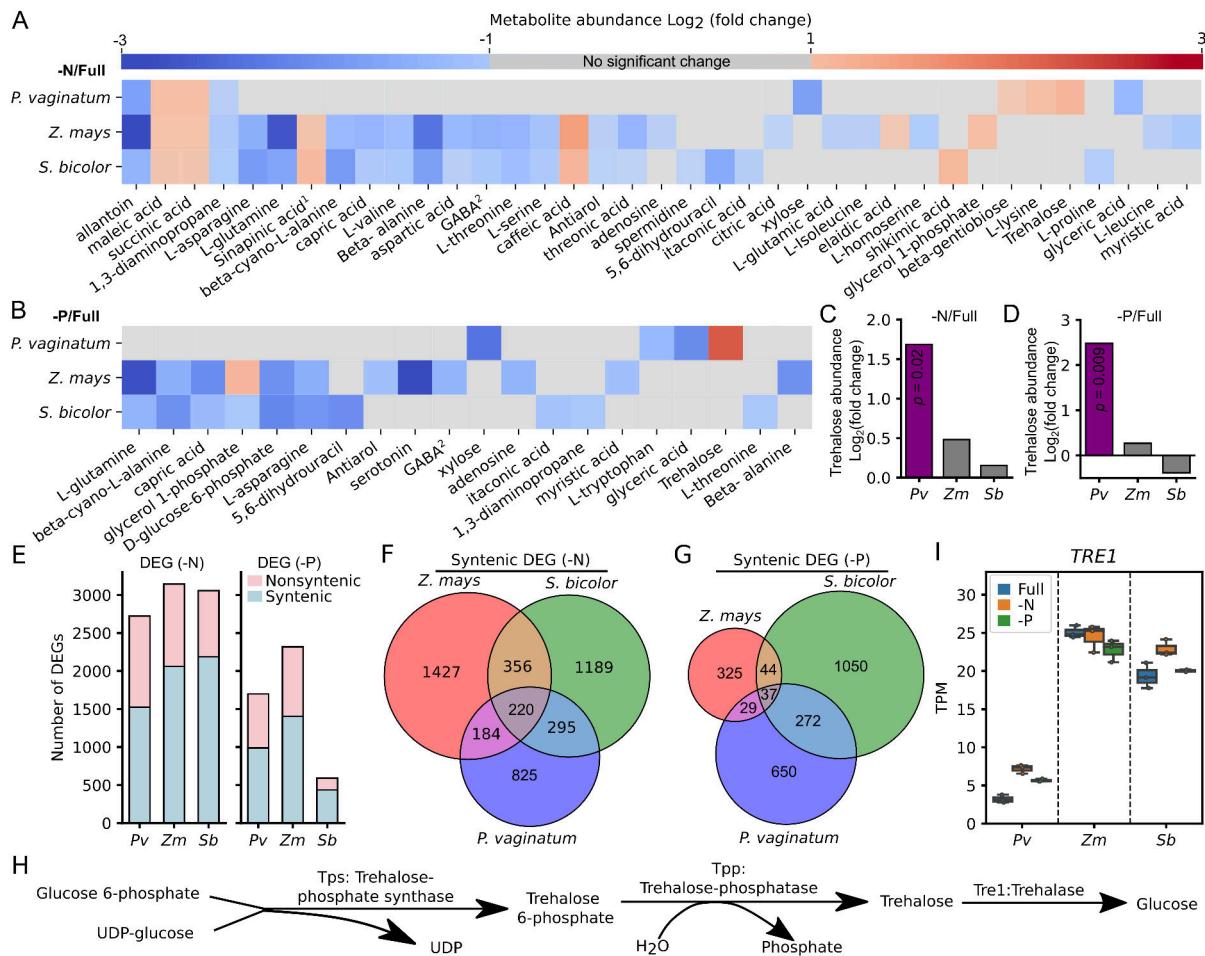


Figure 3. Primary metabolic and transcriptomic responses of maize (*Zea mays*), sorghum (*Sorghum bicolor*), and paspalum (*Paspalum vaginatum*) to nutrient-deficit stress. (A-B) Changes in the abundance of metabolites in the roots of maize, sorghum seedlings, and paspalum ramets grown under -N conditions (A) and -P condition (B) at 21 dap relative to plants grown under Full condition. Only the metabolites with a statistically significance change in abundance ($p < 0.05$; t-test) and an absolute fold change > 2 in at least one of the three species evaluated are shown. Cell marked in gray were not significantly different between conditions and/or exhibited an absolute fold change less than 2.

¹3,5-dimethoxy-4-hydroxycinnamic acid; ²Gamma-aminobutyric acid. Raw data for fold change and t-test results are shown in Supplemental Document 2. (C-D) Change in trehalose abundance in the roots of 3-week-old maize, sorghum seedlings, and paspalum ramets under -N condition (C) and -P condition (D) relative to plants grown under Full condition. Statistically significant changes are indicated in purple (t-test), and non-statistically significant changes are indicated in gray. (E) Number of significantly differentially expressed genes in paspalum (*Pv*), maize (*Zm*) and sorghum (*Sb*) identified in comparisons between roots of 3-week old plants grown under either -N or -P conditions and Full condition. Shading indicates the proportion of differentially expressed genes (DEGs) in each species that are syntenically conserved across species, or present at a unique location in the genome of the individual species evaluated. (F-G) Number of syntenically conserved orthologous triplets exhibiting shared or species-specific differential expression in response to -N and -P conditions (G) in maize (*Z. mays*), sorghum (*S. bicolor*), and paspalum (*P. vaginatum*). (H) Simplified diagram for trehalose metabolic pathway. (I) Expression levels of trehalase-encoding genes in paspalum (*Pv*), maize (*Zm*) and sorghum (*Sb*) in roots of 3-week-old plants grown in nutrient optimal (Full), N-deficient (-N) and P-deficient (-P) conditions.

172 and L-threonine (Figure 3A). This observation is consistent with the decreases in amino acid metabolism
 173 observed under N-limited conditions^{42,43}. A conserved increase in the abundance of caffeic acid was
 174 detected in both maize and sorghum in response to N-deficit conditions, which is consistent with previous

175 reports from rice grown under similar N-limited conditions⁴⁴ (Figure 3A).

176 All three species exhibited decreases in 1,3-diaminopropane and allantoin levels under N-deficit
177 conditions (Figure 3A). Allantoin acts as a pool of relocatable N that can be catabolized into ammonia
178 for N assimilation and amino acid biogenesis⁴⁵. In addition, the abundance of both succinic acid and maleic
179 acid (MaA) increased in all three species in response to N-deficit stress (Figure 3B). Maleic acid produced
180 and secreted in response to another abiotic stress (drought) in holm oak (*Quercus ilex*)⁴⁶. As we examined
181 internal metabolite abundance but did not profile root exudates in the current study, it is not possible to
182 determine whether the internal accumulation of maleic acid resulted in additional secretion in these three
183 grass species. Succinate, the anion of succinic acid, forms part of the tricarboxylic acid (TCA) cycle. The
184 increase in succinic acid levels, combined with the decreased abundance of gamma-aminobutyric acid
185 (GABA), is consistent with these species employing the GABA shunt pathway, which was proposed to act
186 as an additional energy source to support cellular metabolism under stress conditions⁴⁷⁻⁴⁹.

187 We observed changes in metabolite abundance in maize and sorghum grown under P-deficient condi-
188 tions relative to the nutrient optimal conditions, including L-asparagine, GABA, L-glutamine, L-alanine,
189 capric acid, D-glucose-6-phosphate and glycerol-1-phosphate. However, none of these metabolites ex-
190 hibited significant changes in abundance in paspalum plants grown under P-deficient and vs. nutrient
191 optimal conditions (Figure 3 B). The abundance of D-glucose-6-phosphate (D-G6P), the primary entry
192 molecule for glycolysis was significantly lower in maize and sorghum plants grown under P-deficient
193 conditions vs. those grown under nutrient optimal conditions (Figure 3A). The reduction in D-G6P
194 levels might reflect the lack of free phosphate available to produce adenosine triphosphate (ATP) to drive
195 the phosphorylation of glucose, as P is a major component of ATP. The abundance of D-G6P did not
196 decrease in paspalum plants grown under P-deficient conditions (Figure 3 B). None of the metabolites
197 that exhibited significant changes in abundance in paspalum between nutrient optimal and P-deficient
198 conditions, including tryptophan, xylose, glyceric acid, and trehalose exhibited changes in abundance
199 in maize or sorghum (Figure 3 A-D). The abundance of trehalose, a di-saccharide that predominantly
200 functions as a signaling molecule in plants in response to abiotic stresses, was significantly higher in
201 paspalum plants grown under N-deficient or P-deficient conditions vs. the nutrient optimal conditions, but
202 this difference was not observed in maize or sorghum (Figure 3 C & D).

203 **Conserved and differential transcriptomic responses of paspalum to nutrient-deficit con-** 204 **ditions**

205 The sequencing, assembly, and annotation of the paspalum genome provided the opportunity to quantify
206 differences and commonalities in how maize, sorghum, and paspalum transcriptionally respond to nutrient-
207 deficit stress. We collected RNA from the root tissues of three biological replicates of each species and
208 used it to generate an average of approximately 40 million high-quality reads per sample. Principal
209 component analysis based on the transcriptomes of each sample showed a clear separation based on growth
210 conditions in maize (Figure S4 A), sorghum (Figure S4 B) and paspalum (Figure S4 C). We identified
211 3,057, 3,144 and 2,723 genes with significantly differential expression levels between nutrient optimal
212 and N-deficit stress conditions in maize, paspalum, and sorghum, respectively. In addition, 591, 2,318
213 and 1,698 genes showed significantly differential expression levels between nutrient optimal and P-deficit
214 stress conditions in maize, paspalum, and sorghum, respectively (Figure 3 E).

215 Most differentially expressed genes (DEGs) identified for each treatment in each species were them-
216 selves syntenically conserved (Figure 3 E). Members of a number of paspalum specific expanded gene
217 families showed significant transcriptional responses to N-deficit stress (Figure S5A) and/or P-deficit
218 stress (Figure S5B). However, consistent with a previous study of transcriptional responses to abiotic
219 stress⁵⁰, the conservation of transcriptional responses was much less common than the conservation of

220 the genes themselves; syntenic genes that showed significant fold changes varied across the three species
221 under N-deficient and P-deficient conditions (Figure 3 F & G).

222 The set of 220 syntenically conserved orthologous gene groups that responded transcriptionally to
223 N-deficit stress in a consistent fashion among maize, sorghum, and paspalum was disproportionately
224 enriched in GO terms related to response to nutrient levels, nitrate assimilation, metal ion transporter
225 activities and divalent inorganic cation transmembrane transporter activity (Figure 3F; Figure S6A). The
226 set of 37 syntenically conserved orthologous gene groups that responded transcriptionally to P-deficit
227 stress in a consistent fashion among the three grasses was disproportionately enriched in GO terms related
228 to lipid metabolic process, phosphate ion transport, response to nutrient levels and cell communication
229 (Figure 3G; Figure S6A). Syntenically conserved orthologous gene groups where a transcriptional response
230 to N-deficit stress was unique to paspalum where enriched in genes involved in proton transport, glycoside
231 biosynthetic process and serine family amino acid metabolic process (Figure 3 F; Figure S6 B)). By
232 contrast, the syntenically conserved orthologous gene groups that were uniquely differentially expressed in
233 paspalum in response to P-deficit stress were involved in processes related to antioxidation, gene regulation
234 and primary metabolism (Figure 3 G; Figure S6 B).

235 The significant accumulation of trehalose in paspalum in response to nitrogen deficient conditions
236 motivated us to examine the expression of genes involved in the trehalose metabolic pathway including
237 the genes encoding enzymes that catalyze three steps in trehalose metabolism: trehalose-6-phosphate
238 synthase, trehalose-6-phosphate phosphatase, and trehalase (Figure 3 H). Two maize genes encoding
239 trehalose-6-phosphate synthase 1 and 12 are syntenic homeologs resulting from the maize whole-genome
240 duplication and are co-orthologous to single gene copies in sorghum and paspalum. These genes formed
241 a clade sister to the well characterized *Arabidopsis thaliana* gene *AtTPSI*⁵¹ which is consistent with
242 the previous study that characterized *ZmTPSI*⁵² (Figure S7 A; Supplementary Note 6). Copies of this
243 gene in both sorghum and paspalum showed a significant increase in mRNA abundance in response to
244 N-deficient treatment, as did the maize gene encoding trehalose-6-phosphate synthase 1 (*ZmTPSI*), which
245 possesses all catalytic domains of TPS (Figure S7 B). Plots of the detectable transcriptional responses of
246 other trehalose-6-phosphate synthase encoding homologs are shown in Figure S7 C - I. Genes annotated
247 as encoding trehalose-6-phosphate phosphatase 6 (*ZmTPP6*) and trehalose-6-phosphate phosphatase 11
248 (*ZmTPP11*) were phylogenetically clustered with *Arabidopsis AtTPPA* (Figure S8 A-C; Supplementary
249 Note 7), and both tended to be differentially expressed between control and stress conditions in all three
250 species. Similar transcriptional responses of homologs encoding other trehalose-6-phosphate phosphatases
251 were observed across all three species (Figure S8 D-I). Trehalase, an enzyme that breaks trehalose down
252 into two molecules of glucose, is encoded by a single gene copy in maize, with conserved syntenic
253 orthologs in sorghum and paspalum. Lower levels of mRNA abundance were associated with the trehalase
254 encoding gene in paspalum than its syntenic orthologs in sorghum or maize (Figure 3 I).

255 **Inhibiting trehalase activity in maize and sorghum recapitulates the paspalum phenotype**

256 As shown above, paspalum exhibited a significant accumulation of trehalose in response to the two types
257 of nutrient-deficit stresses while maize and sorghum did not (Figure 3 C & D). However, as equivalent
258 P-deficient treatments introduced notably smaller changes in P abundance in paspalum relative to maize
259 and sorghum, we elected to focus exclusively on N-deficit stress in all subsequent experiments.

260 In an attempt to phenocopy the reduced plasticity in response to N-deficient treatment originally ob-
261 served in paspalum, we treated maize and sorghum plants with validamycin A (β -d-glucopyranosilvalidoxylamine,
262 ValA) – a specific inhibitor of trehalase activity^{53–55}. Visibly better growth under both optimal and nitro-
263 gen deficient conditions was observed in maize (Figure 4A) and a slightly better growth under nitrogen
264 deficient condition was observe in sorghum but no obvious changes in growth under both conditions was

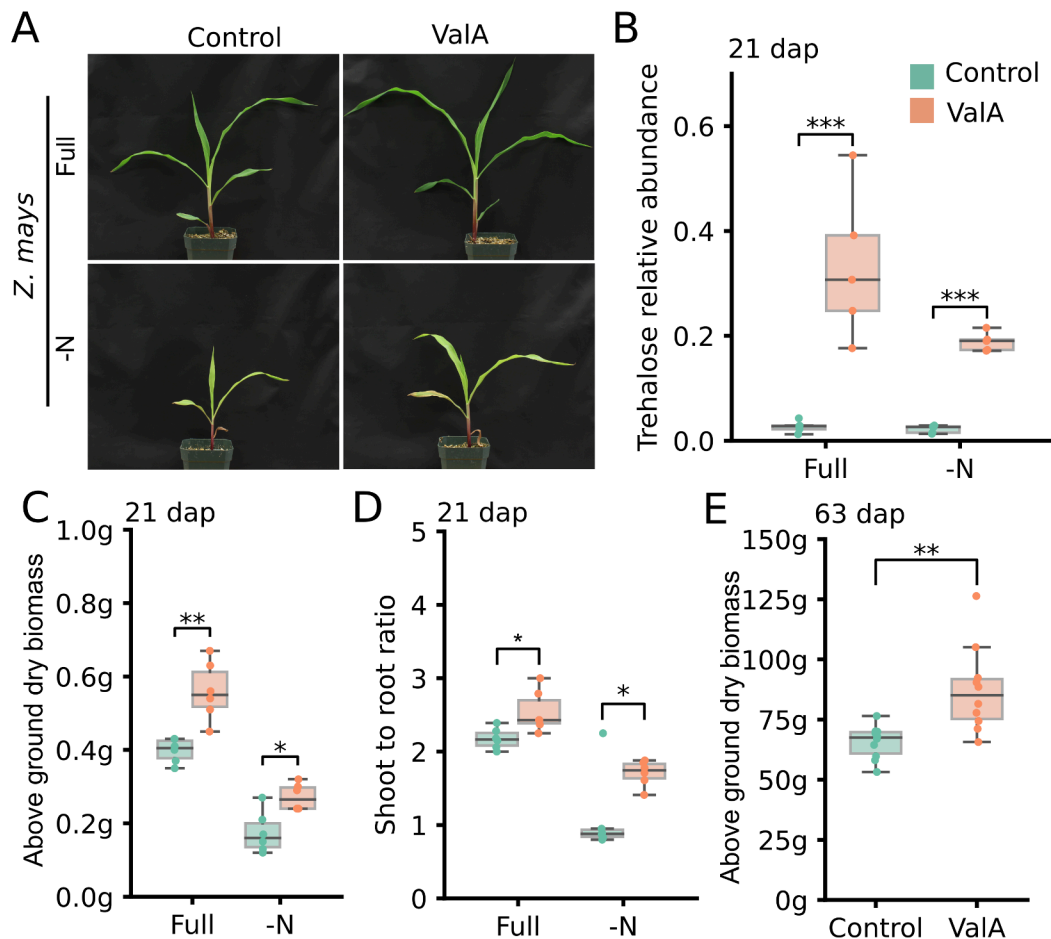


Figure 4. Validamycin A treatment is associated with increased trehalose accumulation and greater biomass production in maize.

(A) Representative images showing maize seedlings at 21 dap grown under Full and -N conditions with (ValA) or without (Control) validamycin A treatment. (B) Changes in observed trehalose abundance – normalized relative to an internal reference (ribitol) – in response to validamycin A (ValA) under Full and -N in maize root tissue from seedlings at 21 dap. (C) Changes in the above ground dry weight of maize seedlings at 21 dap in response to validamycin A (ValA) treatment under Full or -N conditions. (D) Ratio of shoot-to-root dry biomass in 3-week-old maize seedlings grown under Full and -N conditions with (ValA) or without (Control) validamycin A treatment. (E) Statistically significant increases in biomass accumulation observed in late vegetative stage (63 dap) validamycin A (ValA) treated maize relative to control (untreated) plants under Full condition. (** = $p < 0.005$; *** = $p < 0.0005$; t-test)

265 observed in paspalum (Figure S9A & E). Metabolic profiling of treated and untreated plants confirmed
 266 that a treatment with 30 μM ValA significantly increased the accumulation of trehalose under both optimal
 267 and N-deficient nutrient conditions in maize and sorghum (Figure 4B; Figure S9B) but failed to increase
 268 trehalose accumulation in paspalum (Figure S9F). ValA treatment produced significant increases in dry
 269 biomass accumulation in maize under both control and N-deficient treatment (Figure 4 C) and in sorghum
 270 only under N-deficient treatment (Figure S9 C). ValA treatment did not significantly alter biomass accu-
 271 mulation in paspalum under either treatment condition (Figure S9 G). Nutrient-deficit stress is known
 272 to alter shoot-to-root biomass ratios, increasing root biomass as a percentage of the total biomass^{56,57}.
 273 Root biomass made up a smaller proportion of the total biomass for both maize and sorghum seedlings
 274 treated with ValA than untreated seedlings under both full-nutrient and N-deficient conditions (Figure 4 D;
 275 Figure S9 D). However, no significant changes in shoot-to-root ratio were observed in paspalum upon

276 ValA treatment irrespective of nutrient conditions(Figure S9 H).

277 To extend our observations beyond the late seedling stage, we grew a cohort of maize plants for for
278 63 days (until the late vegetative stage) under either control or ValA treated conditions. ValA treated
279 plants accumulated significantly more biomass than control plants grown as part of the same experiment
280 (control mean = 65.6 grams/plant, ValA mean = 87.3 grams/plant; $p = 0.002$; t-test) (Figure 4 E). In
281 a preliminary experiment, a smaller number of maize plants were grown under either control or ValA
282 treated conditions to reproductive stage (Figure S10 A & B). ValA treated plants flowered earlier (Figure
283 S10 C) and produced larger tassels (Figure S10 B & D) and leaves than their untreated siblings (Figure
284 S10 E). In previous studies, genetically modifying trehalose metabolic pathway altered photosynthesis
285 and nutrient partitioning in maize reproductive tissues, thereby affecting yields, via its effect on SnRK1
286 activity^{58,59}. However, in the current smaller experiment, ValA induced differences in above ground
287 biomass accumulation, including both tassels and ear shoots as well as vegetative tissues at the reproductive
288 stage, were not statistically significant (Figure S10 F). Perhaps this was due to an earlier transition to
289 reproductive development in ValA treated plants, or perhaps because of low statistical power, we failed
290 to detect differences between such small numbers of plants. A set of 27 genes associated with trehalose
291 metabolism exhibited significantly more rapid rates of protein sequence evolution in paspalum than did
292 the orthologs of these same genes in foxtail millet (*S. italica*, $p = 0.002$), sorghum (*S. bicolor*, $p = 0.014$)
293 and *Oropetium* (*O. thomaeum*, $p = 0.025$) (Figure 4 E). These data are consistent with, but not conclusive
294 evidence for, a role for trehalose metabolism in the reduced phenotypic plasticity paspalum exhibits in
295 response to a range of abiotic stresses such as salinity⁶⁰.

296 **ValA treatment is associated with increased autophagy in maize**

297 A number of potential mechanisms could explain the association between the ValA associated increases
298 in trehalose accumulation and increased growth under nutrient deficient conditions. Relative to other
299 disaccharides, trehalose accumulates to only low levels and is thought to act as a signal rather than a
300 carbon source^{66,67}. The precursor to trehalose, trehalose-6-phosphate, has been shown to regulate cell
301 growth by inhibiting SNRK1 activity⁶⁸⁻⁷¹. Hence, one potential model to explain the observed result is
302 that treatment with ValA, which inhibits trehalase activity and increases trehalose accumulation⁵³, might
303 also increase the abundance of trehalose-6-phosphate, one step earlier in the pathway^{69,72}. Consistent with
304 this model, the maize ortholog of the Arabidopsis gene encoding trehalose-6-phosphate phosphatase A
305 was significantly downregulated in ValA treated plants relative to control samples under both full-nutrient
306 and N-deficient treatment conditions (Figure 5 A; Figure S8 A; Supplementary Note 6). The trehalose-
307 6-phosphate synthase encoding maize gene *ZmTPSI*⁵² also exhibited significant declines in expression
308 in response to ValA treatment under both full-nutrient and N-deficient treatment conditions (Figure 5 B;
309 Supplementary Note 7). Expression change of the genes encoding other redundant TPSs and TPPs did not
310 show specific patterns (Figure S11 A). Furthermore, the maize gene encoding the SNRK1 alpha subunit A
311 (*Zm00001d038745*)⁶⁴ was upregulated in response to ValA treatment under both Full and -N conditions
312 (Figure 5 C). In addition, the known SNRK1 induced gene in maize *ZmAKIN11* (*Zm00001d028733*)
313 (Figure S11 B) was significantly up-regulated, the known SNRK1 repressed genes in maize *ZmMDH3*
314 (*Zm00001d044042*) (Figure S11 C), *ZmMDH6* (*Zm00001d031899*) (Figure S11 D) and (*ZmBZIP11*)
315 (Figure S11 E)^{73,74} were significantly down-regulated. While abundance of trehalose-6-phosphate was
316 not directly assayed, these transcriptional changes observed in current study were consistent with an
317 increased SNRK1 activity in ValA treated plants and, hence, inconsistent with increases in the abundance
318 of trehalose-6-phosphate which inhibits SNRK1 activity^{68,69}. The expression of a number of ammonium
319 and nitrate transporters in root tissues from maize seedlings treated with ValA were upregulated relative to
320 untreated seedlings whether grown under Full and -N treatment conditions (Figure 5D).

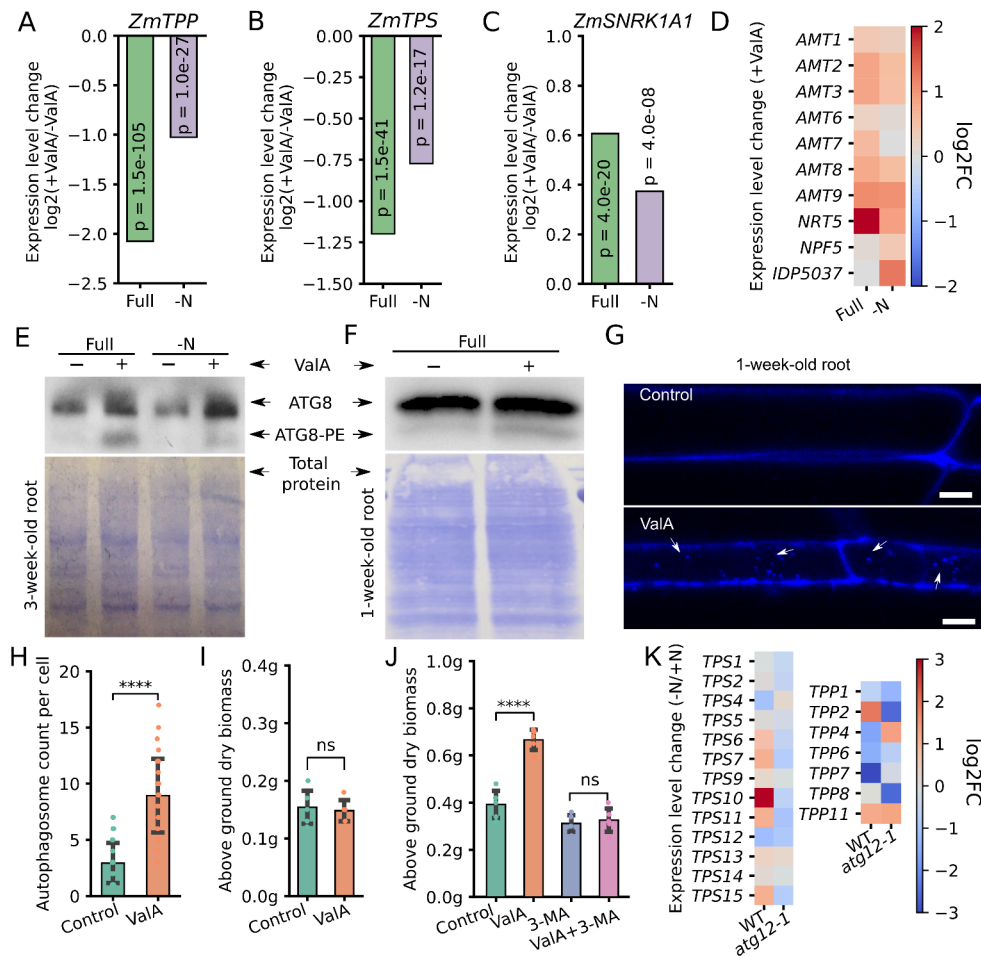


Figure 5. Evidence for increased autophagy in maize seedlings treated with validamycin A (A-B) Decrease in the expression of the trehalose-6-phosphate synthase encoding gene *ZmTPS1* (A)⁵² and the trehalose-6-phosphate phosphatase encoding maize gene *ZmTRPP6*^{58,61,62} (B) in root tissues from ValA treated maize seedlings relative to control seedlings under both Full and -N conditions at 21 dap. (C) Increase in the expression of the SNRK1 alpha subunit encoding maize gene *SNRK1A1*^{63,64} in roots from three week old ValA treated maize seedlings relative to control seedlings under both full nutrient and N-deficient treatment conditions. (D) Uniform upregulation of genes encoding ammonium and nitrate transporters. (E-F) Immunoblot measuring the abundance of both free ATG8 (upper band) and the ATG8-PE conjugate (lower band) in root samples collected from 3-week-old maize seedlings grown under Full and -N conditions with or without ValA treatment and 1-week-old maize seedlings grown under Full conditions with or without ValA treatment. Total protein loading control is shown in the lower panel. (G-H) Microscopy images (G) and counts (H) of autophagosomes stained by 40 μM MDC (monodansylcadaverine) in root tips of 1-week-old maize seedlings grown under Full condition with or without ValA treatment (**** = $p < 0.00005$, t-test). (I) Above ground dry biomass accumulated in 1-week-old maize seedling grown under Full condition with or without ValA treatment (ns = $p > 0.05$, t-test) (J) Accumulation of above ground dry biomass for 3-week-old control seedlings, seedlings treated with 3 mM 3-MA (3-methyladenine), 30 μM ValA or both 3 mM 3-MA and 30 μM ValA. (* = $p < 0.05$; **** = $p < 0.00005$; ns = No significance; t-test) (K) Detectable changes in the expression levels of annotated maize genes encoding trehalose-6-phosphate synthase (TPS) or trehalose-6-phosphate phosphatase (TPP) in response to nitrogen deficient treatment in both the wild type and *atg12-1* mutant backgrounds⁶⁵

321 SNRK1 is an upstream promoter of autophagy^{61,63,75-77} and more rapid turnover of damaged or
 322 unneeded cellular components and proteins allows for more growth with a fixed quantity of N supply
 323 (Figure (S11F)). During autophagy, the protein ATG8 becomes conjugated to phosphatidylethanolamine
 324 (PE). Increases in the abundance of both free ATG8 and ATG8-PE are associated with autophagy acti-

325 vation^{78–80}. In maize, N-deficit stress did not produce any obvious change in the accumulation of either
326 free ATG8 or ATG8-PE; however, under both control and N-deficit stress conditions, two independently
327 replicated plants treated with ValA accumulated more free ATG8 and more ATG8-PE (Figure 5E & Figure
328 S11G). Similar results were observed in two independently replicated one week-old-seedlings (Figure
329 5F & Figure S11H). Consistent to an increased autophagy activity, the abundance of autophagosomes
330 stained by Monodansylcadaverine (MDC) in root tip cells from ValA treated plants was approximately
331 twice as high as the untreated plants (Figure 5G & H). At this stage of growth, no differences in growth
332 could be observed (Figure 5I) as the seedlings were still utilizing the nutrients stored in the cotyledon and
333 therefore the increase in autophagy under nutrient optimal conditions did not result from possible nutrient
334 deficiency stress caused by higher nutrient consumption in faster growing ValA-treated plants, instead,
335 was due to ValA treatment.

336 We treated seedlings grown under optimal nutrient conditions with 3-methyladenine (3-MA, a phos-
337 phatidylinositol 3-kinase (PI3K) inhibitor) that prevents autophagosome formation^{80,81}, ValA, or both.
338 Treatment with 3-MA alone slightly reduced seedling growth. As previously observed, treatment with
339 ValA alone produced significant increases in biomass accumulation. However, in the presence of 3-MA,
340 no significant change in biomass accumulation was observed in response to ValA treatment (Figure 5
341 J). In an *atg12-1* mutant background, maize genes encoding TPS were predominately down-regulated
342 in response to N-deficient treatment, while in wild-type controls, several of the same genes exhibited
343 increased expression in response to N-deficient treatment (Figure 5 K)⁶⁵. In data taken from the same
344 experiment, maize genes encoding TPP showed varying responses to both N-deficit stress and genetic
345 background (Figure 5 K)⁶⁵.

346 Discussion

347 In paspalum, a crop wild relative that is resilient to numerous abiotic stresses, nutrient-deficit stress was
348 associated with substantial accumulation of trehalose. The sequencing of a reference genome for this
349 species allowed us to perform comparative evolutionary analyses, which identified accelerated protein
350 sequence evolution of genes involved in trehalose metabolism in paspalum (Figure 4 J). Treating maize
351 and sorghum exposed to N-deficit stress with a specific inhibitor of trehalase resulted in higher internal
352 trehalose accumulation and recapitulated a number of paspalum phenotypes including reduced decreases
353 in biomass accumulation in response to N-deficit stress, and increased allocation of biomass to shoots
354 under N-deficit stress (Figure 4; Figure S9).

355 Imposing equivalent stress treatment protocols across species presents numerous challenges. One
356 potential concern with the initial finding that paspalum is less phenotypically plastic in response to nutrient-
357 deficient treatment than maize is that the slower baseline accumulation of biomass in paspalum may
358 deplete the modest reserves of nitrate and phosphate in soil more slowly than they would be used by maize.
359 Here comparison of paspalum to sorghum may be more informative than comparison of paspalum to maize.
360 Under nutrient replete conditions, individual paspalum ramets accumulated approximately equivalent
361 amounts of biomass to sorghum seedlings, while sorghum exhibited greater phenotypic plasticity in
362 response to nutrient deficit stress. In addition, several lines of evidence indicate that the three species
363 experienced nutrient deficit stress in response to the nutrient deficient treatment protocols employed in
364 this study: the depletion of the nitrate storage compound allantoin under the N-deficit conditions as well
365 as other metabolic changes (Figure 3 A & B); transcriptional evidence of increased starch biosynthesis in
366 paspalum shoots (Figure 2 H); and significant declines in the abundance of N and P in the above-ground
367 tissue all three species when grown under N- and P-deficient conditions (Figure 2 E & G).

368 In many flowering plant species, the abundance of trehalose is quite low⁸². In *Arabidopsis thaliana*,

369 trehalose accumulation was observed under high and low temperature stress^{83,84}, high light intensity⁸⁵,
370 high cadmium levels⁸⁶ and dehydration⁸⁷, but no increase was observed under N-deficient conditions^{82,88}.
371 A non-targeted metabolic profiling of six legume plants in the *Lotus* genus under drought conditions
372 revealed a significant increase in trehalose abundance across all species tested⁸⁹. In maize, external
373 trehalose treatment enhanced antioxidant activities under high salinity and P-deficient conditions, thus
374 achieving better seedling growth⁹⁰. Genetically modified rice plants that over-expressed a fusion gene
375 encoding both *Escherichia coli* Trehalose-6-phosphate synthase and Trehalose-6-phosphate phosphatase,
376 which are responsible for trehalose biosynthesis, exhibited 200-fold greater accumulation of trehalose
377 and significantly higher tolerance to drought, salinity, and cold stress⁹¹. Associations between trehalose
378 and nutrient-deficit stress appear to have been largely uninvestigated, although one study found that the
379 exogenous application of trehalose to *Nicotiana benthamiana* leaves partially rescued the N-deficiency
380 phenotypes under N-limited conditions⁹². The transgenic expression of trehalose-6-phosphate phosphatase
381 in developing maize ears was associated with increased yield under control and drought stressed condi-
382 tions^{59,71}. By contrast, the increased accumulation of trehalose in response to nutrient-deficit stresses in
383 wild-type plants is, to our knowledge, specific to paspalum. Given the environment that paspalum has
384 adapted to during evolution, paspalum has an extraordinary ability to tolerate high-salinity stress^{19,29,93}.
385 Trehalose accumulation in paspalum might also act to ameliorate osmotic stress caused by a larger amount
386 of salt uptake from the soil driven by a higher transpiration when nutrient deficient plants seek to increase
387 nutrient uptake.

388 Trehalose has been recognized as an autophagy activator in animals⁹⁴⁻⁹⁶ and plants⁷⁶. Autophagy
389 plays pivotal roles in proteome remodeling, lipid turnover^{65,76}, nitrogen remobilization⁹⁷⁻¹⁰⁰, nitrogen
390 use efficiency¹⁰¹, and abiotic stress responses in a variety of plant species (as reviewed previously¹⁰²).
391 In the resurrection plant *Tripogon loliiformis*, trehalose abundance correlated with an increase in ATG8
392 lipidation and the number of autophagosomes⁷⁹. Here, we pharmaceutically inhibited trehalase activity
393 with ValA to increase trehalose abundance in maize and observed increases in both ATG8 protein
394 abundance and lipidation in both 3-week-old and 1-week-old maize seedlings (Figure 5 E & F). The effect
395 of ValA treatment on biomass accumulation in maize was autophagy dependent as inhibiting autophagy
396 via treatment with 3-MA restored the wild-type phenotype of ValA treated plants (Figure 5 H). In addition,
397 we observed upregulation of both ammonium and nitrate transporter expression in the seedlings treated
398 with validamycin A under both Full and -N conditions (Figure 5 D) suggesting that validamycin A treated
399 seedlings may performed better not only as a result of nitrogen recycling and remobilization, but could
400 also exhibit increased nitrogen uptake due to the upregulated transporter activities in roots. However, the
401 reversion of validamycin A treated seedlings to wild type levels of biomass accumulation with treated
402 with an autophagy inhibitor suggests that the role of increased nitrogen uptake, if any, is likely also
403 autophagy dependent. The triggering of trehalose accumulation in response to nutrient deficit is specific to
404 paspalum (Figure 3 A-D). However, caution should be taken in interpreting these results as multiple genes
405 encoding enzymes in the trehalose biosynthetic pathway were also reported to be associated with changes
406 in autophagy activity in different systems^{68,103}. Trehalase activity was initially observed in tissue cultures
407 generated from a range of plant species more than three decades ago¹⁰⁴. Inhibiting trehalase activity with
408 ValA can control wheat Fusarium head blight (FHB) and inhibit Deoxynivalenol (DON) contamination¹⁰⁵.
409 Over-expression of *OsTRE1* in rice was associated with improved salt tolerance¹⁰⁶ and over-expression
410 of *AtTRE1* in Arabidopsis was associated with improved drought tolerance¹⁰⁷. However, these known
411 phenotypic consequences of alterations in trehalase activities would not necessarily predict an association
412 between inhibition of trehalase activity and decreased plasticity in response to nutrient deficiency stress,
413 as was observed here.

414 The maize experiments described in this paper would not have been conducted in the absence of the

415 observation that paspalum accumulates trehalose in response to nutrient deficient treatments. However,
416 at the same time it must be noted that the work linking trehalose accumulation to increased biomass
417 accumulation via an autophagy dependent mechanism was conducted entirely in maize. Hence, while
418 the maize data certainly suggests that an increase in autophagy induced by the increased accumulation
419 of trehalose in paspalum observed under nutrient deficient conditions is also responsible for the low
420 degree of phenotypic plasticity paspalum exhibits in response to nutrient deficit stress, strong tests of this
421 model would require experimentation which is not yet practical, such as the transgenic overexpression of
422 trehalase in paspalum. In any case, these results suggest that the manipulation of trehalose accumulation
423 in maize and sorghum, as well as potentially on other domesticated grasses, whether chemically or via the
424 modification of the expression of the endogenous trehalase enzyme, may increase agricultural productivity
425 per unit of nitrate and phosphate fertilizer applied. Finally, the observation of autophagy dependent
426 increases in biomass accumulation in even maize plants grown under nutrient-replete conditions suggests
427 that current maize lines may exhibit a suboptimal level of autophagy in roots. However, again, caution
428 should be taken in interpreting these results as, while increases in biomass accumulation were observed
429 not only in seedlings but in late stage vegetative plants (Figure 4E), all data presented in this study
430 were generated in controlled environmental conditions and changes in regulation or metabolism that are
431 beneficial in the greenhouse may or may not generalize to the field.

432 **Materials and methods**

433 **Determination of DNA content via flow cytometry and genome size estimation**

434 One leaf per plant of paspalum (PI 509022) and sorghum (BTx623) were harvested and kept on ice until
435 processing. A CyStain Propidium Iodide Absolute P kit (Sysmex, Milton Keynes, United Kingdom)
436 was used to extract and stain the nuclei from a 1 cm² piece of leaf tissue following the manufacturer's
437 instructions. To reduce the amount of cellular debris in the extracts, samples were passed through a 30 µm
438 filter (CellTrics®-Sysmex Partec, Goerlitz, Germany) and centrifuged at 600×g before final staining.
439 Sorghum was used as an internal standard to reduce the staining variability between samples. The stained
440 samples were then analyzed on a CytoFLEX flow cytometer (Beckman Coulter, Brea, CA, USA) following
441 a two-hour incubation at 4 °C. The propidium iodide was excited with a yellow-green 561 nm laser and
442 detected with a 585/42 emission filter. The genome size was calculated for a total of six samples. The
443 2C genome size for BTx623 is 1.67 pg DNA¹⁰⁸; therefore the formula to calculate the DNA content of
444 paspalum was (median fluorescence_{sample nuclei} / median fluorescence_{standard nuclei}) X 1.67 pg. The mean
445 and standard error of these six samples were calculated, and the mean was converted to a 1C genome size
446 using the conversion factor 1 pg = 980 Mbp.

447 **Paspalum genome assembly and annotation**

448 The paspalum genome assembly was generated using an error-corrected 74.3x coverage of PacBio reads
449 with an average read length of 9,523 bp. The reads were assembled using MECAT¹⁰⁹ and polished using
450 QUIVER¹¹⁰. Comparisons with the genome of *Panicum hallii* var. HAL2 (v2) and two paspalum F₂
451 genetic maps (see Supplemental Methods for map generation) were used to identify and split 15 misjoins
452 in the initial assembly. The resulting scaffolds were ordered and orientated using the two paspalum
453 genetic maps. A total of 357 scaffolds were assembled into 10 pseudomolecules representing 75%
454 of the overall assembled genome. A set of six F₁ maps (total of 8,861 markers)³⁰ were used to refine the
455 order/orientation of the contigs. The final numbering and orientation were verified using *S. bicolor* cDNAs
456 obtained from Phytozome (<https://phytozome-next.jgi.doe.gov/>). Heterozygous SNP/InDel phasing errors
457 were corrected using both 74.3X raw PacBio data and 78X Illumina data (San Diego, CA, USA) (2x150 bp

458 reads, 400 bp target insert size). A detailed genome assembly methods and assembly integrity assessment
459 is provided in Supplementary Note 1.

460 The v3.0 paspalum genome assembly was annotated using a combination of an alignment of assembled
461 transcripts from paspalum and protein sequences from other plant species. Prior to its annotation, the
462 genome assembly was first repeat-masked using both known repeats from RepBase and *de novo* identified
463 repetitive sequences from RepeatModeler^{111,112}. Transcript assemblies were generated via a two-stage
464 assembly process utilizing PERTRAN followed by PASA¹¹³. A total of 112,258 RNAseq transcript
465 assemblies were generated from approximately 1.6 billion 2x150 bp strand-specific Illumina sequencing
466 reads. Protein sequences from Arabidopsis, soybean, sorghum, Kitaake rice (*Oryza sativa Kitaake*),
467 green foxtail (*Setaria viridis*), grape (*Vitis vinifera*), and the Swiss-Prot proteomes were aligned to the
468 repeat-masked genome using EXONERATE¹¹⁴. Independent sets of gene models were predicted using
469 FGENESH+, FGENESH_EST, EXONERATE and AUGUST as implemented in BRAKER1, and the
470 in-house PASA assembly open reading frames (ORFs; in-house homology constrained ORF finder) tool
471 from JGI^{114–116}. For each locus, the prediction with the best score based on the expressed sequence
472 tag (EST) and protein support and a lack of overlap with repeats was selected. The best prediction for
473 each locus was further improved using PASA to add untranslated regions, correct splice sites, and add
474 alternative transcripts. Improved transcripts were assessed based on both the C-score (ratio of the BLASTP
475 alignment score to the mutual best hit BLASTP alignment score) and protein coverage. Transcripts were
476 retained if any one of three criteria were met: 1) Transcripts where the C-score and protein coverage score
477 were each ≥ 0.5 and less than 20% of the transcript overlapped with sequence annotated as repetitive.
478 2) Transcripts supported by EST coverage and less than 20% of the transcript overlapped with sequence
479 annotated as repetitive. 3) Transcripts with a Cscore ≥ 0.9 and a protein coverage score ≥ 0.7 , regardless
480 of the proportion of overlap with annotated repeat sequences. Sequences that satisfied one or more of
481 the above three criteria and where more than 30% of predicted protein sequence was covered by Pfam
482 domains annotated as belonging to transposable elements were also removed. Short single exon (predicted
483 coding sequence <300 bp) genes without protein domain support and expression data, incomplete gene
484 models and those with low homology support (sum of Cscore and coverage <1.5 for complete, <1.8 for
485 incomplete) and without full transcriptome support (CDS and intron coverage supported by any transcript
486 assemblies) were removed. Gene models that passed all the criteria described above were included in the
487 gene model annotations for paspalum. The GO terms assignment was based on the InterProScan results¹¹⁷.

488 **Plant materials and growth conditions**

489 The maize (*Zea mays* ssp. *mays*), sorghum (*Sorghum bicolor*), and seashore paspalum (*Paspalum*
490 *vaginatum*) genotypes used to create the reference genomes for each species were: accessions B73,
491 BTx623, and PI 509022, respectively^{118,119}. Maize and sorghum seeds were surface sterilized in 2%
492 bleach for 40 minutes, rinsed, and imbibed overnight in deionized distilled water (ddiH₂O). The seeds
493 were sown in a mixture of 20% MitroMix200, 30% sterilized sand and 50% fine vermiculite(v/v) and
494 grown under greenhouse conditions (temperature: 22-29°C with a 14-h light: 10-h dark photoperiod). The
495 heterozygous reference clone PI 509022 was obtained from the USDA National Plant Germplasm Service
496 and propagated via rhizome cuttings using the same growth medium and conditions used for sorghum and
497 maize. All plants were watered with sterilized ddiH₂O until three days after emergence (usually 4-5 days
498 after planting). For each trial, three days after emergence, the seedlings were divided evenly into three
499 trial groups. The first group received Hoagland nutrient solution (Supplementary Note 8) and ddiH₂O on
500 alternating days. The second group received Hoagland nutrient solution in which the potassium nitrate
501 and calcium nitrate were substituted with potassium sulfate and calcium chloride, respectively, to remove
502 nitrate. The third group received Hoagland nutrient solution in which the monopotassium phosphate was

503 substituted with potassium sulfate to remove phosphate. The nutrient treatments continued every other day
504 until harvest. For the ValA treatment assay, plants grown under nutrient-optimal or N-deficit conditions
505 were treated with 30 μM ValA dissolved in nutrient solutions beginning at 7 days after planting; the plants
506 were treated at 6 PM every other day.

507 **Plant phenotyping and root sampling**

508 On the date of harvest and phenotyping, the plants were taken to a dark room illuminated solely by green
509 light, separated from the potting media and cleaned in a two stage process. The roots were washed in a
510 0.05% bleach solution and then were rinsed with warm running water and dried with paper towels. The
511 root samples used for RNA extraction and metabolite analyses were flash frozen in liquid nitrogen. The
512 roots were scanned using an EPSON scanner (Perfection V550, setting at 120 dpi; Epson, Suwa, Japan)
513 with a green film covering the scanning surface to avoid exposing the roots to non-green light. Fresh
514 biomass measurements were taken for the whole seedlings, after which they were divided into shoot and
515 root fractions and weighted separately. Dry weight measurements of shoots and roots were taken after 48
516 h of freeze-drying. For paspalum, the weight of the original rhizome cutting was subtracted from the final
517 whole-plant fresh biomass to estimate biomass accumulation.

518 **Species phylogeny construction**

519 A set of 7,728 single-copy syntenic orthologs from the *Zea mays*, *Sorghum bicolor*, *Setaria italica*, *Oropetium*
520 *thomaeum*, *Brachypodium distachyon* and, *Oryza sativa* genomes was extracted from the syntenic gene
521 sets identified among the seven species. Of the 7,728 orthologs with primary transcript CDSs longer than
522 500 bp, 6,151 were aligned using the codon-based aligner ParaAT¹²⁰. Subsequently, the 6,151 multiple
523 sequence alignments, each consisting of one gene each from each of the seven species were trimmed to re-
524 move poorly aligned or highly divergent regions using Gblocks(v0.91b)¹²¹ with the following parameters:
525 minimum number of sequences for a conserved position set at 5; minimum number of sequences for a
526 flank position set at 6; maximum number of contiguous nonconserved positions set at 8; and minimum
527 length of a block set at 10. The resulting nucleotide alignments were used to construct phylogenies using
528 RAxML(v8.2) with the parameters '-f a -N 1000 -m GTRGAMMA -x 1234 -p 1234' and a clade containing
529 rice and *Brachypodium* as an outgroup¹²². In 292 cases, it was not possible to form a monophyletic clade
530 containing rice and *Brachypodium*. The remaining 5,859 trees were analyzed using Densitree to generate
531 a consensus tree¹²³. IQ-TREE was used to construct maximum likelihood phylogeny estimate branch
532 lengths using a super gene concatenated from the trimmed nucleotide sequence alignments of the 5,859
533 single copy syntenic genes used for consensus tree analysis¹²⁴. Divergence time estimates were then
534 performed using these branch lengths, a previously estimated divergence date for *B. distachyon* and *O.*
535 *sativa* of 54 Myr ago¹²⁵ and an estimated divergence date for *Z. mays* and *S. bicolor* of 12 Myr ago¹²⁶ as a
536 reference with r8s software¹²⁷.

537 **Syntenic and substitution rate analysis**

538 Syntenic orthologous gene pairs were identified between the sorghum and paspalum genomes using
539 sequence similarity data from LAST¹²⁸ and a Python implementation of MCSScan, JCVI^{129,130}. This
540 analysis was run using the command 'python -m jcvicompara.catalog ortholog paspalum sorghum -
541 no_strip_names'. The LAST results were filtered using a Cscore setting of ≥ -0.7 . Raw synteny gene
542 pairs were polished using a previously described approach⁵⁰. Sorghum-paspalum orthologous gene pairs
543 were merged into a published sorghum referenced synteny list⁵⁰ for maize (B73_RefGen_V4)¹¹⁸, sorghum
544 v3.1¹¹⁹, foxtail millet v2.2³⁴, *Oropetium* v2.0, rice v7¹³¹ and *Brachypodium* v3.1¹³². The final synteny
545 list and the scripts used to generate it are hosted at <https://github.com/gsun2unl/PaspalumNutrientStress>.

546 Codon-level multiple sequence alignments of syntenic orthologous gene groups were generated with
547 ParaAT2.0¹²⁰. Synonymous nucleotide substitution rates (Ks), and non-synonymous nucleotide substi-
548 tution rates (Ka) were estimated from these multiple sequence alignments using the ‘codeml’ package
549 implemented in PAML¹³³. The estimation was conducted using the maximum-likelihood method and
550 the parameters runmode=0, Codon-Freq=2, model=1. The known phylogenetic relationships of the six
551 included species were used as a known input tree. Syntenic orthologous groups containing any genes with
552 a Ks greater than 2, a Ka greater than 0.5, and a Ka/Ks ratio greater than 2 were removed.

553 **Gas chromatography–mass spectrometry (GC-MS) metabolite profiling**

554 Root samples from maize, sorghum, and paspalum seedlings grown as described above were collected
555 in a dark room illuminated solely by a green bulb and ground into a fine powder in liquid nitrogen.
556 Approximately 50 ± 0.5 mg of the ground powder was used for metabolite extraction and derivatization as
557 described previously^{134,135}. A 1 μ L sample of the derivatized material was analyzed in splitless mode
558 using a 7200 GC-QTOF system (Agilent Technologies, Santa Clara, CA, USA). A solution of fatty acid
559 methyl esters (C8 to C30) was added to each sample during derivatization to determine the retention
560 index. The raw data were acquired using MassHunter Workstation v.08 (Agilent Technologies), while peak
561 detection, deconvolution and identification were performed using MassHunter Unknown Analysis software
562 (Agilent Technologies) using the Fiehn GC/MS Metabolomics RTL Library (Agilent Technologies) as a
563 reference. Peak areas of the identified metabolites were computed using MassHunter Quantitative Analysis
564 software (Agilent Technologies). Peak area was normalized by the precise sample fresh weight and the
565 peak area of the ribitol added to each samples as an internal standard to calculate the relative levels of
566 metabolites.

567 **Genetic map construction for genome assembly validation**

568 Nine genetic maps generated from two populations were employed to order, and orient the scaffolds into
569 pseudomolecules, and to validate the assembly. The first population employed was an F₁ population
570 of 184 individuals derived from a cross between paspalum accessions PI 509022 and HI33, previously
571 described in Qi *et al.*³⁰. The second population was generated by crossing two F₁ sibs from the PI 509022
572 x HI33 population. Only 52 progeny of this cross were validated and ultimately used for map construction.
573 Genotyping-by-sequencing (GBS), single nucleotide polymorphism (SNP) calling, and mapping of the F₁
574 population were previously described³⁰. Essentially the same protocols were used for marker development
575 and genetic mapping in the F₂ population, except that the restriction enzymes *Pst*I and *Msp*I were used
576 for GBS library preparation. SNPs in the F₁ population were called from GBS reads both independently
577 of the genome assembly and by alignment to an early draft of the paspalum genome assembly. SNPs in
578 the F₂ population were called from GBS reads aligned to seashore paspalum assembly v2.0. Because the
579 mapping software MAPMAKER¹³⁶ does not have an algorithm to deal with outcrossing species, the three
580 sets of SNPs were further split into HA sets (comprising markers heterozygous in the female parent and
581 homozygous in the male parent), AH sets (homozygous in the female parent and heterozygous in the male
582 parent) and HH sets (heterozygous in both parents), leading to a total of six F₁ datasets³⁰ and three F₂
583 datasets (Supplementary Note 2). For the F₂ population, information from the grandparents was used to
584 rescore the progeny using the rules listed in Table S2 to ensure that all markers were in the same linkage
585 phase.

586 To assist with scaffold ordering and assessment of the quality of the assembly, 500 bp on either side
587 of mapped SNP markers were excised from the assembly used for GBS read alignment and mapped to
588 consecutive improved versions of the assembly using BLASTN. The sequences and location of the mapped
589 F₁ and F₂ markers on the seashore paspalum version 3.0 assembly reported here as determined by the

590 top BLASTN hit are provided in Supplementary Note 2. Discrepancies between marker orders in any
591 two of the nine maps and the order and orientation of scaffolds in the pseudomolecules triggered manual
592 examination and in some cases error correction

593 **Gene family analysis in various crop species**

594 Protein sequences of the primary transcripts for seven species were retrieved from Phytozome: *Zea mays*,
595 *Sorghum bicolor*, *Setaria italica*, *Paspalum vaginatum*, *Oropetium thomeaum*, *Brachypodium distachyon*,
596 and *Oryza sativa*¹³⁷. These sequences were used as inputs for orthoFinder^{138,139} to generate clusters
597 of genes representing gene families. Family expansion and contraction were determined with CAFE5
598 using default settings¹⁴⁰. Significantly expanded gene families in paspalum were defined as those with
599 significantly different lambda value ($p < 0.05$) that showed increases in gene copy numbers in the lineage
600 leading to paspalum as estimated by CAFE5¹⁴⁰.

601 **RNA isolation, sequencing, and quantification**

602 Root samples were homogenized by grinding to a fine powder in liquid nitrogen. Approximately 50 mg of
603 homogenized root tissue per sample was mixed with 1 mL of TRIzol reagents by robust vortexing and,
604 incubated at room temperature (25°C) for 10 minutes. The samples were mixed with 200 μ L chloroform
605 and incubated for 15 minutes at room temperature until a clear separation of three layers was observed. The
606 tubes containing the mixtures were centrifuged at 12,000 rpm for 15 minutes to achieve phase separation.
607 The top layer was transferred to a new set of tubes containing 400 μ L isopropanol and incubated on ice for
608 at least 30 minutes. RNA precipitation was achieved by centrifugation at 12,000 rpm for 15 minutes at
609 4°C. Following the removal of the supernatants, the precipitates were washed with 75 % ethanol three
610 times before being dissolved in 40 μ L of 65°C DEPC treated water.

611 The quality of individual RNA samples was assessed using an Agilent 2100 Bioanalyzer. Samples
612 with RNA Integrity Number (RIN) values >5 were used to isolate mRNA and construct RNA sequencing
613 libraries using a TrueSeq v2 kit from Illumina¹⁴¹. Paired-end sequence data (2x75 bp) were generated
614 using an Illumina NextSeq 500 platform. The overall quality of the RNAseq reads was assessed using
615 FASTQC¹⁴² (Figure S4A). Demultiplexed reads were filtered and quality trimmed using Trimmomatic
616 (v0.33) with the parameters "-phred33 LEADING:3 TRAILING:3 slidingwindow:4:15 MINLEN:36
617 ILLUMINACLIP:TruSeq3-PE.fa:2:30:10"¹⁴³. Trimmed reads were mapped to the reference genomes of
618 their respective species using STAR/2.7¹⁴⁴ with two rounds of mapping; the first round of mapping was run
619 with the parameters "-alignIntronMin 20 -alignIntronMax 20000 -outSAMtype None -outSJfilterReads
620 Unique -outSJfilterCountUniqueMin 10 3 3 3 -outSJfilterCountTotalMin 10 3 3 3" and the second
621 round of mapping was run after a new genome index was built based on the known and novel splicing
622 sites recognized by the first round of mapping with parameters "-alignIntronMin 20 -alignIntronMax
623 20000 -limitBAMsortRAM 5000000000 -outSAMstrandField intronMotif -alignSJoverhangMin 20
624 -outSAMtype BAM SortedByCoordinate". Maize reads were mapped to B73_RefGen_V4¹¹⁸. Sorghum
625 reads were mapped to v3.1 of the BTx623 reference genome downloaded from Phytozome¹¹⁹. Paspalum
626 reads were mapped to the paspalum genome assembly described and released as a part of this paper. A
627 Transcripts Per Million (TPM) table was generated using Kallisto¹⁴⁵. Syntenic orthologous genes across
628 paspalum, maize and sorghum with a mean TPM value higher than 50 were log transformed prior to
629 principal component analysis (Figure S4B). For each individual sequencing library, the read counts were
630 determined using the software package HTSeq (version 0.9) with the parameter settings "-r pos -s no -t
631 exon -i gene_id", the overlap mode used was the default ("union")¹⁴⁶. Statistically significant DEGs were
632 identified from the read count matrix generated by HTSeq using DESeq2 (v1.22.2)¹⁴⁷ (Figure S4B). Genes
633 were considered to be significantly differentially expressed when an absolute \log_2 fold change >1 and an

634 adjusted p value lower than 0.05 were both observed. Total RNA of paspalum shoot was extracted using
635 the same method and sequenced using the same library preparation protocol and sequencing platform as
636 other samples described in this study, only genes with TPM higher than 5 and syntenically conserved were
637 examined. Statistical significance of expression level changes was calculated by DESeq2¹⁴⁷.

638 **MDC staining of samples and confocal microscopy**

639 Microscopy visualization of autophagosomes by Monodansylcadaverine (MDC) staining was performed
640 as described by Contento et al. (2005)¹⁴⁸. Root tissues from maize seedlings one week after germination
641 were gently rinsed with sterilized ddiH₂O and submerged in 40 μM MDC solution for 30 minutes in the
642 dark. Confocal microscopy imaging was performed with a Nikon A1 laser scanning confocal mounted on
643 a Nikon 90i compound microscope (software version: NIS Elements 4.13). Excitation/emission for MDC
644 detection was set to 488 nm/505–550 nm. Aperture and light source intensity were kept the same for all
645 images taken. MDC stained autophagosomes from ten different cells in root tips were counted. Three
646 biological samples were examined.

647 **Gene ontology enrichment analysis**

648 Gene ontology (GO) enrichment analysis of the DEGs was performed using GOATOOLS¹⁴⁹. To ensure
649 consistency in cross species comparisons, the same population of syntenically conserved genes in maize,
650 sorghum, and paspalum was used as the population set for enrichment analysis in each species. Similarly,
651 to avoid bias introduced by the use of different GO term annotation pipelines, the same set of GO terms
652 was assigned to each syntenic ortholog in each of the three species. These annotations were taken from
653 the GO terms assigned to the maize copy of each conserved syntenic gene group by Maize-GAMER¹⁵⁰.
654 As the whole genome duplication in maize introduced bias into the background gene set (genes retained as
655 duplicate homeologous gene pairs are enriched in the annotations transcription factor, "responds to X"
656 and protein complex subunit) only a single copy from maize1 subgenome of each maize gene pair was
657 retained for both the background population set and the DEG defined set.

658 **Immunoblot detection of free ATG8 and ATG8-PE conjugate**

659 ATG8 and ATG8-PE conjugate were detected as previously described with slight modifications¹⁵¹. Maize
660 seedlings were grown under full-nutrient or N-deficient conditions for three weeks with or without the 30
661 μM validamycin A treatment described above. Root tissues were collected in a dark room solely illuminated
662 by green light and ground to a fine powder in liquid nitrogen. The ground root tissues were homogenized
663 in lysis buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride, 10 mM
664 iodoacetamide, and 1 X complete protease inhibitor cocktail [Sigma Aldrich, St. Louis, MO, USA]) and
665 centrifuged at 2000 Xg, 4°C for 5 min. The extracted protein samples were quantified using a Bradford
666 assay, and 25 μg protein was loaded onto a 15% SDS-PAGE (polyacrylamide gel electrophoresis) gel
667 containing 6 M urea. Immunoblotting was performed with affinity-purified anti-At ATG8 antibodies
668 (1:1000 dilution)(Agrisera, Vännäs, Sweden; AS14 2769). The ATG8-PE (lipidation) band was confirmed
669 by incubating protein samples at 37°C for 1 hour with *Streptomyces chromofuscus* phospholipase D
670 (Thermo Fisher Scientific, Waltham, MA, USA; 525200-250U; 250 units mL⁻¹ final concentration) as
671 previously described⁷⁸.

672 **Data availability statement**

673 The genome sequence and annotation is accessible via Phytozome v13: <https://phytozome-next.jgi.doe.gov/info/Pvagina>
674 RNAseq data for root tissues of paspalum, maize and sorghum under three nutrient conditions are avail-
675 able at NCBI under the BioProject: PRJNA746310. RNAseq data for root tissues of maize seedlings

676 under three nutrient conditions with or without validamycin A treatment are available at NCBI under the
677 BioProject: PRJNA746310. RNAseq data for paspalum shoots/rhizome is available at NCBI with SRA
678 id SRR10230104; SRR10230108; SRR10230122; SRR10230130. RNAseq data of maize wild type and
679 atg12 mutant is available at NCBI with Accession ID: PRJNA449498. Illumina sequence of paspalum
680 genome is available at NCBI with Accession ID: PRJNA234783. All of the scripts and raw data used for
681 figures can be accessed at github: <https://github.com/gsun2unl/PaspalumGenome>

682 Acknowledgements

683 This manuscript is based upon work supported by award 2016-67013-24613 from the USDA National
684 Institute of Food and Agriculture to JCS, by National Science Foundation Award OIA-1826781 to JS
685 and JCS and OIA-1557417 to CZ, TO, BS, BY and JCS, by National Health Institute Award GM127414
686 to BY, and by USDA-SCRI award UFDSP00011194 to KMD. This work was completed utilizing the
687 Holland Computing Center of the University of Nebraska, which receives support from the Nebraska
688 Research Initiative. The work in this manuscript was enabled by the UNMC DNA Sequencing Core
689 Facility which receives partial support from the Nebraska Research Network In Functional Genomics
690 NE-INBRE P20GM103427-14, The Molecular Biology of Neurosensory Systems CoBRE P30GM110768,
691 The Fred & Pamela Buffett Cancer Center - P30CA036727, and the Nebraska Research Initiative. The
692 work conducted by the U.S. Department of Energy Joint Genome Institute is supported by the Office of
693 Science of the U.S. Department of Energy under Contract no. DE-AC02-05CH11231. We thank the Plant
694 Editors for their work and feedback on an early draft of the manuscript.

695 Author contribution

696 J. C. S., J. S., and G. S., conceived this research. J. A. S., and G. S., designed and directed the study. T. O.,
697 N. W., and L. B., profiled primary metabolites. C. C., L. S., Y. Y., C. D., K. B., and R. O directed and
698 performed paspalum genome sequencing. J. S., C.P., and J. J., directed and performed paspalum genome
699 assembly. K. D., P. Q., and T. G., constructed genetic maps used for genome assembly. S. S., performed
700 the annotation of the genome assembly. B. Y., and B. Z., designed and conducted the quantification
701 of ATG8 and ATG8-PE. C. Z., A.L. and H. Y assisted with the RNAseq analysis. B. S., designed and
702 conducted flow cytometry experiments. J. C. S., and G. S., drafted the manuscript. The final version of the
703 manuscript was generated with input and contributions from N. W., S. S., J. J., B. Z., P. Q., H. Y., C. Z., K.
704 D., B. S., B. Y., T. O., J. S., All authors approved the final version of the manuscript.

705 References

- 706 ¹ FAO, I., UNICEF *et al.* Wfp and who. 2018. the state of food security and nutrition in the world 2018.
707 building climate resilience for food security and nutrition. rome, fao (2019).
- 708 ² Roser, M. & Ritchie, H. Fertilizers. *Our World in Data* (2020). <https://ourworldindata.org/fertilizers>.
- 709 ³ Erisman, J. W., Sutton, M. A., Galloway, J., Klimont, Z. & Winiwarter, W. How a century of ammonia
710 synthesis changed the world. *Nature Geoscience* **1**, 636–639 (2008).
- 711 ⁴ Mancus, P. Nitrogen fertilizer dependency and its contradictions: a theoretical exploration of social-
712 ecological metabolism. *Rural Sociology* **72**, 269–288 (2007).
- 713 ⁵ Cordell, D., Drangert, J.-O. & White, S. The story of phosphorus: global food security and food for
714 thought. *Global environmental change* **19**, 292–305 (2009).

- 715 ⁶ Service, U. N. A. S. *USDA National Agricultural Statistics Service. NASS - Quick Stats. Accessed*
716 *2020-08-26* (2007). URL <https://data.nal.usda.gov/dataset/nass-quick-stats>.
- 717 ⁷ PIT. “*Fertilizer Outlook 2019 - 2023*. International fertilizer association (IFA) Annual Conference,
718 Montreal (2019). URL <https://www.ifastat.org/market-outlooks>.
- 719 ⁸ Schnitkey, G. Fertilizer costs in 2017 and 2018. *farmdoc daily* **7** (2017).
- 720 ⁹ Cameron, K., Di, H. J. & Moir, J. Nitrogen losses from the soil/plant system: a review. *Annals of*
721 *applied biology* **162**, 145–173 (2013).
- 722 ¹⁰ Smith, V. H., Tilman, G. D. & Nekola, J. C. Eutrophication: impacts of excess nutrient inputs on
723 freshwater, marine, and terrestrial ecosystems. *Environmental pollution* **100**, 179–196 (1999).
- 724 ¹¹ Hart, M. R., Quin, B. F. & Nguyen, M. L. Phosphorus runoff from agricultural land and direct fertilizer
725 effects: A review. *Journal of environmental quality* **33**, 1954–1972 (2004).
- 726 ¹² Bennett, E. M., Carpenter, S. R. & Caraco, N. F. Human impact on erodible phosphorus and
727 eutrophication: a global perspective: increasing accumulation of phosphorus in soil threatens rivers,
728 lakes, and coastal oceans with eutrophication. *BioScience* **51**, 227–234 (2001).
- 729 ¹³ Lightfoot, D. A. Developing crop varieties with improved nutrient-use efficiency. In *Engineering*
730 *Nitrogen Utilization in Crop Plants*, 1–11 (Springer, 2018).
- 731 ¹⁴ Gramma, V., Kontbay, K. & Wahl, V. Crops for the future: on the way to reduce nitrogen pollution.
732 *American Journal of Botany* **n/a** (2020). URL <https://bsapubs.onlinelibrary.wiley.com/doi/abs/10.1002/ajb2.1527>.
733 <https://bsapubs.onlinelibrary.wiley.com/doi/pdf/10.1002/ajb2.1527>.
- 734
- 735 ¹⁵ Fernie, A. R. *et al.* Synchronization of developmental, molecular and metabolic aspects of source–sink
736 interactions. *Nature Plants* 1–12 (2020).
- 737 ¹⁶ Tollenaar, M. & Lee, E. Yield potential, yield stability and stress tolerance in maize. *Field crops*
738 *research* **75**, 161–169 (2002).
- 739 ¹⁷ Mueller, S. M. & Vyn, T. J. Maize plant resilience to n stress and post-silking n capacity changes over
740 time: a review. *Frontiers in plant science* **7**, 53 (2016).
- 741 ¹⁸ Duncan, R. Environmental compatibility of seashore paspalum (saltwater couch) for golf courses and
742 other recreational uses. ii. management protocols. *International Turfgrass Society Research Journal* **8**,
743 1216–1229 (1997).
- 744 ¹⁹ Wu, P. *et al.* Comparative transcriptome profiling provides insights into plant salt tolerance in seashore
745 paspalum (*paspalum vaginatum*). *BMC genomics* **21**, 131 (2020).
- 746 ²⁰ Huang, B., Duncan, R. & Carrow, R. Drought-resistance mechanisms of seven warm-season turfgrasses
747 under surface soil drying: Ii. root aspects. *Crop science* **37**, 1863–1869 (1997).
- 748 ²¹ Huang, B., Duncan, R. & Carrow, R. Drought-resistance mechanisms of seven warm-season turfgrasses
749 under surface soil drying: I. shoot response. *Crop Science* **37**, 1858–1863 (1997).
- 750 ²² Shahba, M. A., Abbas, M. S. & Alshammary, S. F. Drought resistance strategies of seashore paspalum
751 cultivars at different mowing heights. *HortScience* **49**, 221–229 (2014).
- 752 ²³ Jiang, Y. & Carrow, R. N. Broadband spectral reflectance models of turfgrass species and cultivars to
753 drought stress. *Crop science* **47**, 1611–1618 (2007).

- 754 ²⁴ Cardona, C., Duncan, R. & Lindstrom, O. Low temperature tolerance assessment in paspalum. *Crop*
755 *science* **37**, 1283–1291 (1997).
- 756 ²⁵ Cyril, J., Powell, G., Duncan, R. & Baird, W. Changes in membrane polar lipid fatty acids of seashore
757 paspalum in response to low temperature exposure. *Crop Science* **42**, 2031–2037 (2002).
- 758 ²⁶ He, Y., Xiao, H., Wang, H., Chen, Y. & Yu, M. Effect of silicon on chilling-induced changes of solutes,
759 antioxidants, and membrane stability in seashore paspalum turfgrass. *Acta physiologiae plantarum* **32**,
760 487–494 (2010).
- 761 ²⁷ Jiang, Y., Duncan, R. R. & Carrow, R. N. Assessment of low light tolerance of seashore paspalum and
762 bermudagrass. *Crop Science* **44**, 587–594 (2004).
- 763 ²⁸ Bamidele, J. & Igiri, A. Growth of seashore paspalum,(paspalum vaginatum l) in soil contaminated
764 with crude petroleum oil. *Journal of Applied Sciences and Environmental Management* **15** (2011).
- 765 ²⁹ Duncan, R. R. & Carrow, R. N. *Seashore paspalum: The environmental turfgrass* (John Wiley & Sons,
766 2000).
- 767 ³⁰ Qi, P. *et al.* High density genetic maps of seashore paspalum using genotyping-by-sequencing and
768 their relationship to the sorghum bicolor genome. *Scientific reports* **9**, 1–10 (2019).
- 769 ³¹ Brosnan, J. & Deputy, J. *Seashore paspalum* (University of Hawaii, 2008).
- 770 ³² Eudy, D., Bahri, B. A., Harrison, M. L., Raymer, P. & Devos, K. M. Ploidy level and genetic diversity
771 in the genus paspalum, group disticha. *Crop Science* **57**, 3319–3332 (2017).
- 772 ³³ II, G. P. W. G. New grass phylogeny resolves deep evolutionary relationships and discovers c4 origins.
773 *New Phytologist* **193**, 304–312 (2012).
- 774 ³⁴ Bennetzen, J. L. *et al.* Reference genome sequence of the model plant setaria. *Nature biotechnology*
775 **30**, 555 (2012).
- 776 ³⁵ Bouchenak-Khelladi, Y., Slingsby, J. A., Verboom, G. A. & Bond, W. J. Diversification of c4 grasses
777 (poaceae) does not coincide with their ecological dominance. *American Journal of Botany* **101**,
778 300–307 (2014).
- 779 ³⁶ Yan, L. *et al.* Parallel natural selection in the cold-adapted crop-wild relative tripsacum dactyloides
780 and artificial selection in temperate adapted maize. *BioRxiv* 187575 (2018).
- 781 ³⁷ Ludewig, U., Vatov, E., Hedderich, D. & Neuhäuser, B. Adjusting plant nutrient acquisition to
782 fluctuating availability: transcriptional co-regulation of the nitrate and phosphate deprivation responses
783 in roots. *Journal of Experimental Botany* **72**, 3500–3503 (2021).
- 784 ³⁸ Ning, P., Yang, L., Li, C. & Fritschi, F. B. Post-silking carbon partitioning under nitrogen deficiency
785 revealed sink limitation of grain yield in maize. *Journal of experimental botany* **69**, 1707–1719 (2018).
- 786 ³⁹ Schlüter, U. *et al.* Maize source leaf adaptation to nitrogen deficiency affects not only nitrogen and
787 carbon metabolism but also control of phosphate homeostasis. *Plant Physiology* **160**, 1384–1406
788 (2012).
- 789 ⁴⁰ RB, G. A., Schwarz-Sommer, Z. & Saedler, H. Molecular analysis of the waxy locus of zea mays. *Mol*
790 *Gen Genet* **203**, 237–244 (1986).
- 791 ⁴¹ Dinges, J. R., Colleoni, C., James, M. G. & Myers, A. M. Mutational analysis of the pullulanase-type
792 debranching enzyme of maize indicates multiple functions in starch metabolism. *The Plant Cell* **15**,
793 666–680 (2003).

- 794 ⁴² Carvalhais, L. C. *et al.* Root exudation of sugars, amino acids, and organic acids by maize as affected
795 by nitrogen, phosphorus, potassium, and iron deficiency. *Journal of Plant Nutrition and Soil Science*
796 **174**, 3–11 (2011).
- 797 ⁴³ Brouwer, R. Nutritive influences on the distribution of dry matter in the plant. Tech. Rep., [sn] (1962).
- 798 ⁴⁴ Chishaki, N. & Horiguchi, T. Responses of secondary metabolism in plants to nutrient deficiency. In
799 *Plant nutrition for sustainable food production and environment*, 341–345 (Springer, 1997).
- 800 ⁴⁵ Lee, D.-K. *et al.* A nitrogen molecular sensing system, comprised of the allantoinase and ureide
801 permease 1 genes, can be used to monitor n status in rice. *Frontiers in plant science* **9**, 444 (2018).
- 802 ⁴⁶ Gargallo-Garriga, A. *et al.* Root exudate metabolomes change under drought and show limited capacity
803 for recovery. *Scientific reports* **8**, 12696 (2018).
- 804 ⁴⁷ Carillo, P. Gaba shunt in durum wheat. *Frontiers in plant science* **9**, 100 (2018).
- 805 ⁴⁸ Hijaz, F. & Killiny, N. Exogenous gaba is quickly metabolized to succinic acid and fed into the plant
806 tca cycle. *Plant signaling & behavior* **14**, e1573096 (2019).
- 807 ⁴⁹ Yoshikawa, M., Hirai, N., Wakabayashi, K., Sugizaki, H. & Iwamura, H. Succinic and lactic acids as
808 plant growth promoting compounds produced by rhizospheric pseudomonas putida. *Canadian journal*
809 *of microbiology* **39**, 1150–1154 (1993).
- 810 ⁵⁰ Zhang, Y. *et al.* Differentially regulated orthologs in sorghum and the subgenomes of maize. *The Plant*
811 *Cell* **29**, 1938–1951 (2017).
- 812 ⁵¹ Eastmond, P. J. *et al.* Trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose
813 synthesis, is essential for arabidopsis embryo maturation. *The Plant Journal* **29**, 225–235 (2002).
- 814 ⁵² Jiang, W., Fu, F.-L., Zhang, S.-Z., Wu, L. & Li, W.-C. Cloning and characterization of functional
815 trehalose-6-phosphate synthase gene in maize. *Journal of Plant Biology* **53**, 134–141 (2010).
- 816 ⁵³ Goddijn, O. J. *et al.* Inhibition of trehalase activity enhances trehalose accumulation in transgenic
817 plants. *Plant physiology* **113**, 181–190 (1997).
- 818 ⁵⁴ Müller, J., Boller, T. & Wiemken, A. Trehalose and trehalase in plants: recent developments. *Plant*
819 *science* **112**, 1–9 (1995).
- 820 ⁵⁵ Müller, J., Aeschbacher, R. A., Wingler, A., Boller, T. & Wiemken, A. Trehalose and trehalase in
821 arabidopsis. *Plant Physiology* **125**, 1086–1093 (2001).
- 822 ⁵⁶ Johnson, I. t. & Thornley, J. A model of shoot: root partitioning with optimal growth. *Annals of botany*
823 **60**, 133–142 (1987).
- 824 ⁵⁷ Ericsson, T. Growth and shoot: root ratio of seedlings in relation to nutrient availability. In *Nutrient*
825 *uptake and cycling in forest ecosystems*, 205–214 (Springer, 1995).
- 826 ⁵⁸ Oszvald, M. *et al.* Trehalose 6-phosphate regulates photosynthesis and assimilate partitioning in
827 reproductive tissue. *Plant Physiology* **176**, 2623–2638 (2018).
- 828 ⁵⁹ Nuccio, M. L. *et al.* Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in
829 well-watered and drought conditions. *Nature biotechnology* **33**, 862 (2015).
- 830 ⁶⁰ Paul, M. J., Watson, A. & Griffiths, C. A. Trehalose 6-phosphate signalling and impact on crop yield.
831 *Biochemical Society Transactions* **48**, 2127–2137 (2020).
- 832 ⁶¹ Liao, C.-Y. & Bassham, D. C. Combating stress: the interplay between hormone signaling and
833 autophagy in plants. *Journal of experimental botany* **71**, 1723–1733 (2020).

- 834 ⁶² Kumar, R., Bishop, E., Bridges, W. C., Tharayil, N. & Sekhon, R. S. Sugar partitioning and source–sink
835 interaction are key determinants of leaf senescence in maize. *Plant, cell & environment* **42**, 2597–2611
836 (2019).
- 837 ⁶³ Baena-González, E., Rolland, F., Thevelein, J. M. & Sheen, J. A central integrator of transcription
838 networks in plant stress and energy signalling. *Nature* **448**, 938–942 (2007).
- 839 ⁶⁴ Wang, J. *et al.* Overexpression of maize sucrose non-fermenting-1-related protein kinase 1 genes,
840 *zmsnrk1s*, causes alteration in carbon metabolism and leaf senescence in *arabidopsis thaliana*. *Gene*
841 **691**, 34–44 (2019).
- 842 ⁶⁵ McLoughlin, F. *et al.* Maize multi-omics reveal roles for autophagic recycling in proteome remodelling
843 and lipid turnover. *Nature plants* **4**, 1056–1070 (2018).
- 844 ⁶⁶ Goddijn, O. J. & van Dun, K. Trehalose metabolism in plants. *Trends in plant science* **4**, 315–319
845 (1999).
- 846 ⁶⁷ Lunn, J. E., Delorge, I., Figueroa, C. M., Van Dijck, P. & Stitt, M. Trehalose metabolism in plants. *The*
847 *Plant Journal* **79**, 544–567 (2014).
- 848 ⁶⁸ Soto-Burgos, J. & Bassham, D. C. *Snrk1* activates autophagy via the tor signaling pathway in
849 *arabidopsis thaliana*. *PloS one* **12**, e0182591 (2017).
- 850 ⁶⁹ Nunes, C. *et al.* Inhibition of *snrk1* by metabolites: tissue-dependent effects and cooperative inhibition
851 by glucose 1-phosphate in combination with trehalose 6-phosphate. *Plant Physiology and Biochemistry*
852 **63**, 89–98 (2013).
- 853 ⁷⁰ Delatte, T. L. *et al.* Growth arrest by trehalose-6-phosphate: an astonishing case of primary metabolite
854 control over growth by way of the *snrk1* signaling pathway. *Plant physiology* **157**, 160–174 (2011).
- 855 ⁷¹ Zhang, Y. *et al.* Inhibition of *snf1*-related protein kinase1 activity and regulation of metabolic pathways
856 by trehalose-6-phosphate. *Plant physiology* **149**, 1860–1871 (2009).
- 857 ⁷² Schluepmann, H. *et al.* Trehalose mediated growth inhibition of *arabidopsis* seedlings is due to
858 trehalose-6-phosphate accumulation. *Plant physiology* **135**, 879–890 (2004).
- 859 ⁷³ Henry, C. *et al.* Differential role for trehalose metabolism in salt-stressed maize. *Plant Physiology* **169**,
860 1072–1089 (2015).
- 861 ⁷⁴ Bledsoe, S. W. *et al.* The role of *tre6p* and *snrk1* in maize early kernel development and events leading
862 to stress-induced kernel abortion. *BMC Plant Biology* **17**, 1–17 (2017).
- 863 ⁷⁵ Chen, Q. *et al.* Autophagy and nutrients management in plants. *Cells* **8**, 1426 (2019).
- 864 ⁷⁶ Janse van Rensburg, H. C., Van den Ende, W. & Signorelli, S. Autophagy in plants: both a puppet and
865 a puppet master of sugars. *Frontiers in Plant Science* **10**, 14 (2019).
- 866 ⁷⁷ Hulsmans, S., Rodriguez, M., De Coninck, B. & Rolland, F. The *snrk1* energy sensor in plant biotic
867 interactions. *Trends in Plant Science* **21**, 648–661 (2016).
- 868 ⁷⁸ Chung, T., Suttangkakul, A. & Vierstra, R. D. The *atg* autophagic conjugation system in maize:
869 *Atg* transcripts and abundance of the *atg8*-lipid adduct are regulated by development and nutrient
870 availability. *Plant physiology* **149**, 220–234 (2009).
- 871 ⁷⁹ Williams, B. *et al.* Trehalose accumulation triggers autophagy during plant desiccation. *PLoS genetics*
872 **11** (2015).

- 873 ⁸⁰ Klionsky, D. J. *et al.* Guidelines for the use and interpretation of assays for monitoring autophagy. *autophagy* **17**, 1–382 (2021).
874
- 875 ⁸¹ Takatsuka, C., Inoue, Y., Matsuoka, K. & Moriyasu, Y. 3-methyladenine inhibits autophagy in tobacco
876 culture cells under sucrose starvation conditions. *Plant and Cell Physiology* **45**, 265–274 (2004).
- 877 ⁸² Obata, T. & Fernie, A. R. The use of metabolomics to dissect plant responses to abiotic stresses.
878 *Cellular and Molecular Life Sciences* **69**, 3225–3243 (2012).
- 879 ⁸³ Kaplan, F. *et al.* Exploring the temperature-stress metabolome of arabidopsis. *Plant physiology* **136**,
880 4159–4168 (2004).
- 881 ⁸⁴ Cook, D., Fowler, S., Fiehn, O. & Thomashow, M. F. A prominent role for the cbf cold response
882 pathway in configuring the low-temperature metabolome of arabidopsis. *Proceedings of the National*
883 *Academy of Sciences* **101**, 15243–15248 (2004).
- 884 ⁸⁵ Wulff-Zottele, C. *et al.* Photosynthesis and metabolism interact during acclimation of arabidopsis
885 thaliana to high irradiance and sulphur depletion. *Plant, cell & environment* **33**, 1974–1988 (2010).
- 886 ⁸⁶ Sun, X. *et al.* The responses of arabidopsis thaliana to cadmium exposure explored via metabolite
887 profiling. *Chemosphere* **78**, 840–845 (2010).
- 888 ⁸⁷ Urano, K. *et al.* Characterization of the aba-regulated global responses to dehydration in arabidopsis
889 by metabolomics. *The Plant Journal* **57**, 1065–1078 (2009).
- 890 ⁸⁸ Tschoep, H. *et al.* Adjustment of growth and central metabolism to a mild but sustained nitrogen-
891 limitation in arabidopsis. *Plant, cell & environment* **32**, 300–318 (2009).
- 892 ⁸⁹ Sanchez, D. H., Schwabe, F., Erban, A., Udvardi, M. K. & Kopka, J. Comparative metabolomics of
893 drought acclimation in model and forage legumes. *Plant, Cell & Environment* **35**, 136–149 (2012).
- 894 ⁹⁰ Rohman, M. *et al.* Trehalose protects maize plants from salt stress and phosphorus deficiency. *Plants*
895 **8**, 568 (2019).
- 896 ⁹¹ Garg, A. K. *et al.* Trehalose accumulation in rice plants confers high tolerance levels to different
897 abiotic stresses. *Proceedings of the National Academy of Sciences* **99**, 15898–15903 (2002).
- 898 ⁹² Lin, Y. *et al.* Exogenous trehalose improves growth under limiting nitrogen through upregulation of
899 nitrogen metabolism. *BMC plant biology* **17**, 247 (2017).
- 900 ⁹³ Trenholm, L., Carrow, R. & Duncan, R. Wear tolerance, growth, and quality of seashore paspalum in
901 response to nitrogen and potassium. *HortScience* **36**, 780–783 (2001).
- 902 ⁹⁴ Sarkar, S., Davies, J. E., Huang, Z., Tunnacliffe, A. & Rubinsztein, D. C. Trehalose, a novel mtor-
903 independent autophagy enhancer, accelerates the clearance of mutant huntingtin and α -synuclein.
904 *Journal of Biological Chemistry* **282**, 5641–5652 (2007).
- 905 ⁹⁵ Yoon, Y.-S. *et al.* Is trehalose an autophagic inducer? unraveling the roles of non-reducing disaccharides
906 on autophagic flux and alpha-synuclein aggregation. *Cell death & disease* **8**, e3091–e3091 (2017).
- 907 ⁹⁶ Chen, X. *et al.* Trehalose, sucrose and raffinose are novel activators of autophagy in human keratinocytes
908 through an mtor-independent pathway. *Scientific reports* **6**, 1–17 (2016).
- 909 ⁹⁷ Li, F. *et al.* Autophagic recycling plays a central role in maize nitrogen remobilization. *The Plant Cell*
910 **27**, 1389–1408 (2015).
- 911 ⁹⁸ Guiboileau, A. *et al.* Autophagy machinery controls nitrogen remobilization at the whole-plant level
912 under both limiting and ample nitrate conditions in arabidopsis. *New Phytologist* **194**, 732–740 (2012).

- 913 ⁹⁹ Fan, T. *et al.* A rice autophagy gene *osatg8b* is involved in nitrogen remobilization and control of grain
914 quality. *Frontiers in Plant Science* **11**, 588 (2020).
- 915 ¹⁰⁰ Wang, Y. *et al.* *Bzr1* mediates brassinosteroid-induced autophagy and nitrogen starvation in tomato.
916 *Plant Physiology* **179**, 671–685 (2019).
- 917 ¹⁰¹ Wada, S. *et al.* Autophagy supports biomass production and nitrogen use efficiency at the vegetative
918 stage in rice. *Plant Physiology* **168**, 60–73 (2015).
- 919 ¹⁰² Avin-Wittenberg, T. Autophagy and its role in plant abiotic stress management. *Plant, Cell &*
920 *Environment* **42**, 1045–1053 (2019).
- 921 ¹⁰³ Kim, B., Lee, Y., Choi, H. & Huh, W.-K. The trehalose-6-phosphate phosphatase *tps2* regulates *atg8*
922 transcription and autophagy in *saccharomyces cerevisiae*. *Autophagy* 1–15 (2020).
- 923 ¹⁰⁴ Kendall, E. J., Adams, R. P. & Kartha, K. K. Trehalase activity in plant tissue cultures. *Phytochemistry*
924 **29**, 2525–2528 (1990).
- 925 ¹⁰⁵ Bian, C. *et al.* Mechanism of validamycin a inhibiting don biosynthesis and synergizing with dmi
926 fungicides against *fusarium graminearum*. *Molecular Plant Pathology* (2021).
- 927 ¹⁰⁶ Islam, M. O. *et al.* Functional identification of a rice trehalase gene involved in salt stress tolerance.
928 *Gene* **685**, 42–49 (2019).
- 929 ¹⁰⁷ Van Houtte, H. *et al.* Overexpression of the trehalase gene *attre1* leads to increased drought stress
930 tolerance in *arabidopsis* and is involved in abscisic acid-induced stomatal closure. *Plant physiology*
931 **161**, 1158–1171 (2013).
- 932 ¹⁰⁸ Price, H. J. *et al.* Genome evolution in the genus *sorghum* (poaceae). *Annals of Botany* **95**, 219–227
933 (2005).
- 934 ¹⁰⁹ Xiao, C.-L. *et al.* Mecat: fast mapping, error correction, and de novo assembly for single-molecule
935 sequencing reads. *nature methods* **14**, 1072 (2017).
- 936 ¹¹⁰ Chin, C.-S. *et al.* Nonhybrid, finished microbial genome assemblies from long-read smrt sequencing
937 data. *Nature methods* **10**, 563–569 (2013).
- 938 ¹¹¹ Smit, A. F. & Hubley, R. Repeatmodeler open-1.0. <http://www.repeatmasker.org> (2008–
939 2015).
- 940 ¹¹² Smit, A., Hubley, R. & Green, P. Repeatmasker open-4.0. 2013–2015 (2015).
- 941 ¹¹³ Haas, B. J. *et al.* Improving the *arabidopsis* genome annotation using maximal transcript alignment
942 assemblies. *Nucleic acids research* **31**, 5654–5666 (2003).
- 943 ¹¹⁴ Slater, G. S. C. & Birney, E. Automated generation of heuristics for biological sequence comparison.
944 *BMC bioinformatics* **6**, 31 (2005).
- 945 ¹¹⁵ Salamov, A. A. & Solovyev, V. V. Ab initio gene finding in *drosophila* genomic dna. *Genome research*
946 **10**, 516–522 (2000).
- 947 ¹¹⁶ Hoff, K. J., Lange, S., Lomsadze, A., Borodovsky, M. & Stanke, M. Braker1: unsupervised rna-seq-
948 based genome annotation with genemark-et and augustus. *Bioinformatics* **32**, 767–769 (2016).
- 949 ¹¹⁷ Quevillon, E. *et al.* Interproscan: protein domains identifier. *Nucleic acids research* **33**, W116–W120
950 (2005).
- 951 ¹¹⁸ Schnable, P. S. *et al.* The b73 maize genome: complexity, diversity, and dynamics. *science* **326**,
952 1112–1115 (2009).

- 953 ¹¹⁹ Paterson, A. H. *et al.* The sorghum bicolor genome and the diversification of grasses. *Nature* **457**, 551
954 (2009).
- 955 ¹²⁰ Zhang, Z. *et al.* Paraat: a parallel tool for constructing multiple protein-coding dna alignments.
956 *Biochemical and biophysical research communications* **419**, 779–781 (2012).
- 957 ¹²¹ Talavera, G. & Castresana, J. Improvement of phylogenies after removing divergent and ambiguously
958 aligned blocks from protein sequence alignments. *Systematic biology* **56**, 564–577 (2007).
- 959 ¹²² Stamatakis, A. Raxml version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies.
960 *Bioinformatics* **30**, 1312–1313 (2014).
- 961 ¹²³ Bouckaert, R. R. Densitree: making sense of sets of phylogenetic trees. *Bioinformatics* **26**, 1372–1373
962 (2010).
- 963 ¹²⁴ Nguyen, L.-T., Schmidt, H. A., Von Haeseler, A. & Minh, B. Q. Iq-tree: a fast and effective stochastic
964 algorithm for estimating maximum-likelihood phylogenies. *Molecular biology and evolution* **32**,
965 268–274 (2015).
- 966 ¹²⁵ Zou, C. *et al.* The genome of broomcorn millet. *Nature communications* **10**, 1–11 (2019).
- 967 ¹²⁶ Swigoňová, Z. *et al.* Close split of sorghum and maize genome progenitors. *Genome research* **14**,
968 1916–1923 (2004).
- 969 ¹²⁷ Sanderson, M. J. r8s: inferring absolute rates of molecular evolution and divergence times in the
970 absence of a molecular clock. *Bioinformatics* **19**, 301–302 (2003).
- 971 ¹²⁸ Kielbasa, S. M., Wan, R., Sato, K., Horton, P. & Frith, M. C. Adaptive seeds tame genomic sequence
972 comparison. *Genome research* **21**, 487–493 (2011).
- 973 ¹²⁹ Wang, Y. *et al.* Mcscanx: a toolkit for detection and evolutionary analysis of gene synteny and
974 collinearity. *Nucleic acids research* **40**, e49–e49 (2012).
- 975 ¹³⁰ Tang, H., Krishnakuar, V. & Li, J. jcvi: Jcvi utility libraries. *Zenodo*.(doi: 10.5281/zenodo.31631)
976 (2015).
- 977 ¹³¹ Ouyang, S. *et al.* The tigr rice genome annotation resource: improvements and new features. *Nucleic
978 acids research* **35**, D883–D887 (2006).
- 979 ¹³² Initiative, I. B. *et al.* Genome sequencing and analysis of the model grass brachypodium distachyon.
980 *Nature* **463**, 763 (2010).
- 981 ¹³³ Yang, Z. Paml: a program package for phylogenetic analysis by maximum likelihood. *Bioinformatics*
982 **13**, 555–556 (1997).
- 983 ¹³⁴ Lisec, J., Schauer, N., Kopka, J., Willmitzer, L. & Fernie, A. R. Gas chromatography mass spectrometry–
984 based metabolite profiling in plants. *Nature protocols* **1**, 387 (2006).
- 985 ¹³⁵ Dhatt, B. K. *et al.* Metabolic dynamics of developing rice seeds under high night-time temperature
986 stress. *Frontiers in Plant Science* **10**, 1443 (2019).
- 987 ¹³⁶ Lander, E. S. *et al.* Mapmaker: an interactive computer package for constructing primary genetic
988 linkage maps of experimental and natural populations. *Genomics* **1**, 174–181 (1987).
- 989 ¹³⁷ Goodstein, D. M. *et al.* Phytozome: a comparative platform for green plant genomics. *Nucleic acids
990 research* **40**, D1178–D1186 (2012).
- 991 ¹³⁸ Emms, D. M. & Kelly, S. Orthofinder: solving fundamental biases in whole genome comparisons
992 dramatically improves orthogroup inference accuracy. *Genome biology* **16**, 157 (2015).

- 993 ¹³⁹ Buchfink, B., Xie, C. & Huson, D. H. Fast and sensitive protein alignment using diamond. *Nature*
994 *methods* **12**, 59 (2015).
- 995 ¹⁴⁰ De Bie, T., Cristianini, N., Demuth, J. P. & Hahn, M. W. Cafe: a computational tool for the study of
996 gene family evolution. *Bioinformatics* **22**, 1269–1271 (2006).
- 997 ¹⁴¹ Schroeder, A. *et al.* The rin: an rna integrity number for assigning integrity values to rna measurements.
998 *BMC molecular biology* **7**, 3 (2006).
- 999 ¹⁴² Andrews, S. *et al.* Fastqc: a quality control tool for high throughput sequence data (2010).
- 1000 ¹⁴³ Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for illumina sequence data.
1001 *Bioinformatics* **30**, 2114–2120 (2014).
- 1002 ¹⁴⁴ Dobin, A. *et al.* Star: ultrafast universal rna-seq aligner. *Bioinformatics* **29**, 15–21 (2013).
- 1003 ¹⁴⁵ Bray, N. L., Pimentel, H., Melsted, P. & Pachter, L. Near-optimal probabilistic rna-seq quantification.
1004 *Nature biotechnology* **34**, 525–527 (2016).
- 1005 ¹⁴⁶ Anders, S., Pyl, P. T. & Huber, W. Htseq—a python framework to work with high-throughput
1006 sequencing data. *Bioinformatics* **31**, 166–169 (2015).
- 1007 ¹⁴⁷ Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for rna-seq
1008 data with deseq2. *Genome biology* **15**, 550 (2014).
- 1009 ¹⁴⁸ Contento, A. L., Xiong, Y. & Bassham, D. C. Visualization of autophagy in arabidopsis using the
1010 fluorescent dye monodansylcadaverine and a gfp-atatg8e fusion protein. *The Plant Journal* **42**, 598–608
1011 (2005).
- 1012 ¹⁴⁹ Klopfenstein, D. *et al.* Goatools: A python library for gene ontology analyses. *Scientific reports* **8**,
1013 1–17 (2018).
- 1014 ¹⁵⁰ Wimalanathan, K., Friedberg, I., Andorf, C. M. & Lawrence-Dill, C. J. Maize go annotation—methods,
1015 evaluation, and review (maize-gamer). *Plant Direct* **2**, e00052 (2018).
- 1016 ¹⁵¹ Srivastava, R. *et al.* Response to persistent er stress in plants: a multiphasic process that transitions
1017 cells from prosurvival activities to cell death. *The Plant Cell* **30**, 1220–1242 (2018).

Table S1. Final summary assembly statistics for chromosome scale assembly

Scaffold total	1903
Contig total	2212
Scaffold sequence total	651.0 Mb
Contig sequence total	648.0 Mb (0.5% gap)
Scaffold L/N50	7 / 44.5 Mb
Contig L/N50	111/ 1.5 Mb
Number of scaffolds >50 Kb	1112
% main genome in scaffolds >50 Kb	95.5 %

Table S2. Rescoring of progeny based on allele information from grandparents

Grandparent 1	Grandparent 2	Parents	Rule (to be applied to the F2 progeny)	Marker suffix
A	H	AH or HA	Keep original scores	-
H	A	AH or HA	Change 'A' to 'H', and 'H' to 'A'	R
H	H	AH or HA	Duplicate marker; keep original scores in one copy (marker suffix 'u'), change 'A' to 'H' and 'H' to 'A' in second copy (marker suffix 'ur')	u / ur
A	B	HH	Keep original scores	-
A	H	HH	Keep original scores	-
H	B	HH	Keep original scores	-
B	A	HH	Change 'A' to 'B', and 'B' to 'A'	r
H	A	HH	Change 'A' to 'B', and 'B' to 'A'	r
B	H	HH	Change 'A' to 'B', and 'B' to 'A'	r
H	H	HH	Duplicate marker; keep original scores in one copy (marker suffix 'u'), change 'H' to '-' and both 'A' and 'B' to 'H' in second copy (marker suffix 'uD')	u / uD

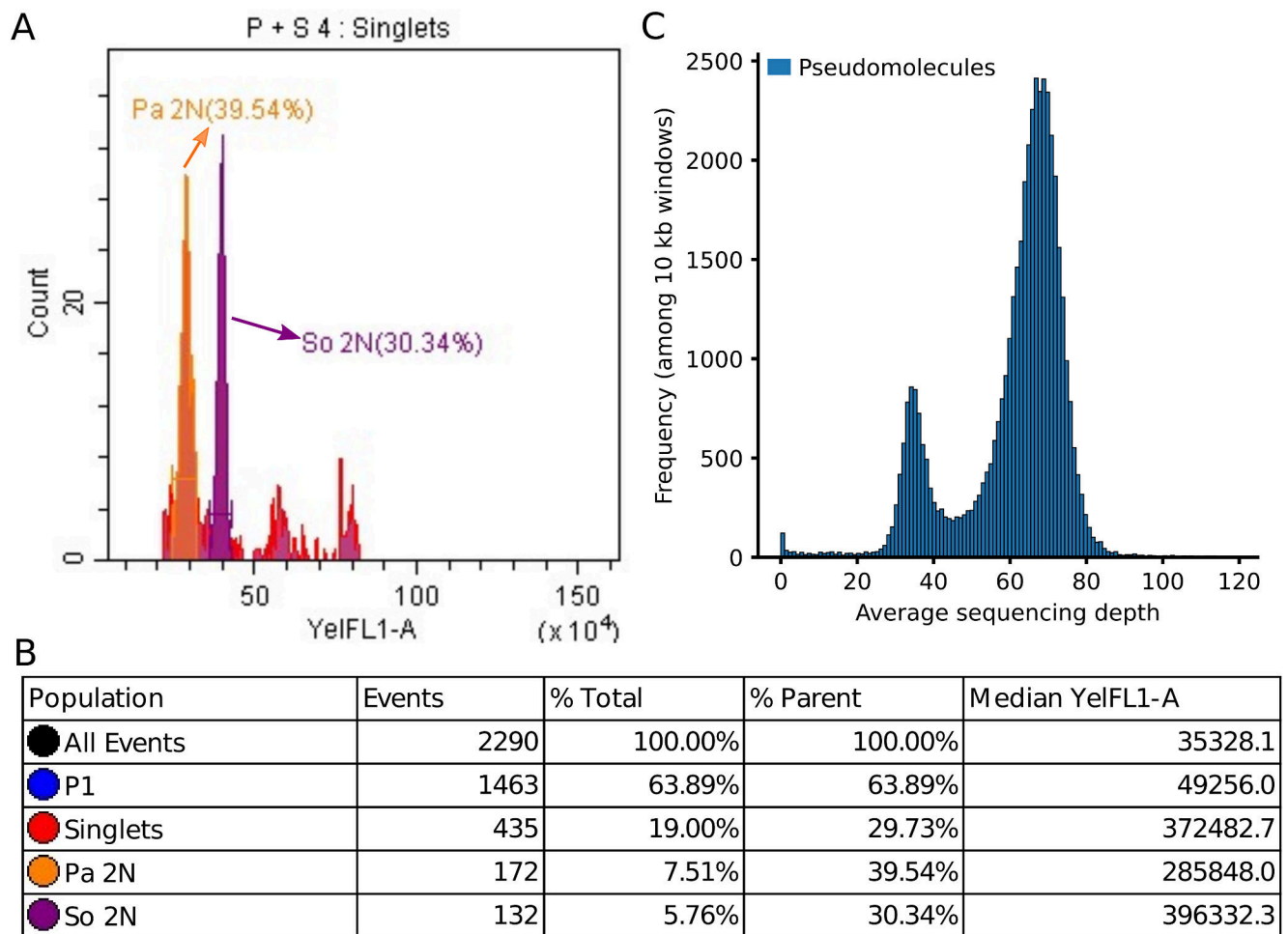


Figure S1. Estimating the genome size of the paspalum accession employed for genome sequencing. (A) Estimation of genome size using flow cytometry. The x axis indicates yellow fluorescence intensity, which is linearly correlated with the genome size. *Sorghum bicolor* BTX623 nuclei (So 2N) were used as an internal control. (B) Statistics of the flow cytometry results for one representative sample. Based on the median yellow fluorescence intensity, the ratio of genome size of *Paspalum vaginatum* (Pa) to *Sorghum bicolor*(So) is 285848:396332. The *Sorghum bicolor* genome size is 818 Mbp¹⁰⁸; therefore, the estimated genome size of *Paspalum vaginatum* is 590 Mbp. (C) Genome wide read coverage of Illumina sequencing reads mapped to the current paspalum genome assembly.

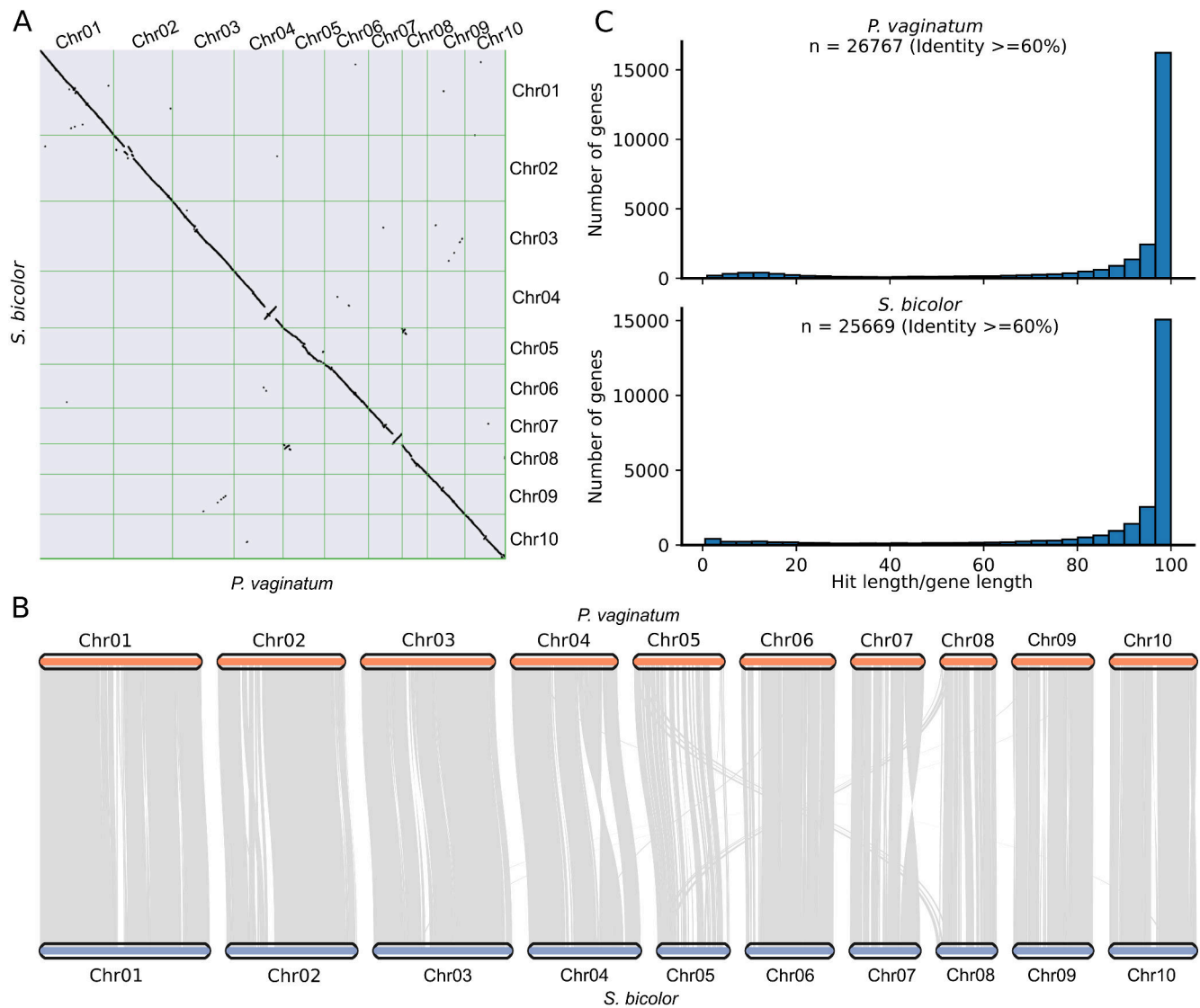


Figure S2. Gene families identified among the seven grass species. (A-B) Syntenic regions conserved between the paspalum genome and sorghum genome. (C) Length of homologs with identity >60% over the annotated length of proteins annotated in paspalum genome when BLAST paspalum proteome against sorghum proteome (upper) and reversely, the length of homologs with identity >60% over the annotated length of proteins annotated in sorghum genome when BLAST sorghum proteome against paspalum proteome (lower).

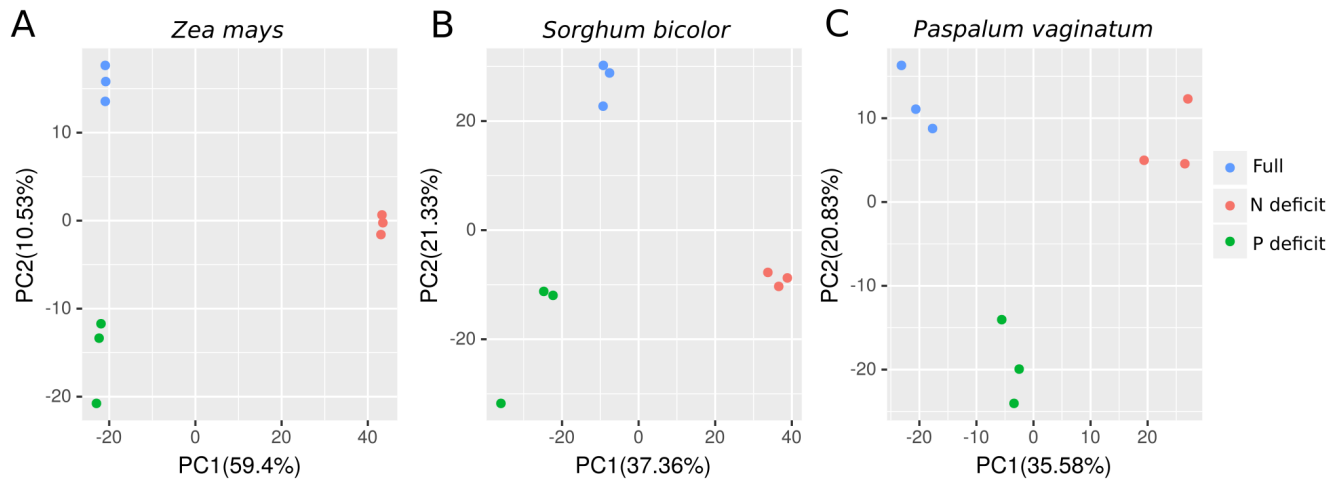


Figure S4. Principal component analysis of biological replicates of root transcriptomes under three experimental nutrient conditions. (A) Principal component analysis based on log transformed expression of syntenic genes in maize (*Zea mays*) (B) Principal component analysis based on log transformed expression of syntenic genes in sorghum (*Sorghum bicolor*) (C) Principal component analysis based on log transformed expression of syntenic genes in paspalum (*Paspalum vaginatum*). Panels (A-C) Nutrient conditions are color coded. PC, principal component.

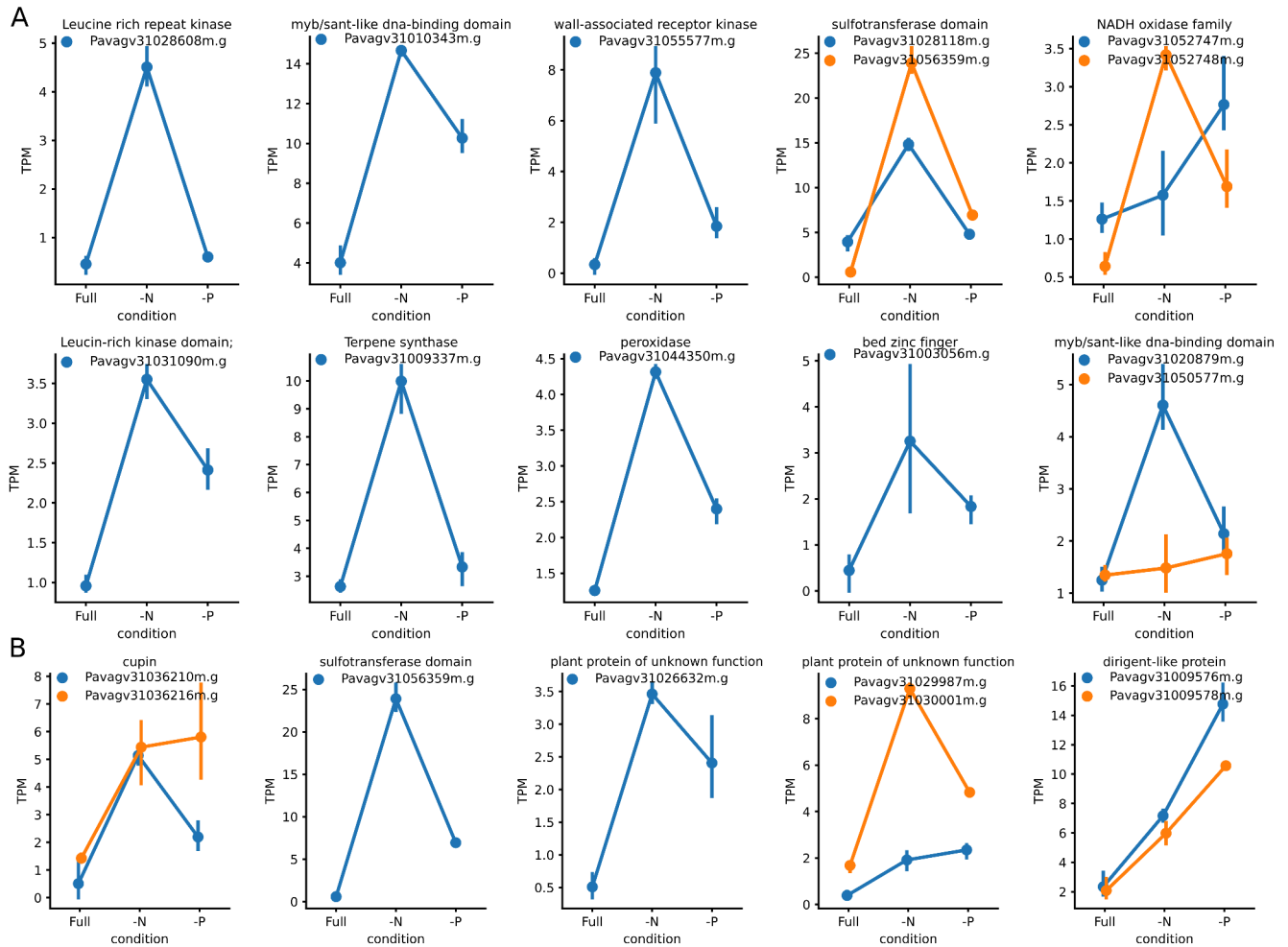
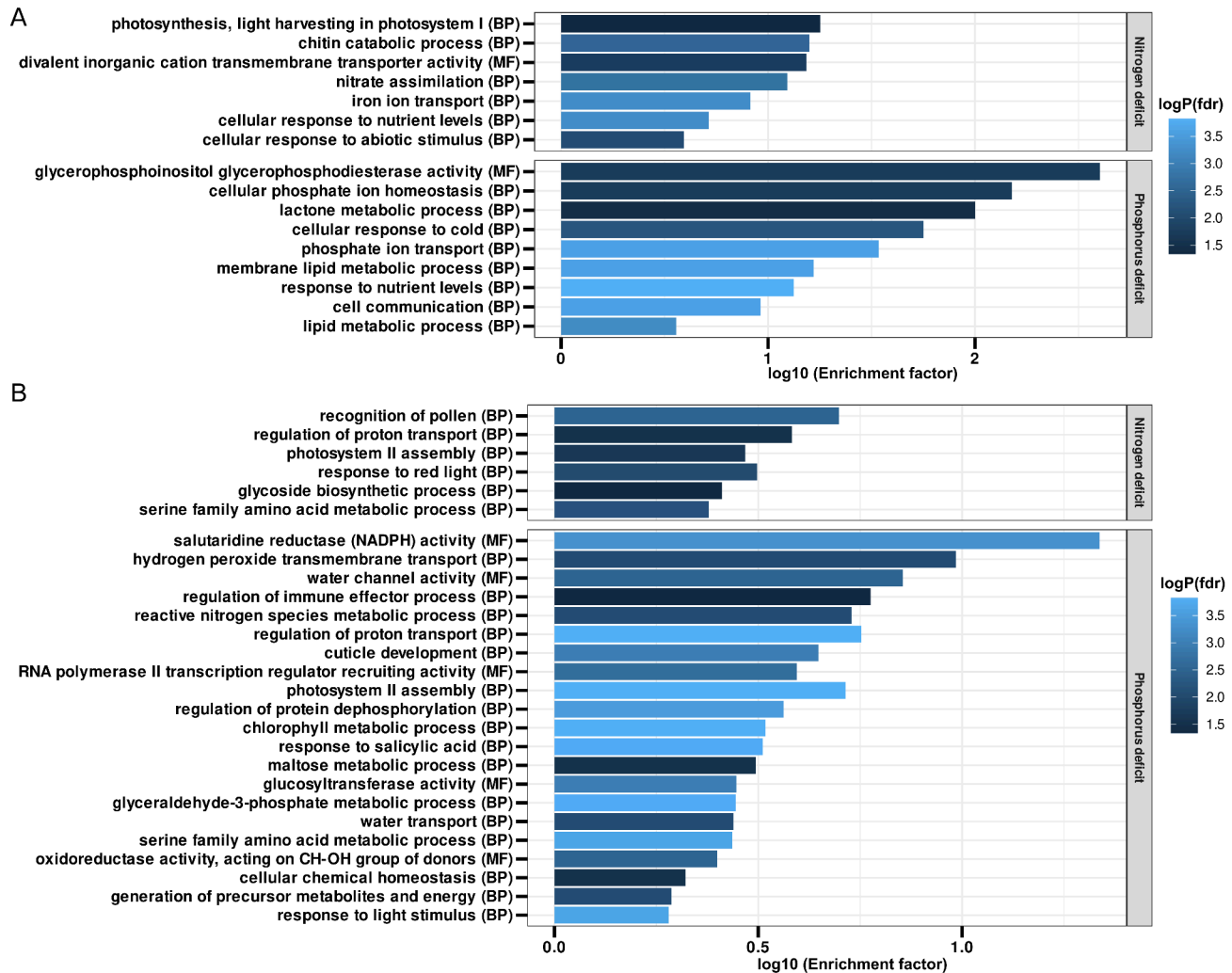


Figure S5. Members of gene families that are transcriptionally responsive to nutrient-deficit conditions. (A) Members of paspalum-specific expanded gene families that are transcriptionally responsive to nitrogen deficiency. (B) Members of paspalum-specific expanded gene families that are transcriptionally responsive to phosphorus deficiency.



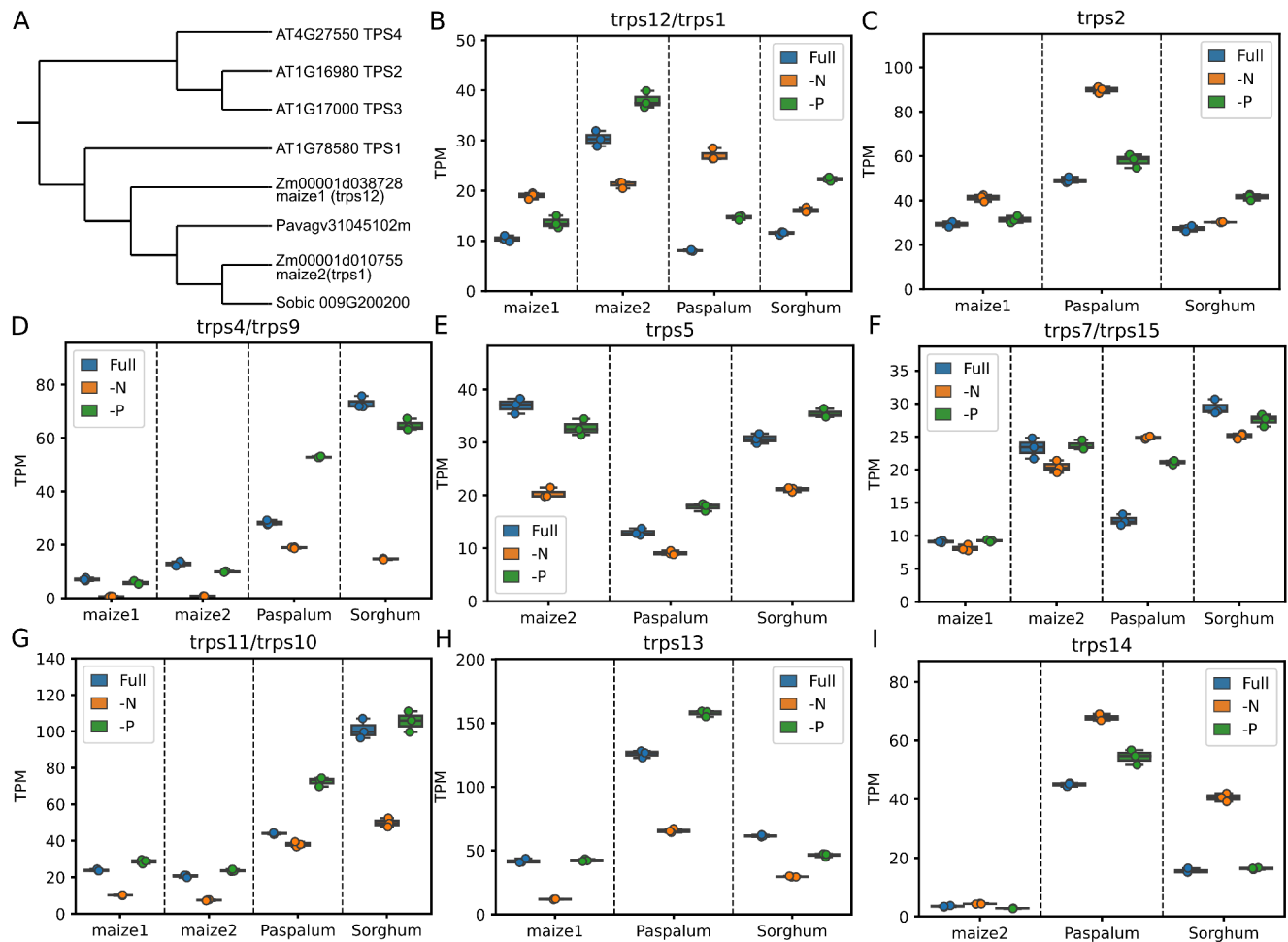


Figure S7. Expression patterns of genes encoding trehalose-6-phosphate synthase in response to nutrient stress across maize (*Zea mays*), sorghum (*Sorghum bicolor*), and paspalum (*Paspalum vaginatum*). (A) Phylogeny of orthologs of Arabidopsis trehalose-6-phosphate synthase 1 (*TPS1*) genes in the three species. (B) Expression pattern of the trehalose-6-phosphate synthase genes in the three species under nutrient-optimal (Full), nitrogen-deficit (-N), and phosphorus-deficit (-P) conditions. "maize1" and "maize2" indicate the two subgenomes that formed in maize after the recent whole-genome duplication event 12–16 million years ago. (C-I) Expression patterns of other syntenic genes annotated as encoding trehalose-6-phosphate synthase that did not cluster with Arabidopsis homologs in the three species under nutrient-optimal (Full), nitrogen-deficit (-N), and phosphorus-deficit (-P) conditions.

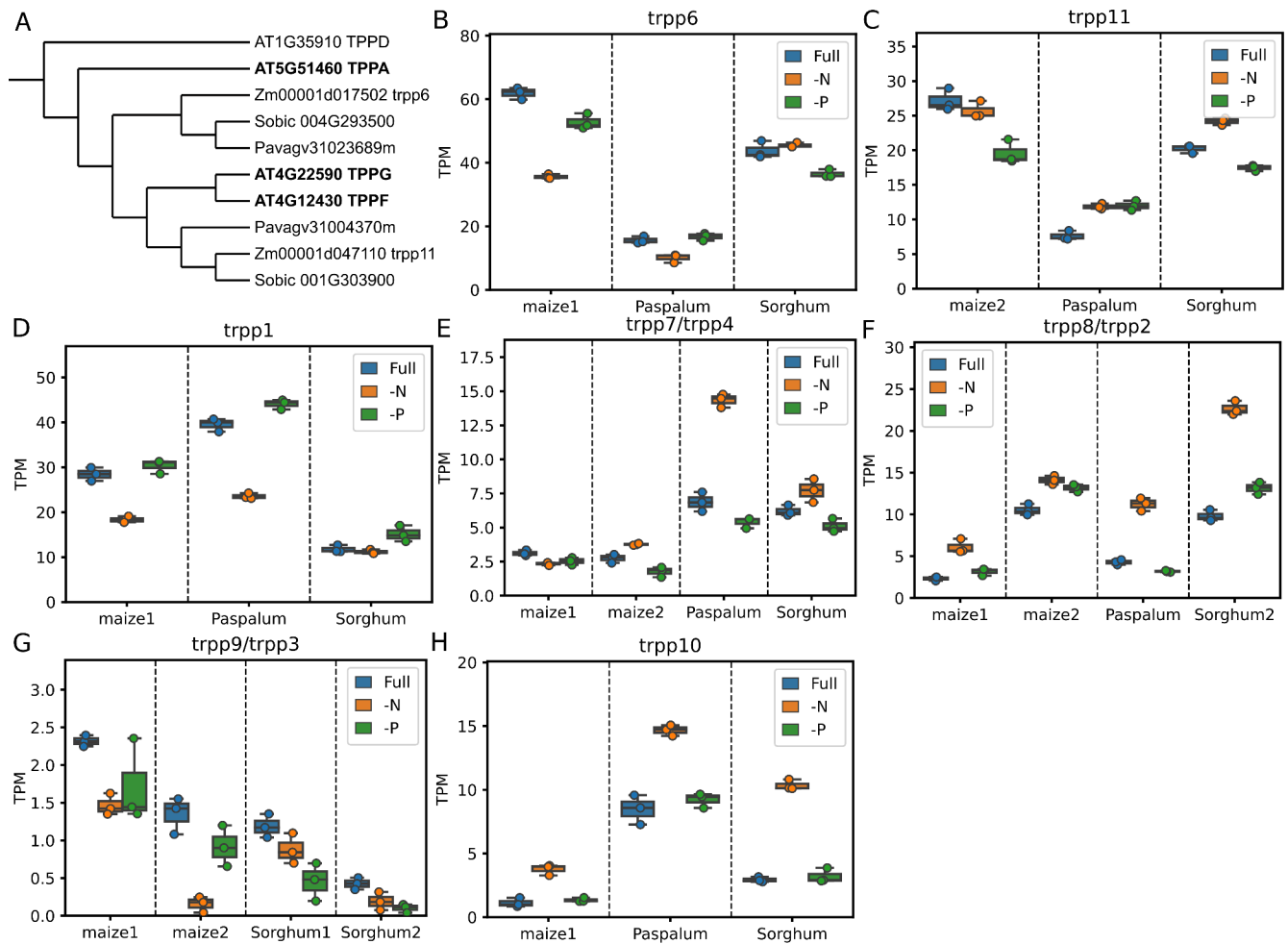


Figure S8. Expression patterns of genes encoding trehalose-6-phosphate phosphatase enzymes in response to nutrient stress across maize (*Zea mays*), sorghum (*Sorghum bicolor*), and paspalum (*Paspalum vaginatum*). (A) Phylogeny of orthologs of characterized Arabidopsis trehalose-6-phosphate phosphatase (TPP) genes in the three species. (B-C) Expression patterns of the trehalose-6-phosphate phosphatase genes (*trpp6* and *trpp11*) that clustered with their Arabidopsis homologous (*TPPA*, *TPPG*, *TPPF*) under nutrient-optimal (Full), nitrogen-deficit (-N), and phosphorus-deficit (-P) conditions. (D-H) Expression patterns of other syntenic genes annotated as trehalose-6-phosphate phosphatase that did not cluster with Arabidopsis homologs in the three species grown under nutrient-optimal (Full), nitrogen-deficit (-N), and phosphorus-deficit (-P) conditions. For panels B to I, "maize1" and "maize2" indicate the two subgenomes that formed in maize after the recent whole-genome duplication event 12–16 million years ago.

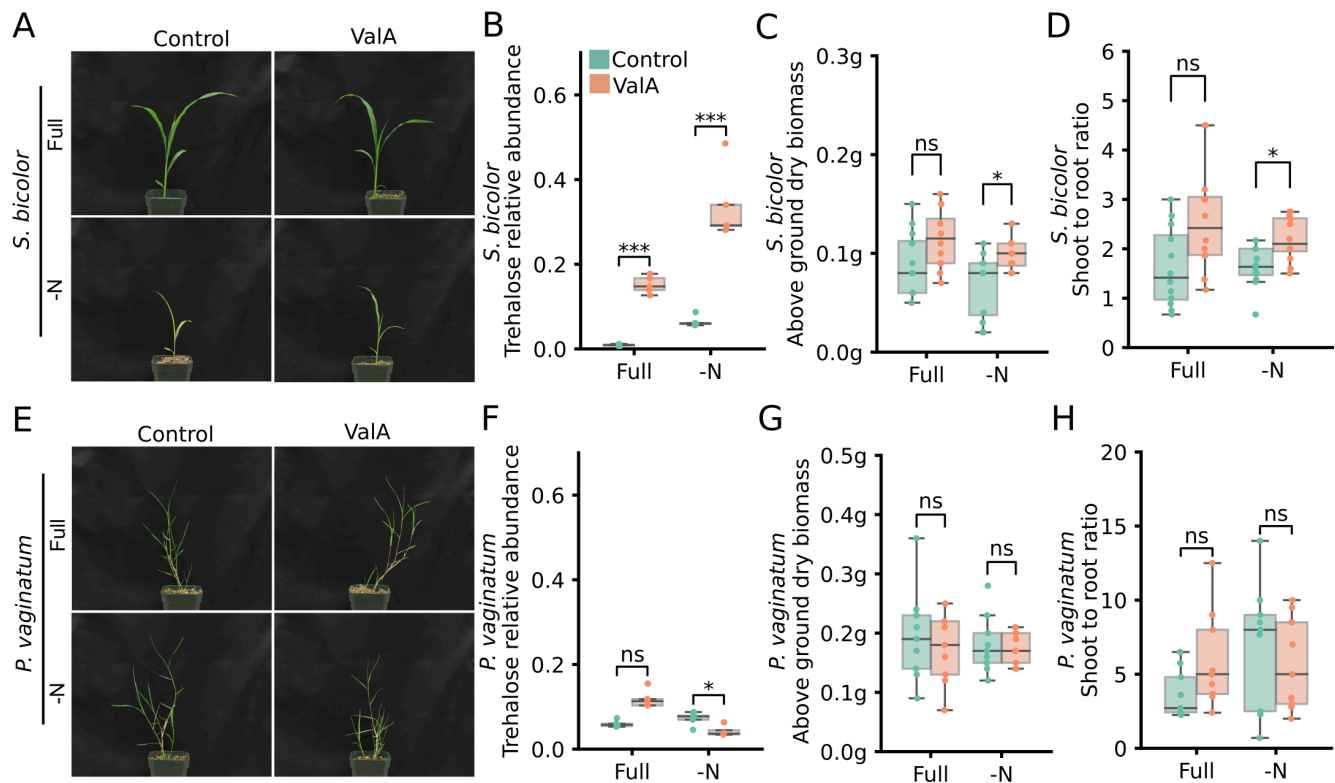


Figure S9. ValA treatment alters biomass accumulation and nutrient reallocation to sorghum (*Sorghum bicolor*)

grown under nutrient-deficient conditions. (A) Representative images of sorghum seedlings grown under nutrient optimal and N-deficit conditions with or without validamycin A (ValA) treatment. Images were taken 21 days after planting. For the ValA treatment, a 30 μ M solution was added at 6 PM on the day that the plants were watered with the indicated nutrient solutions. (B) Changes in observed trehalose abundance – normalized to an internal reference (ribitol) – in response to validamycin A and/or nutrient conditions in sorghum root tissues. Error bars are standard deviations. **Student's t-test** (* = $p < 0.05$; ** = $p < 0.005$; *** = $p < 0.0005$). (C) Dry weight of the above-ground tissue of sorghum seedlings grown under nutrient-optimal and nitrogen-deficit conditions harvested at 3 weeks after planting. Plant tissues were freeze-dried for 48 hours after harvesting. (D) Shoot-to-root ratio calculated from the dry weight of above-ground tissues and roots of the same sorghum seedlings. (E) Representative images of paspalum seedlings at 3 weeks after planting grown under nutrient optimal (Full) and nitrogen-deficient (-N) conditions with (ValA) or without (Control) validamycin A treatment. (F) Lack of significant increases in trehalose abundance (normalized to an internal reference [ribitol]) in response to validamycin A treatment (ValA) in 3-week-old paspalum seedlings under either full-nutrient or N-deficient conditions. (G) No significant change observed in above ground dry weight of 3-week-old paspalum seedlings in response to validamycin A treatment (ValA) under full-nutrient or N-deficient conditions. (H) Ratio of shoot-to-root dry weight in 3-week-old paspalum seedlings grown with or without validamycin A under full-nutrient or N-deficient conditions.

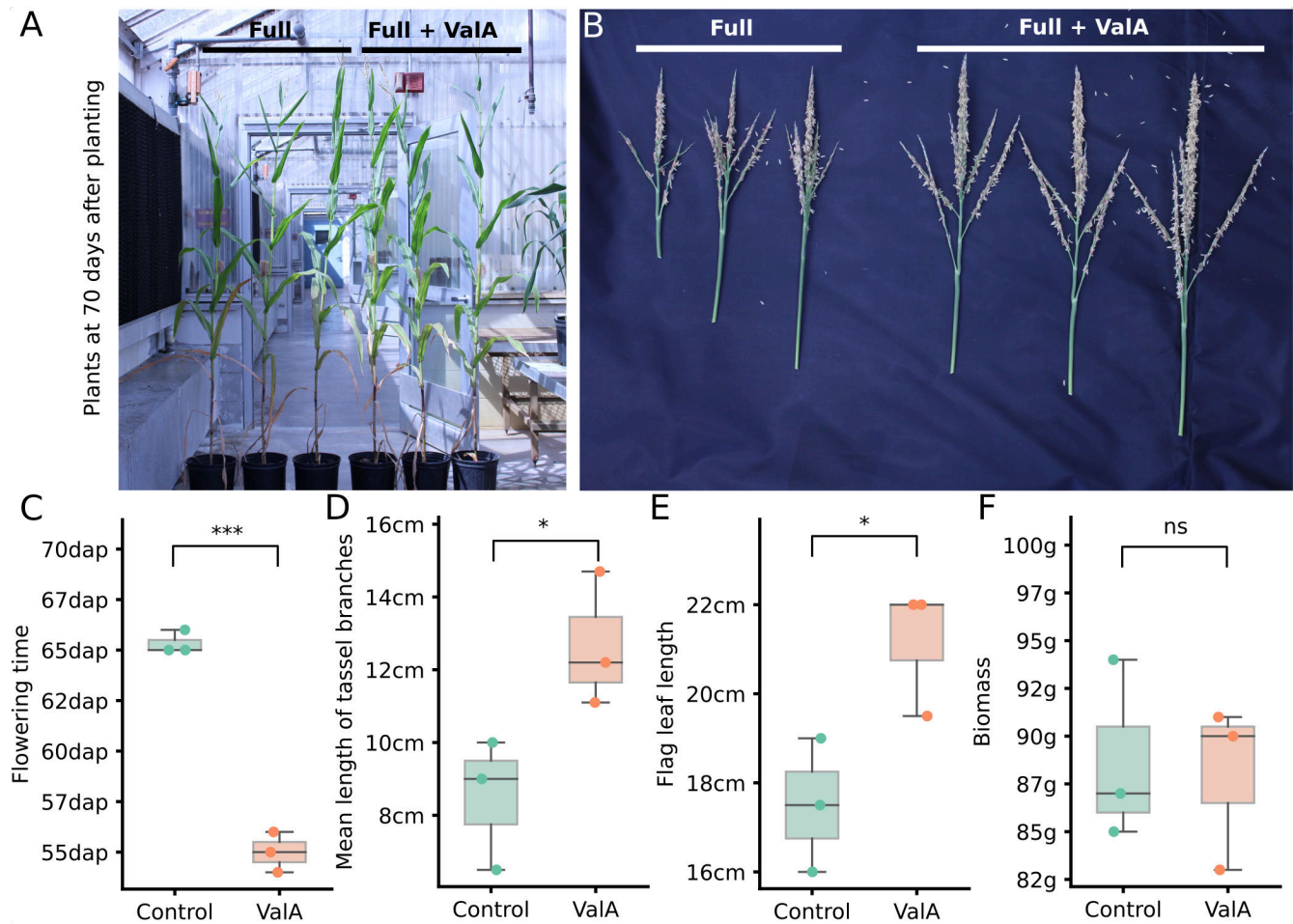


Figure S10. Validamycin A treatment improves important agronomic traits in adult maize plants. (A-B) Images of whole plants (A) and tassels (B) taken 70 days after planting. The left three plants were grown under full-nutrient conditions and the right three were grown under full-nutrient conditions with a weekly 30 μM ValA treatment. (C-F) Flowering time (C), mean length of tassel branches (D), flag leaf length (E) and above ground dry biomass (F) of plants with or without ValA treatment. **Student's t-test** (* = $p < 0.05$; ** = $p < 0.005$; *** = $p < 0.0005$).

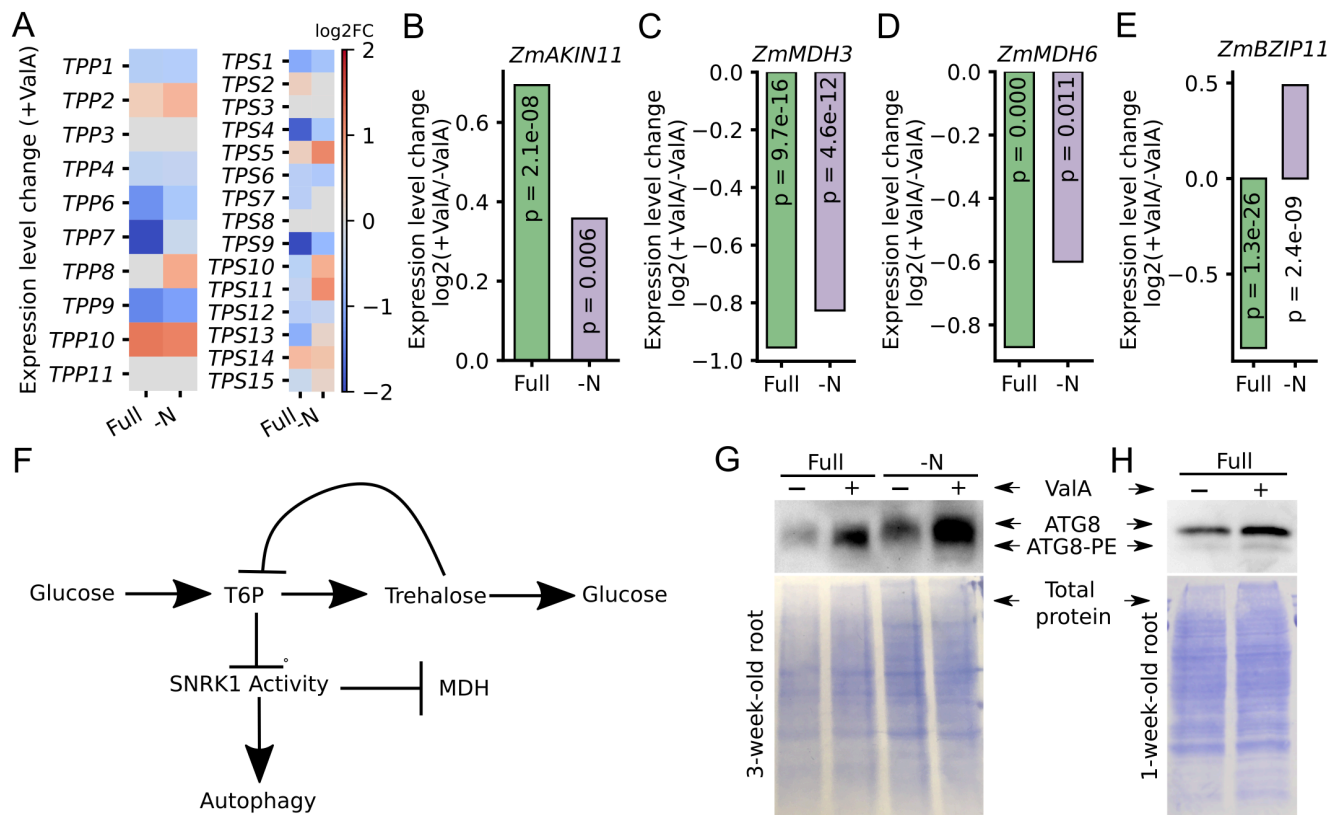


Figure S11. Transcriptional responses of SNRK1 target genes to Validamycin A treatment (A) Expression level fold changes of the genes encoding trehalose-6-phosphate synthases (TPS) and trehalose-6-phosphate phosphatases (TPP) relative to the seedlings treated with validamycin A under Full and -N conditions. (B-E) Expression level fold changes of the *ZmAKIN2* (A), *ZmMDH3* (B), *ZmMDH6* (C) and *ZmBZIP11* with or without validamycin A treatment under full nutrient and N-deficient conditions. p values were calculated by DESeq2 after correction for false discovery rate lower than 0.05. (F) Trehalose accumulation might lead to a lower T6P level, resulting in the release of inhibition of SNRK1 activity. The active status of SNRK1 would promote autophagy and *ZmAKIN11* expression while repressing the expression of MDH and bZIP genes. (G-H) A biological replicate of the immunoblot measuring the abundance of both free ATG8 (upper band) and the ATG8-PE conjugate (lower band) in root samples collected from 3-week-old maize seedlings grown under optimal nutrient (Full) and nitrogen-deficit (-N) conditions with or without ValA treatment (G) and in root samples collected from 1-week-old maize seedlings grown under optimal nutrient conditions with or without ValA treatment (H). Total protein loading control is shown in the lower panel.

