

## Supplementary Figures:

**Figure S1.** Inferred three dimensional structure of some *Brachypodium* dehydrins. All species' dehydrins showed  $\alpha$ -helices (1 to 4) whereas only some of them presented  $\beta$ -sheets (*Bdhn1* two in all species except *B. distachyon*, *Bdhn2* two to three in *B. sylvaticum*, and *Bdhn6* and *Bdhn9* three in *B. hybridumS* and *B. sylvaticum*): (A) *B. distachyon Bdhn4*; (B) *B. hybridumD Bdhn3*; (C) *B. stacei Bdhn1*; (D) *B. hybridumS Bdhn10*; (E): *B. sylvaticum Bdhn1b*. Complete sets of 3D structures for all *Brachypodium* species and proteins are available in the following links:

*B. distachyon* ([https://galactus.uma.es/triticae/pipeline\\_web/production/compartida/raptorx/distachyon.php](https://galactus.uma.es/triticae/pipeline_web/production/compartida/raptorx/distachyon.php));  
*B. hybridumD* ([https://galactus.uma.es/triticae/pipeline\\_web/production/compartida/raptorx/hybridumD.php](https://galactus.uma.es/triticae/pipeline_web/production/compartida/raptorx/hybridumD.php))  
*B. stacei* ([https://galactus.uma.es/triticae/pipeline\\_web/production/compartida/raptorx/stacei.php](https://galactus.uma.es/triticae/pipeline_web/production/compartida/raptorx/stacei.php));  
*B. hybridumS* ([https://galactus.uma.es/triticae/pipeline\\_web/production/compartida/raptorx/hybridumS.php](https://galactus.uma.es/triticae/pipeline_web/production/compartida/raptorx/hybridumS.php));  
*B. sylvaticum* ([https://galactus.uma.es/triticae/pipeline\\_web/production/compartida/raptorx/sylvaticum.php](https://galactus.uma.es/triticae/pipeline_web/production/compartida/raptorx/sylvaticum.php)).

**Figure S2.** BES1/BZR1 (Basic leucine zipper; green), MYB124 (Myb gene protein; blue), and ZAT (C2H2 zinc finger; red) *cis*-regulatory elements found in 5'-upstream promoter region (-500-to-+200 bp) of the *Brachypodium Bdhn* genes. **(a)** Distributions of *cis*-motifs per species and reference genomes (BD, *B. distachyon* Bd21; BS, *B. stacei* ABR114; BHD, *B. hybridum* subgenome-D ABR113; BHS, *B. hybridum* subgenome-S ABR113; BSY, *B. sylvaticum* Ain1) and per *Bdhn* gene promoter (*Bdhn1* to *Bdhn10*). Values indicate the number of each type of *cis*-motif found in the promoter region of each gene (see color codes in the chart). Analysis of *cis*-regulatory element discovery with Rsat::plants tools in the 5-upstream promoter region (-500-to-+200 bp) of 47 *Bdhn* genes from four *Brachypodium* species. **(b)** Maximum k-mer significance values from each analysis are shown for *Bdhn* sequences from each species **(c)** Maximum number of sites from each background analysis are shown for *Bdhn* sequences from each species. In each case, another 47 random gene sequences from the respective reference genome were analyzed ten times as controls (grey bars; C1-C10).

**Figure S3.** Chromosomal location of the 10 *Bdhn* genes in 54 *B. distachyon* ecotypes. *Bdhn* genes (clusters) detected in each ecotype were compared against the dehydrins of the reference genome (Bd21 v3) and assigned to the cluster with highest score using global pairwise alignment (Needleman-Wunsch with BLOSUM62). *Bdhn* color codes and the accuracy of the annotations are indicated in the charts.

**Figure S4.** Maximum likelihood *B. distachyon* dehydrin trees obtained from the aligned exon and intron sequences of each independent *Bdhn* gene (*Bdhn1* to *Bdhn10*). Trees were constructed with IQTREE using

the *B. stacei* outgroup sequence to root the tree. Bootstrap support is indicated on branches. Accession codes correspond to those indicated in Supplementary Table 4.

**Figure S5.** Bidimensional PCA plot of 54 *B. distachyon* ecotypes obtained from 19 climate variables (see Supplementary Table 6). PC1 and PC2 axes comprise 48.5% and 22.4% of the variance, respectively. Ecotype codes correspond to those indicated in Supplementary Table 4. Ellipses include ecotypes classified within cold (aquamarine), mesic (green), and warm (red) climate classes according to their PC1 values.

**Figure S6.** Boxplots and Wilcoxon pairwise significance tests of differential gene expression values (normalized transcript per million, TPM) of the four expressed dehydrin genes (*Bdhn1a*, *Bdhn2*, *Bdhn3*, *Bdhn7*) under joint and separately analyzed drought and temperature stress conditions in 32 *B. distachyon* ecotypes. Averaged expression values for C (Cool), H (Hot), W (Watered), and D (Drought) treatments and their combinations (see text). **(a)** Pairwise comparative tests of combined CD-CW-HD-HW treatments; all dehydrins were significantly differentially expressed in all CD vs CW and HD vs HW tests, by contrast they were not significantly different in all CD vs HD tests and in two CW vs HW tests (*Bdhn1a*, *Bdhn2*); **(b)** Pairwise comparative tests of D vs W treatments; all dehydrins were significantly differentially expressed; **(c)** Pairwise comparative tests of C vs H treatments; none of the dehydrins were significantly differentially expressed.

**Figure S7.** Differentially expressed *Bdhn1a*, *Bdhn2*, *Bdhn3* and *Bdhn7* dehydrin genes (normalized transcript per million, TPM) across 32 ecotypes of *B. distachyon* under drought (D, red) vs watered (W, blue) conditions. Different letters in the boxplots indicate significant group differences (Tukey tests).

**Figure S8.** Linear model regression plots of pairwise dehydrine *Bdhn* expression values (normalized transcript per million, TPM) under joint drought and watered conditions.

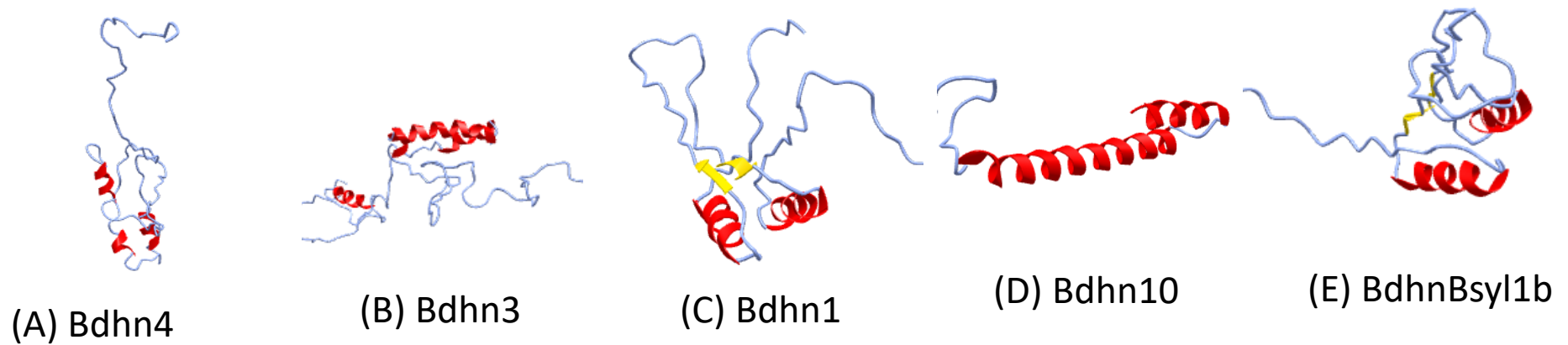
**Figure S9.** Physical locations of orthologous *Brachypodium distachyon* and *Triticum aestivum* water stress responsive dehydrin genes. Drought-induced wheat dehydrin genes are highlighted in colors. *Brachypodium* chromosomes are drawn at 10x scale with respect to wheat chromosomes.

**Figure S10.** Drought-response phenotypic changes as a function of drought treatment averaged across 32 *B. distachyon* ecotypes [leaf\_rwc (relative water content in leaf); leaf\_wc (water content in leaf); lma (leaf mass per area); pro (leaf proline content); abvrgd (above ground biomass); blwgrd (below ground biomass); tlmass (total mass); rmr (root mass ratio); delta13c (carbon isotope, a proxy for lifetime integrated WUE); leafc (leaf carbon content); leafn (leaf nitrogen content); cn (leaf carbon/nitrogen ratio)]. Asterisks above boxes indicate Wilcoxon pairwise significant difference among drought (D, red) and watered (W, blue) conditions (p-value<0.001, \*\*\*).

**Figure S11.** Linear model regression plots of dehydrin *Bdhn1a*, *Bdhn2*, *Bdhn3* and *Bdhn7* expression values (normalized transcript per million, TPM) and drought-response phenotypic traits changes under total dry (D) and watered (W) conditions.

**Figure S12.** Maximum Likelihood *B. distachyon* nuclear species tree cladogram showing the relationships of 30 ecotypes. Phyloheatmaps of normalized values for different sets of variables: (a) dehydrin (*Bdhn1*, *Bdhn2*, *Bdhn3*, *Bdhn7*) gene expression values under watered (W) and drought (D) conditions; (b) drought-response phenotypic traits (leaf\_rwc; leaf\_wc; lma; pro; abvrgd; blwgrd; ttlmass; rmr; delta13c; leafc; leafn; cn) values under watered (W) and drought (D) conditions; (c) climate niche PCA1 values. Traits showing significant phylogenetic signal are highlighted with dotted lines (see Supplementary Table 13a).

Fig.S1



Complete sets of 3D structures for all *Brachypodium* species and proteins are available in the following links:

**Brachypodium distachyon:** <http://zeta.uma.es/public/journal/brachy/raptorx/distachyon.html>

**Brachypodium hybridum D:** <http://zeta.uma.es/public/journal/brachy/raptorx/hybridumD.html>

**Brachypodium stacei:** <http://zeta.uma.es/public/journal/brachy/raptorx/stacei.html>

**Brachypodium hybridum S:** <http://zeta.uma.es/public/journal/brachy/raptorx/hybridumS.html>

**Brachypodium sylvaticum:** <http://zeta.uma.es/public/journal/brachy/raptorx/sylvaticum.html>

Fig.S2

(a)

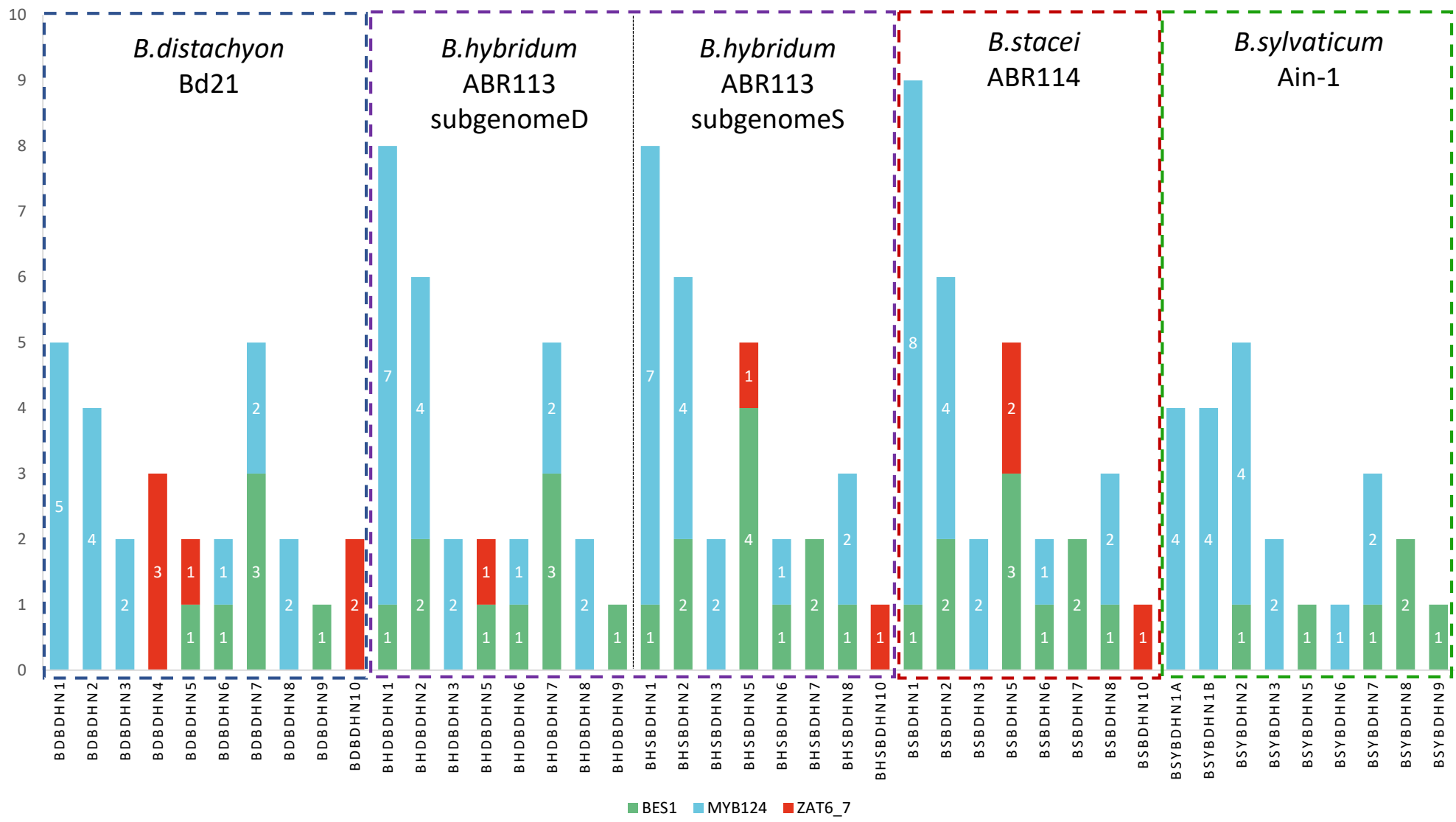
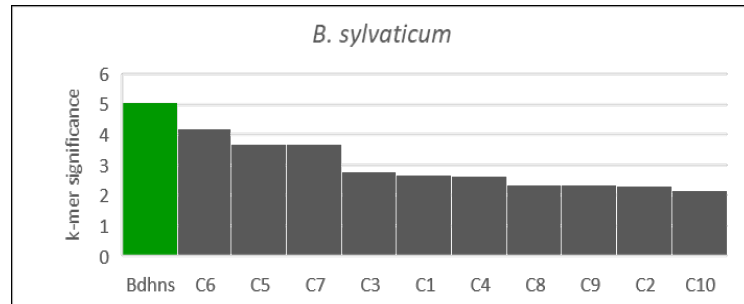
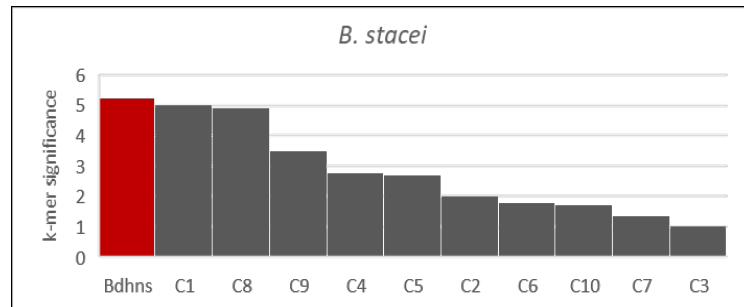
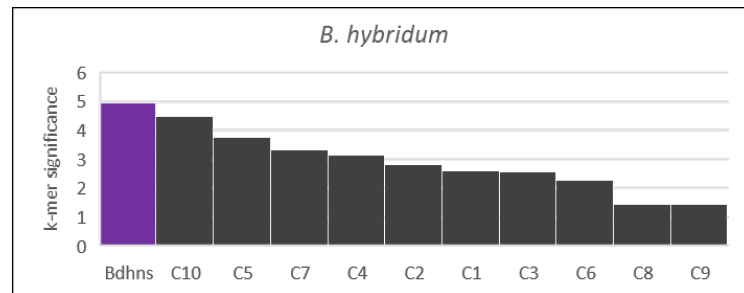
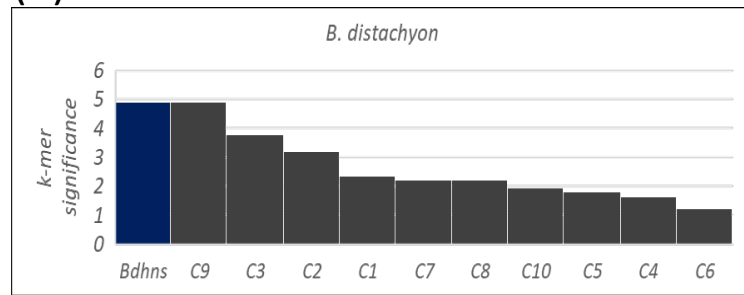


Fig.S2

(b)



(c)

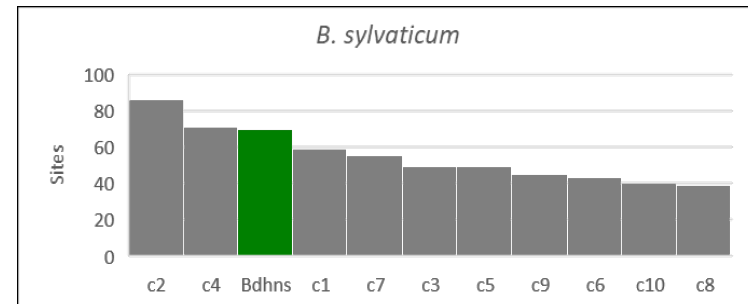
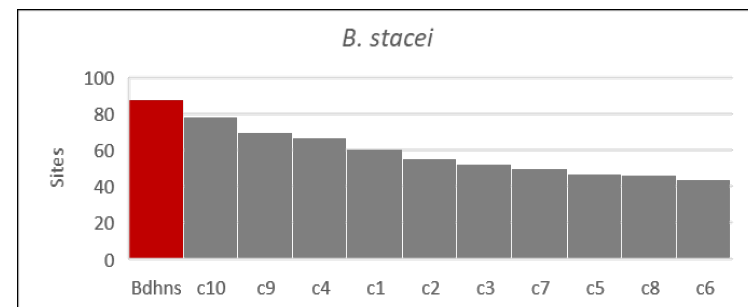
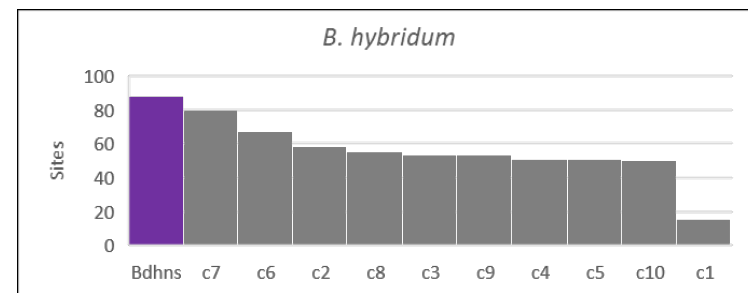
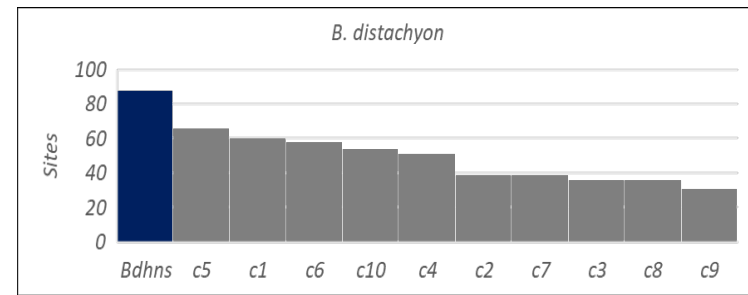


Fig.S3

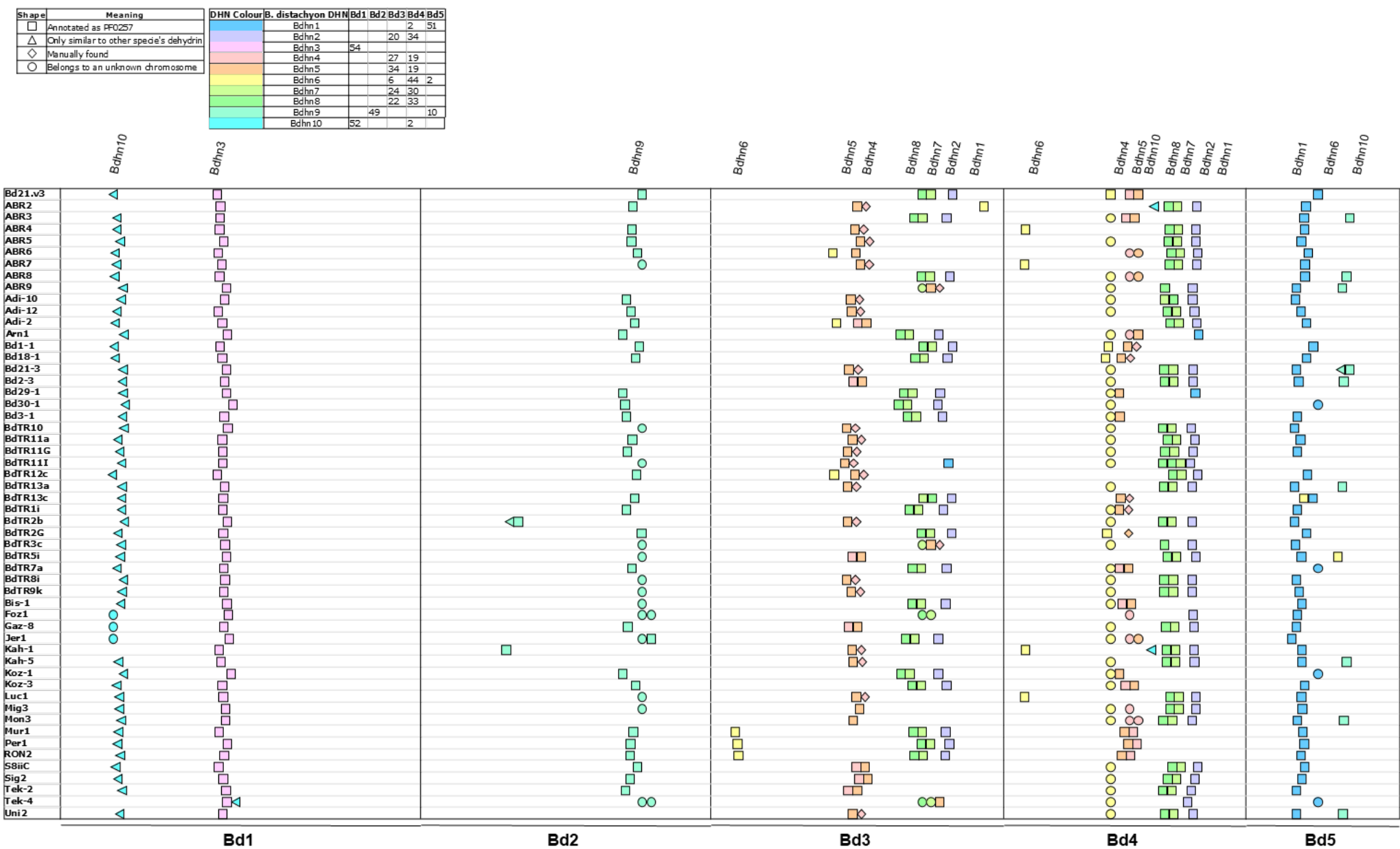


Fig.S4

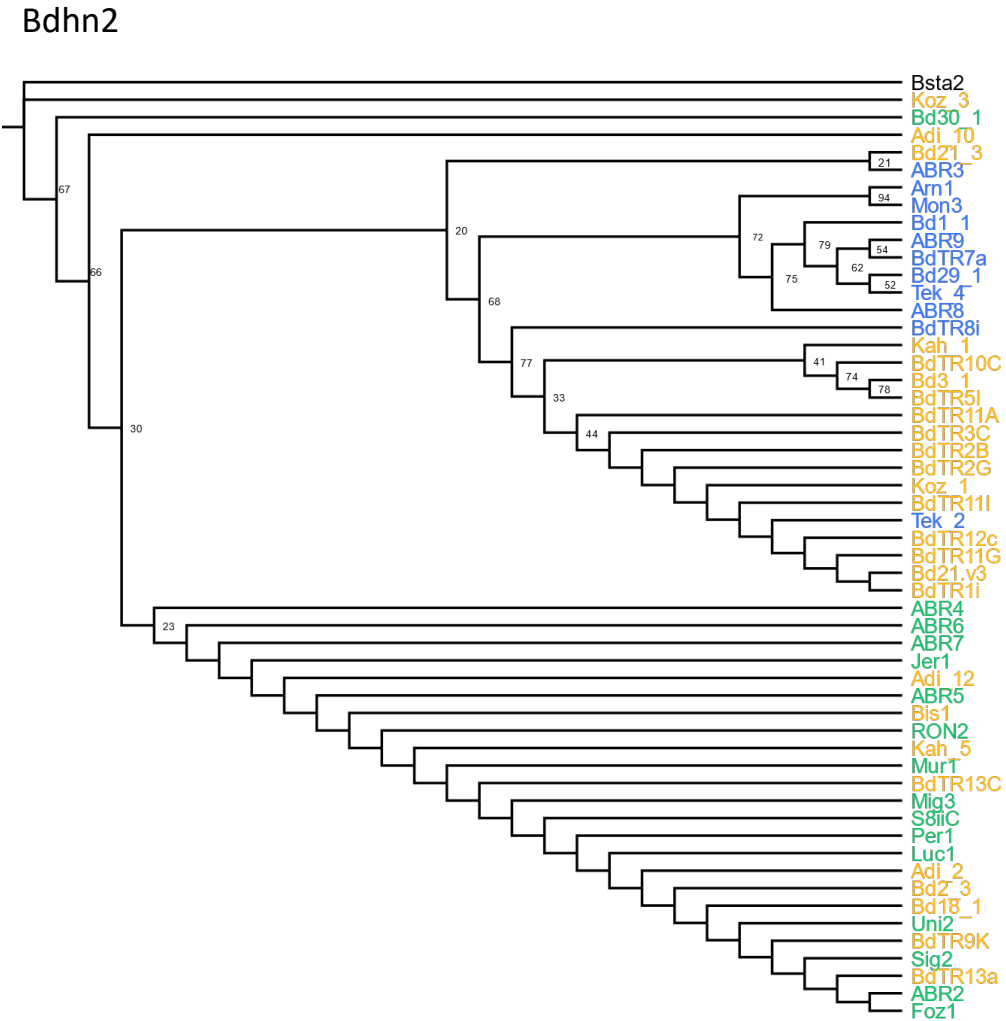
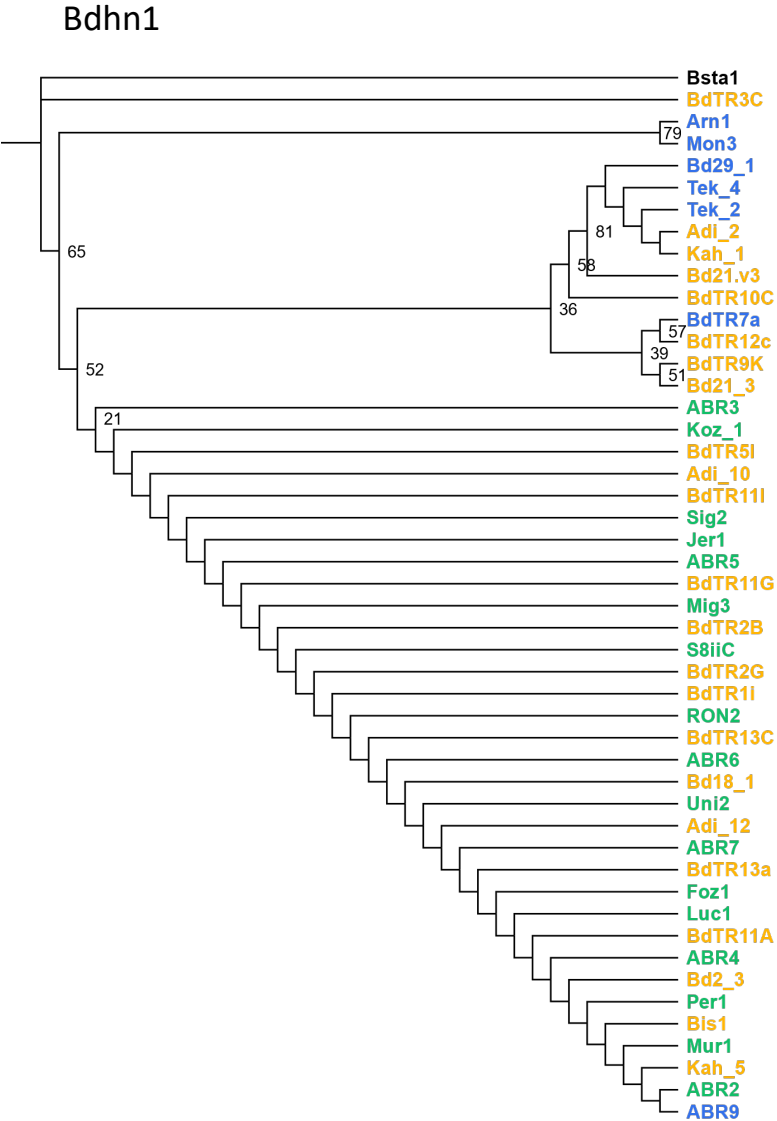




Fig.S4

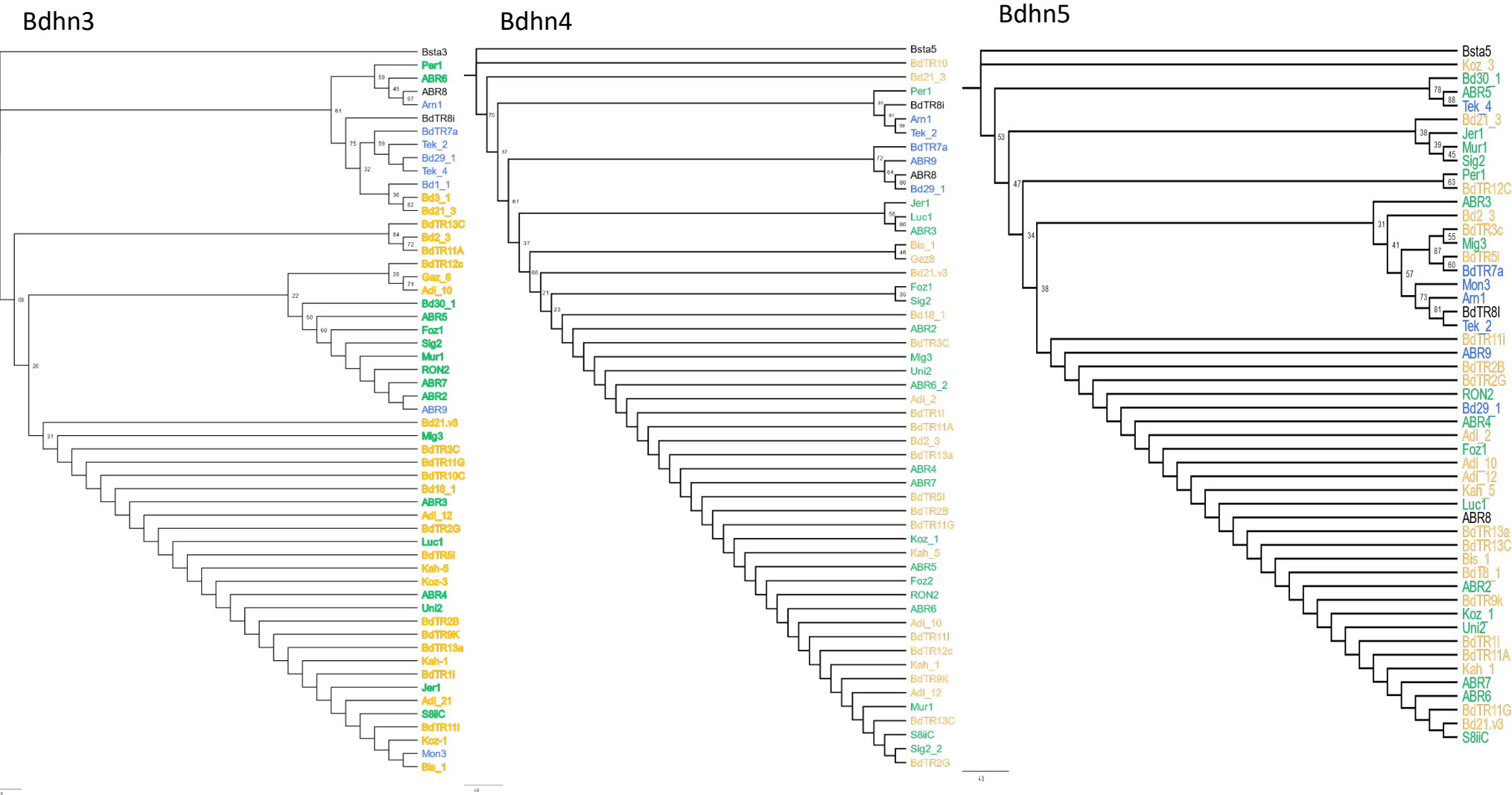


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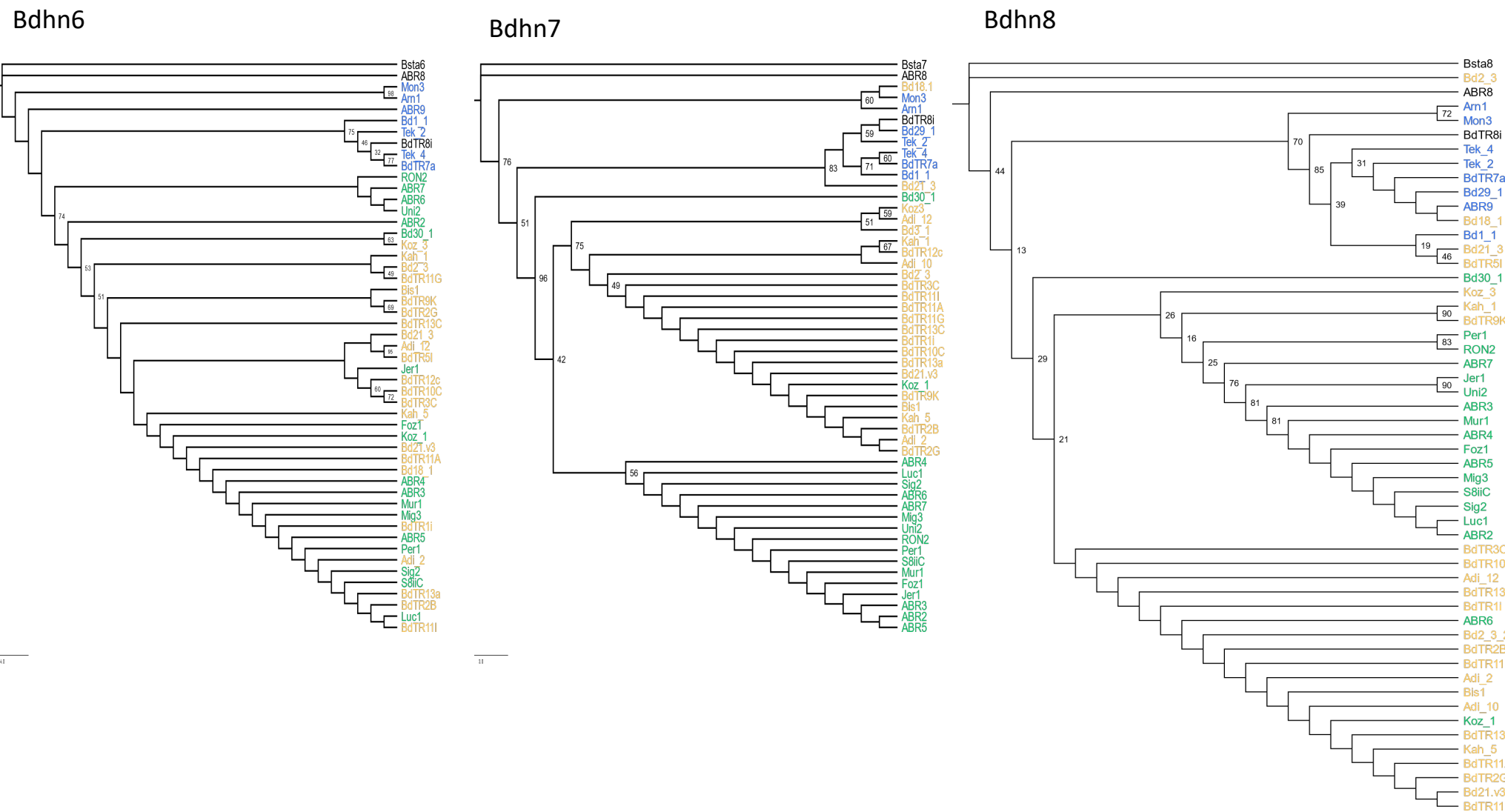


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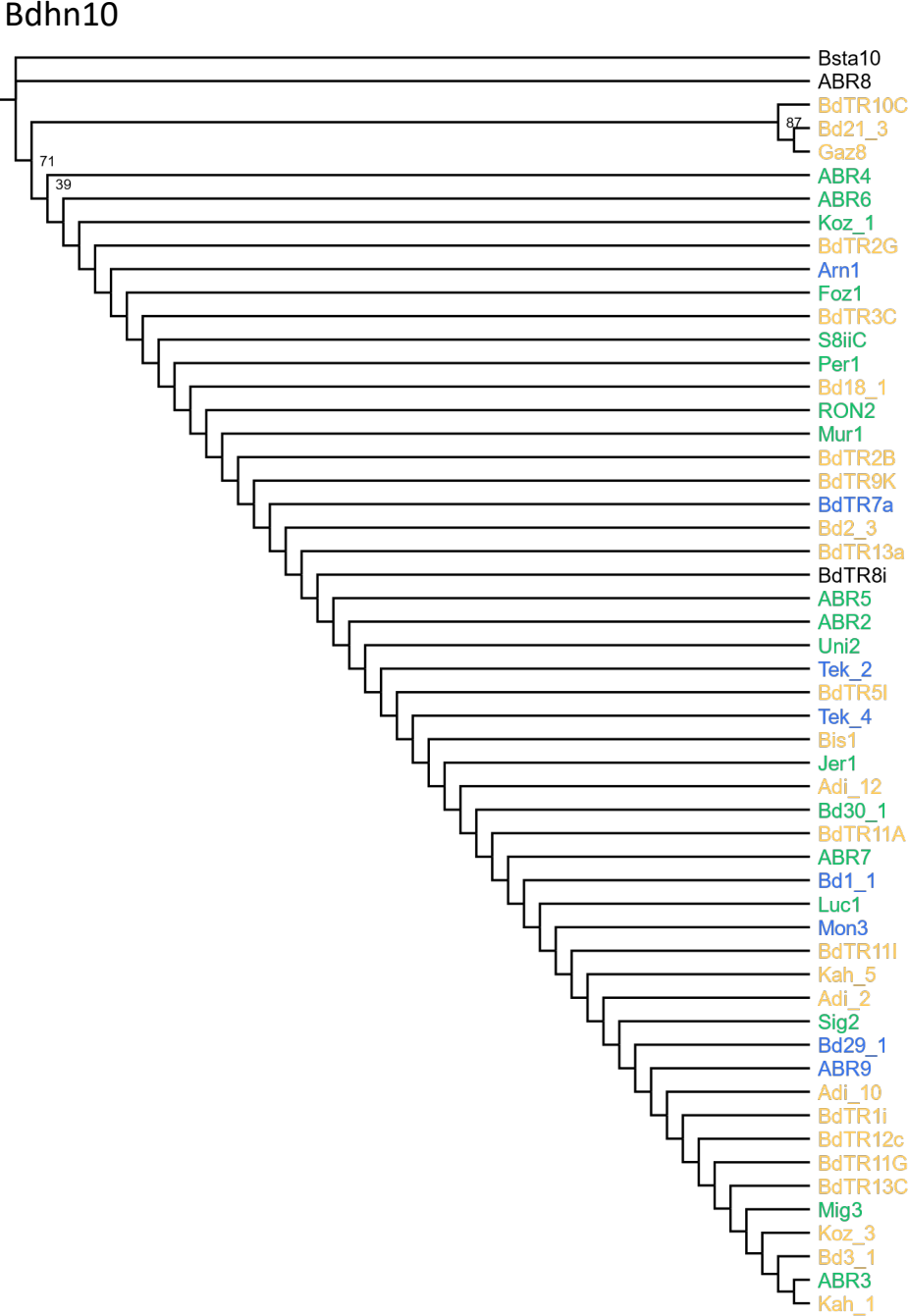
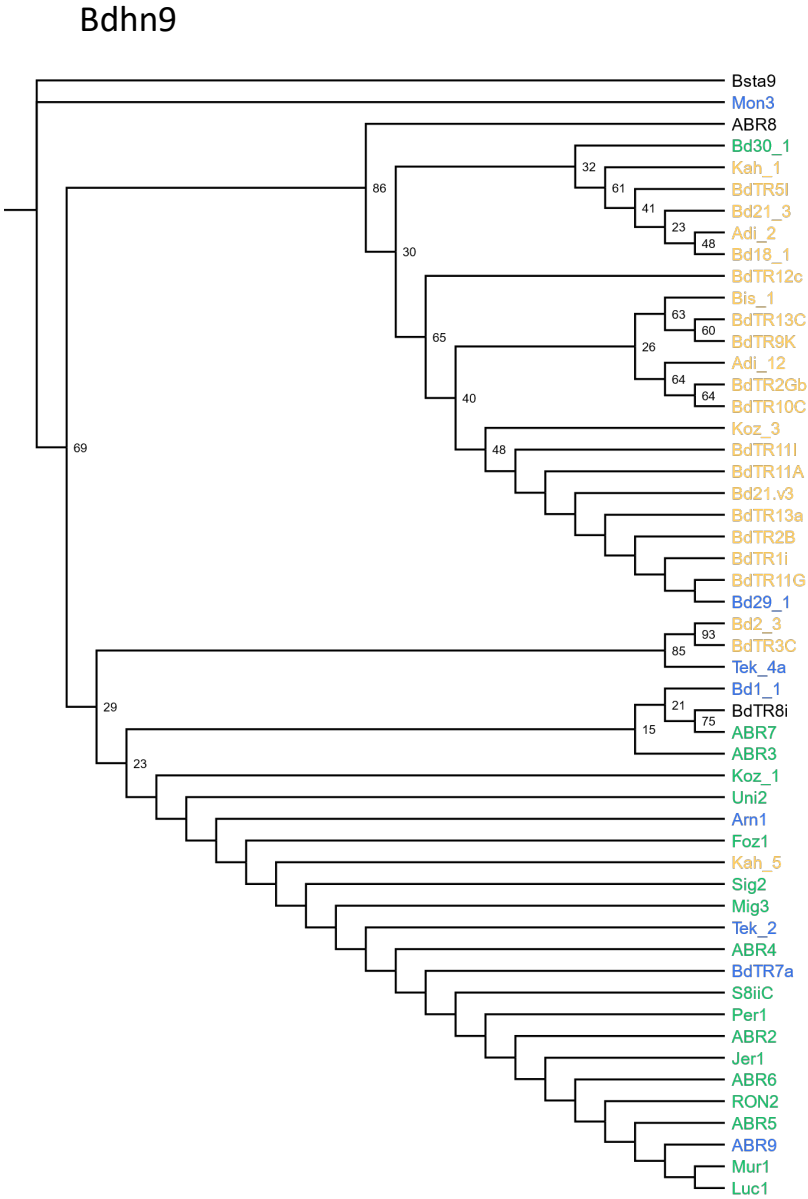


Fig.S5

#	Ecotypes	PCA1
1	ABR2	2.579269
2	ABR3	4.86108471
3	ABR4	5.69006454
4	ABR5	5.39529274
5	ABR6	3.40042965
6	ABR8	2.17586982
7	Adi10	-1.75668741
8	Adi12	-1.75668741
9	Adi2	-1.75668741
10	Bd1-1	-0.5973436
11	Bd18-1	0.73085546
12	Bd2-3	-5.71768222
13	Bd21-3	-3.51663046
14	Bd21control	-3.51663046
15	Bd3-1	-3.51663046
16	Bd30-1	-0.45762855
17	BdTR10c	0.88782452
18	BdTR11g	1.03334719
19	BdTR11i	0.04350879
20	BdTR1i	-0.28516673
21	BdTR2b	1.56035254
22	BdTR2g	2.60379124
23	BdTR5i	2.60379124
24	BdTR9k	1.12002182
25	Bis1	-3.66257669
26	Kah1	-3.54231265
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28	Koz1	-3.2693441
29	Koz3	-3.2693441
30	RON2	5.47816162

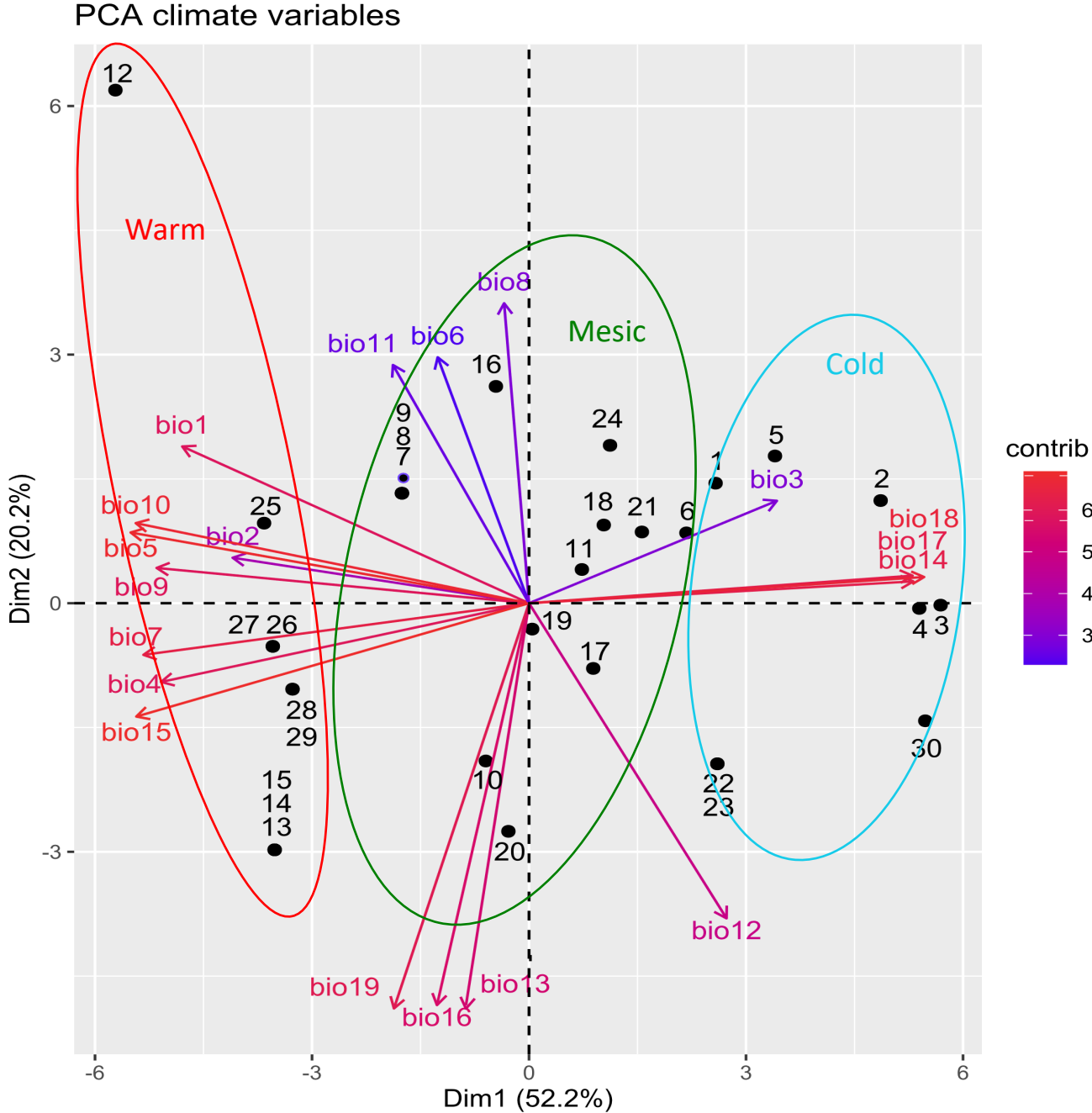
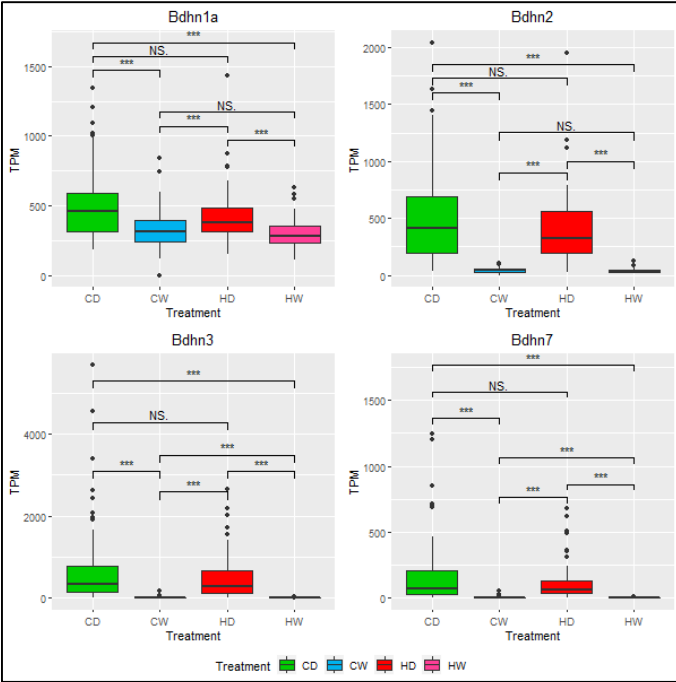
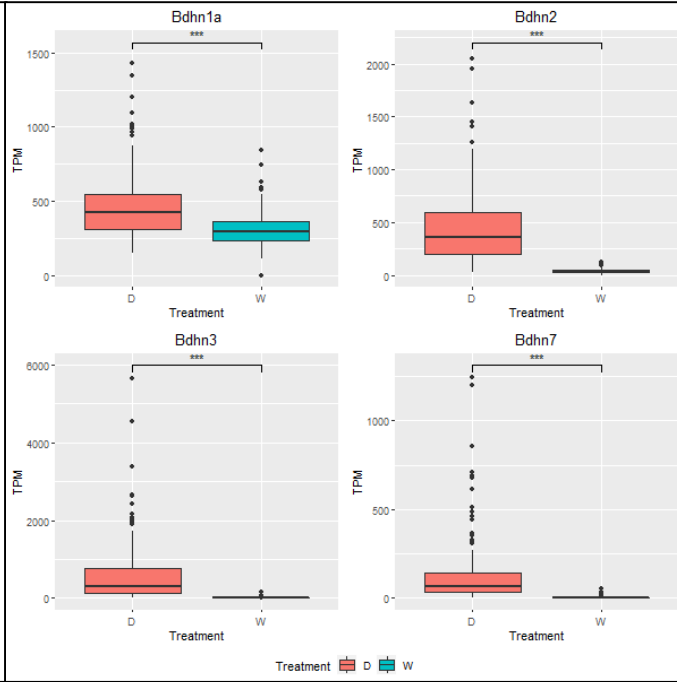


Fig.S6

(a)



(b)



(c)

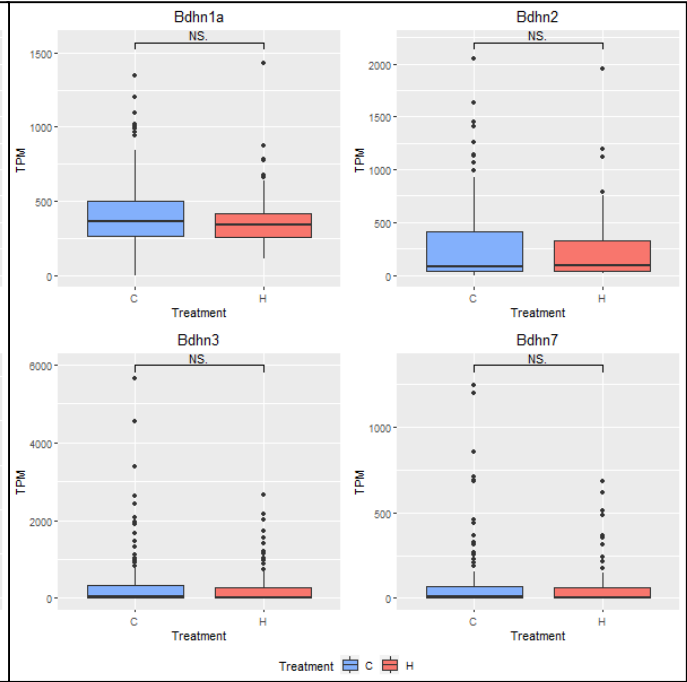
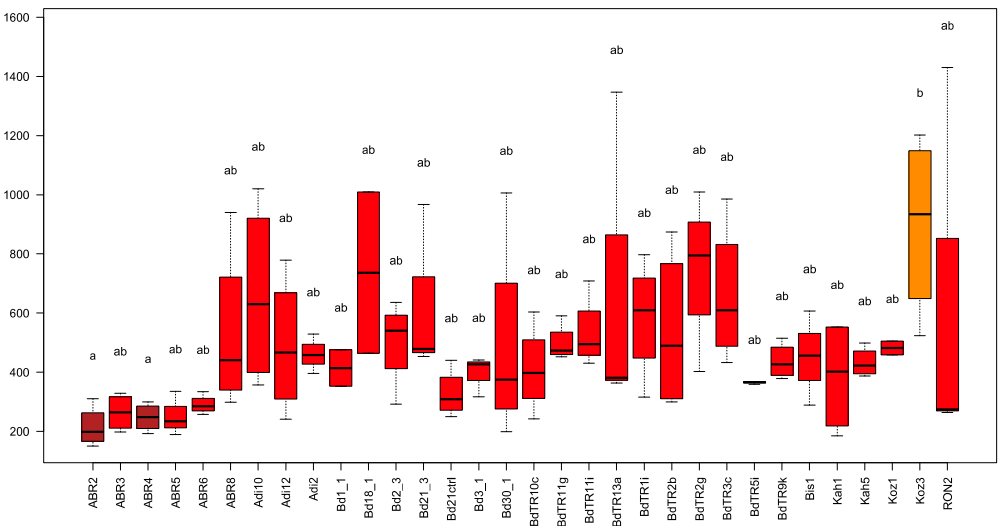
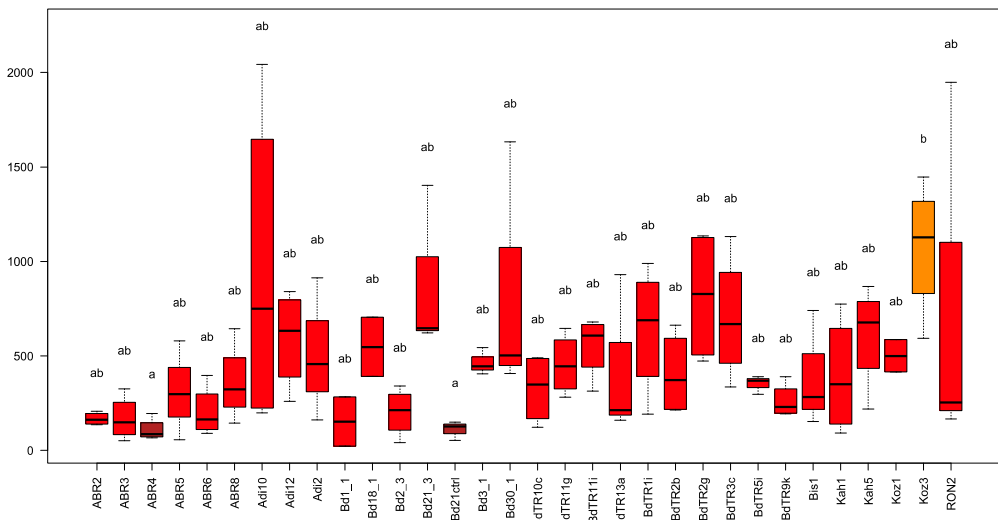


Fig.S7

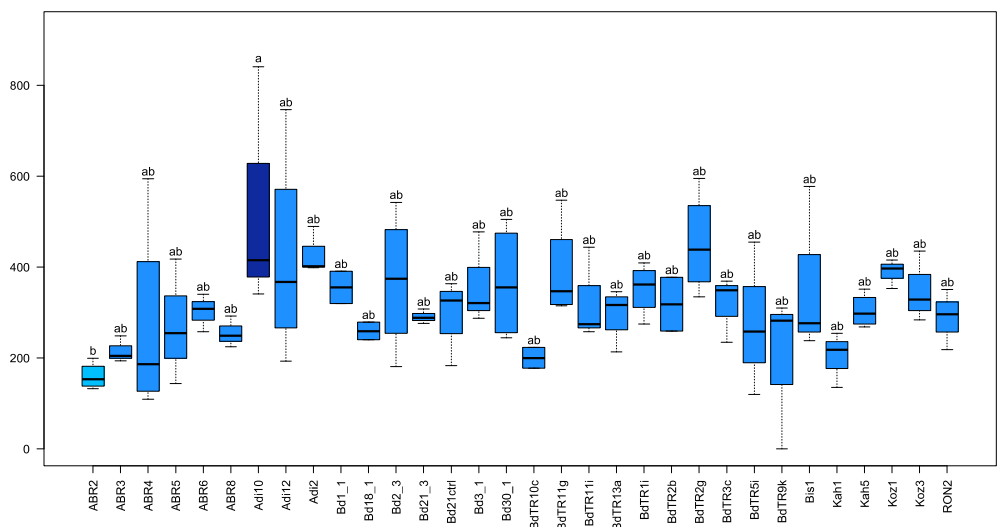
Bdhn1a D



Bdhn2 D



Bdhn1a W



Bdhn2W

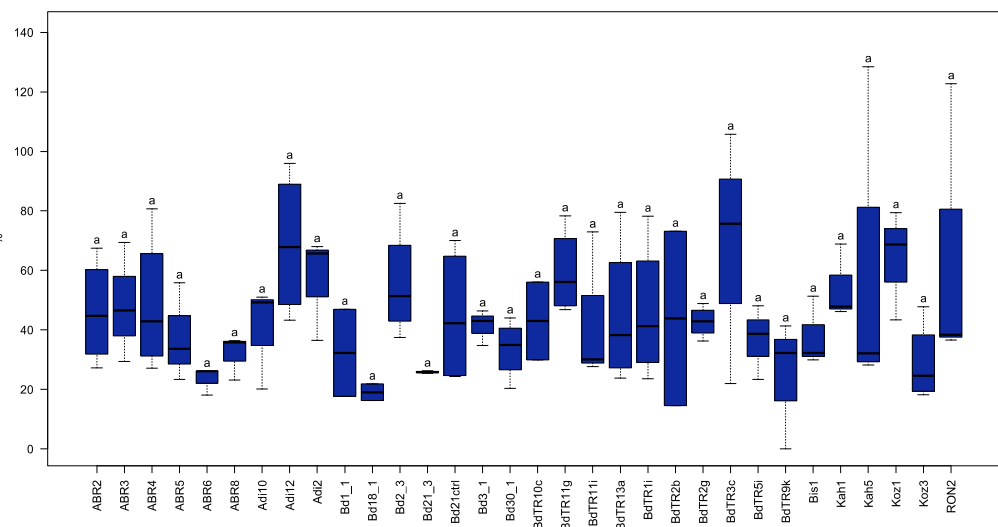


Fig.S7

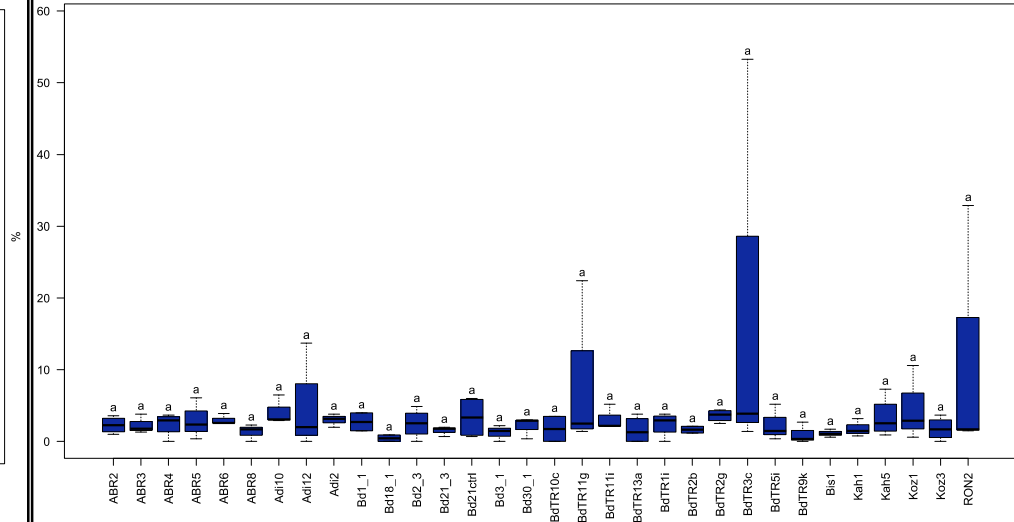
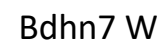
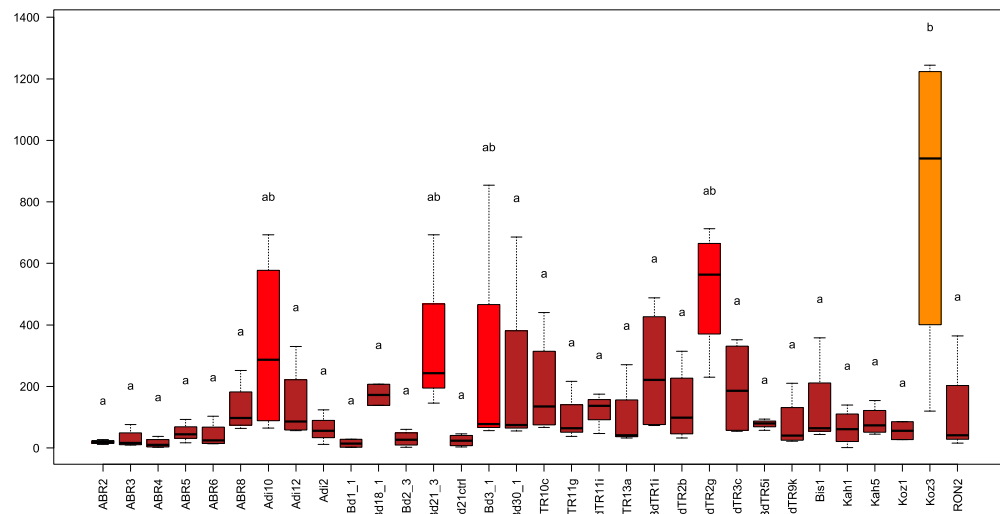
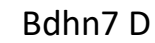
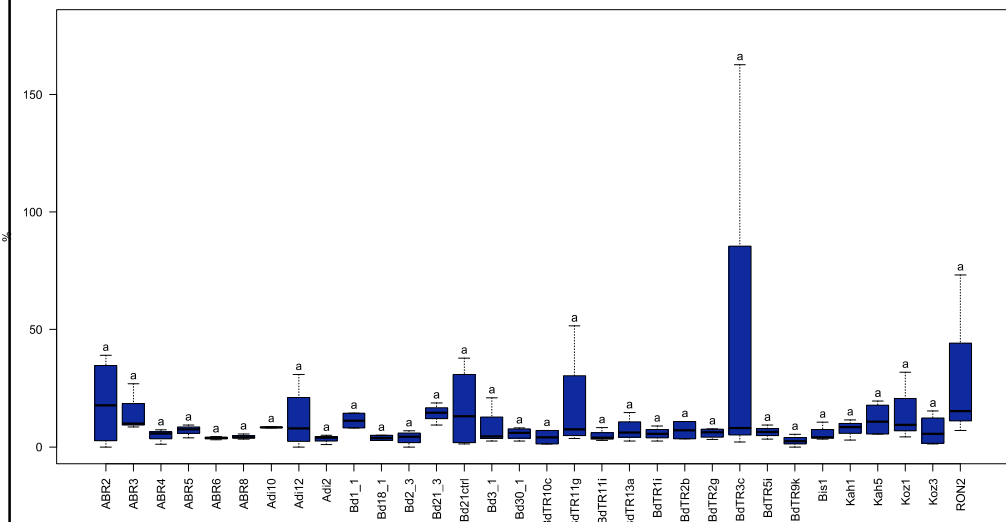
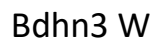
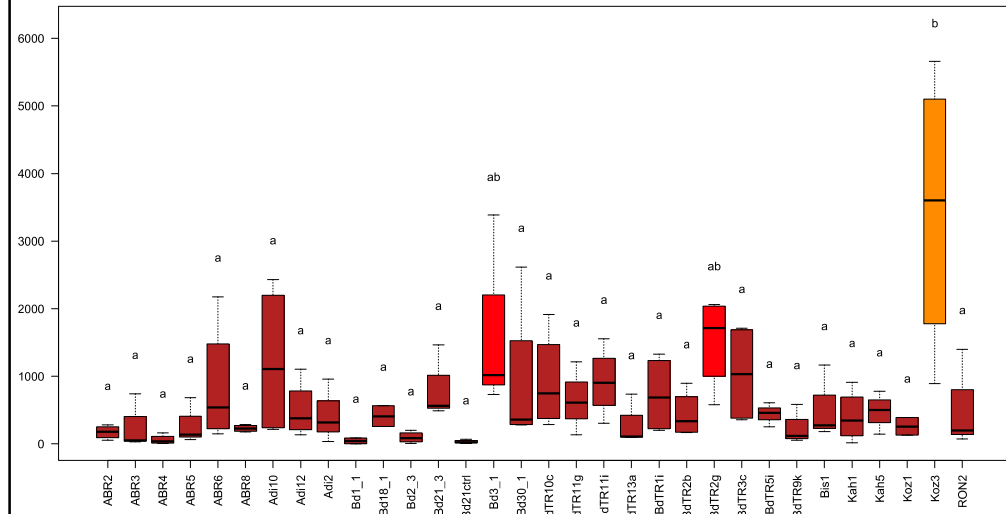
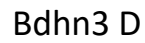


Fig.S8

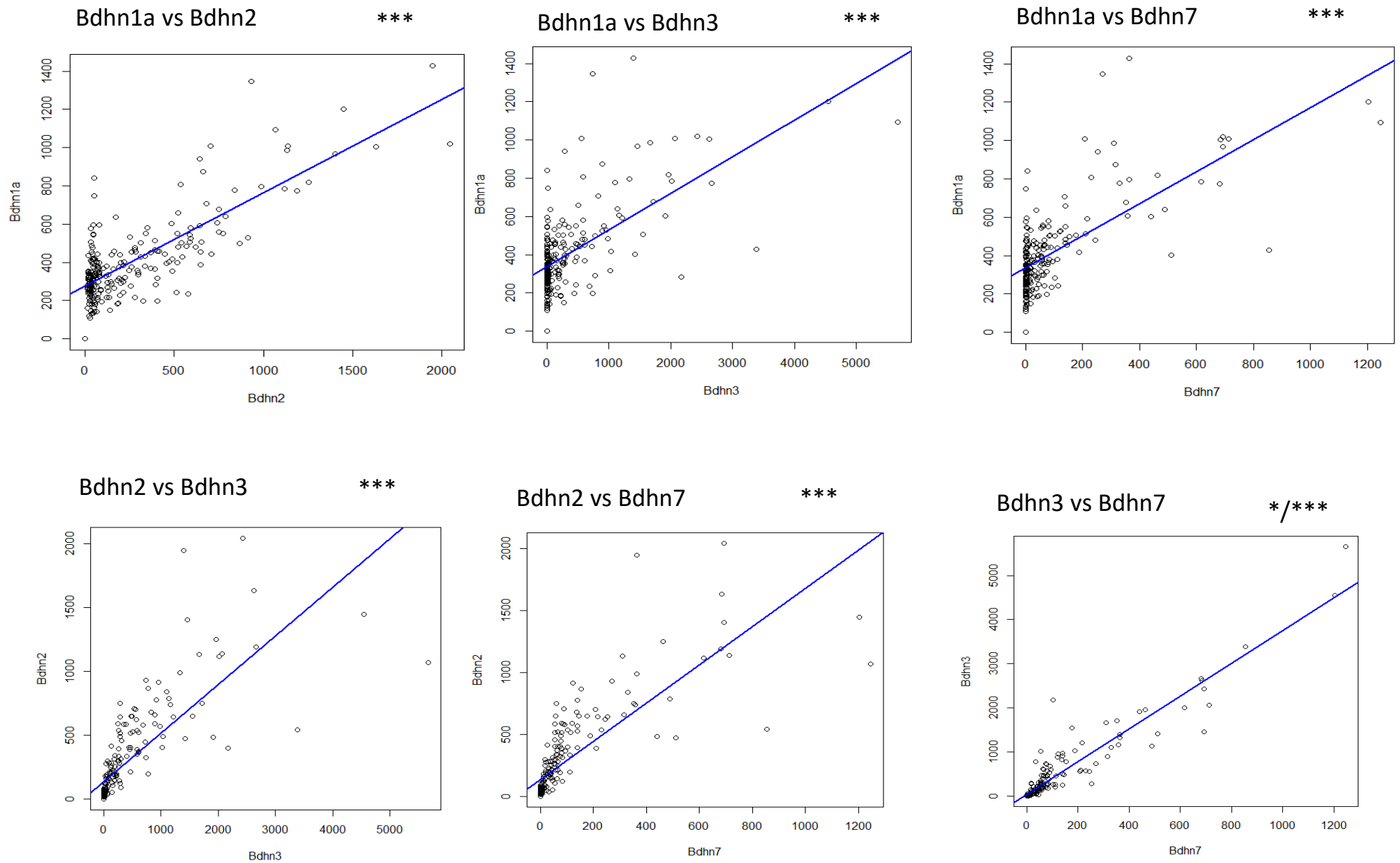




Fig.S9

*Triticum aestivum*

*Brachypodium distachyon*

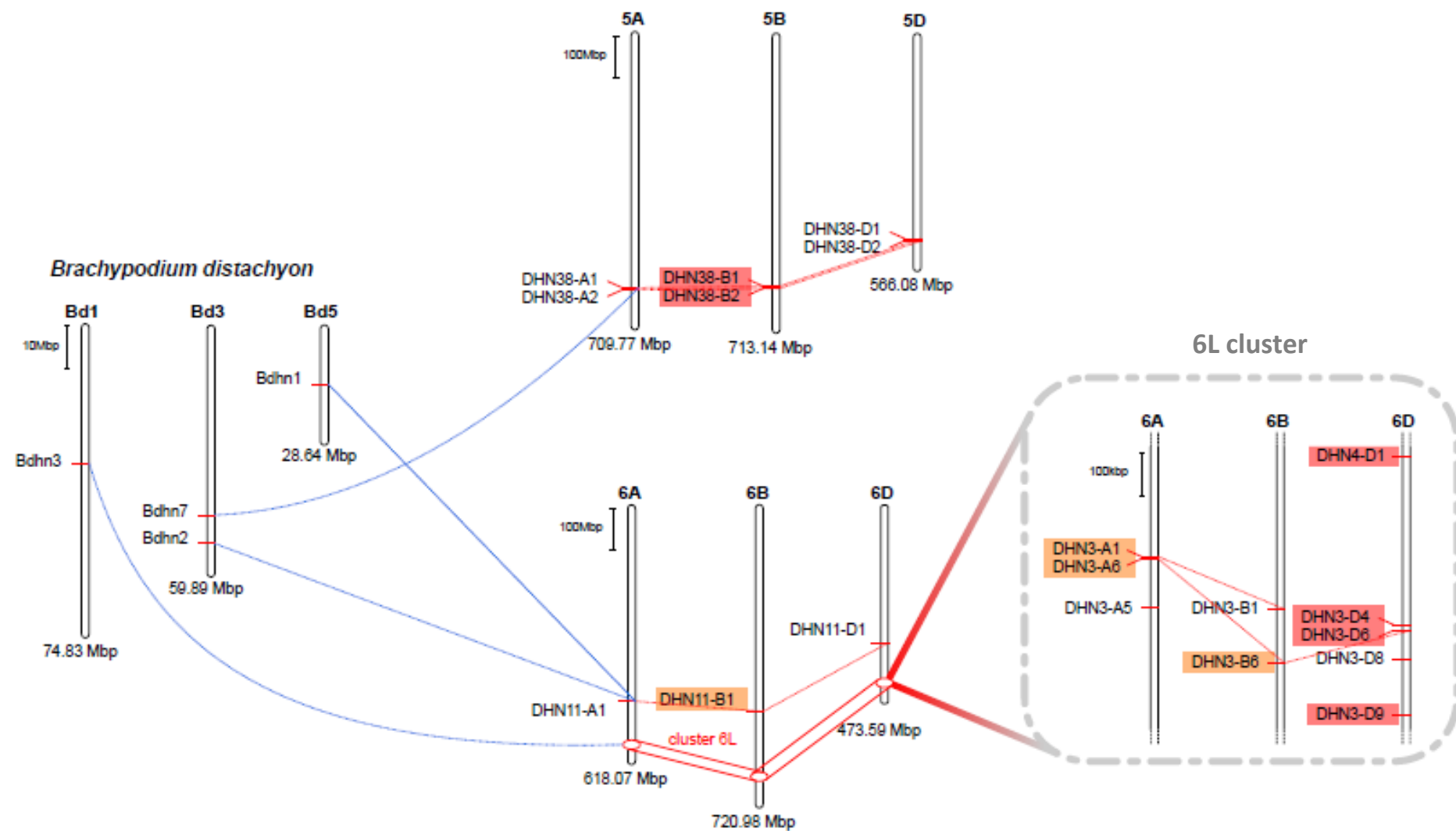


Fig. S10

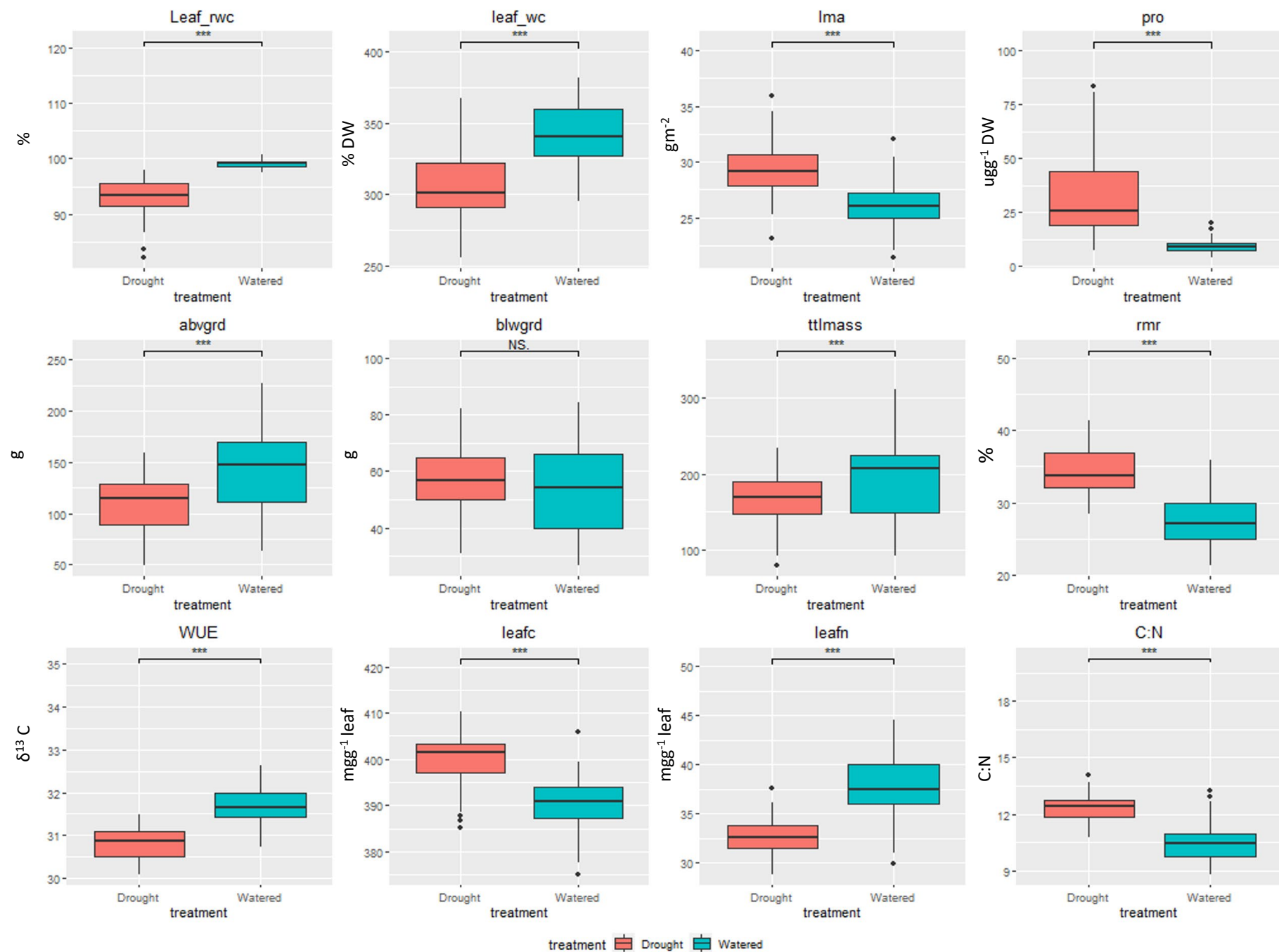


Fig.S11

Bdhn1a vs phenotypic traits

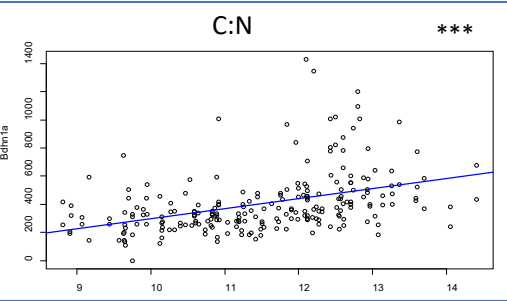
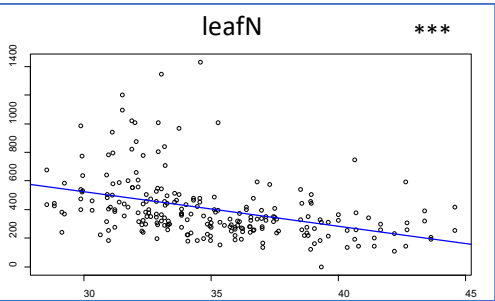
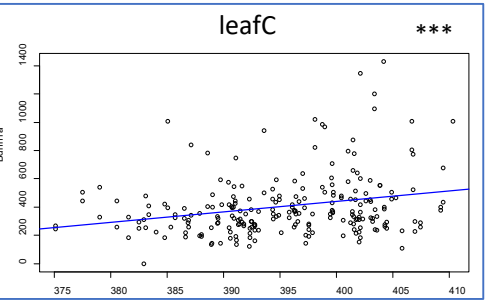
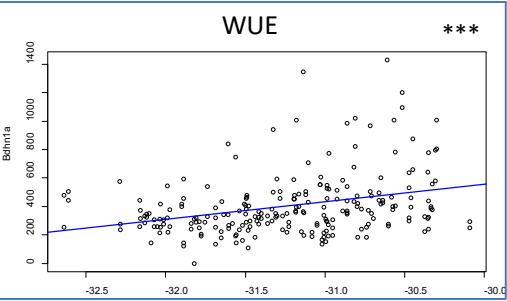
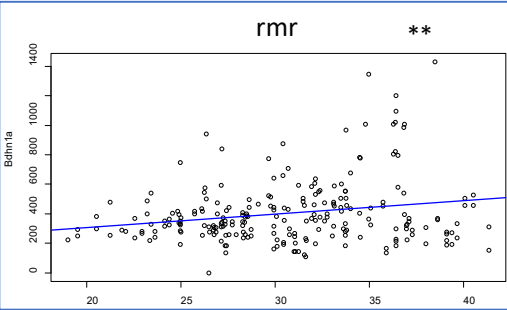
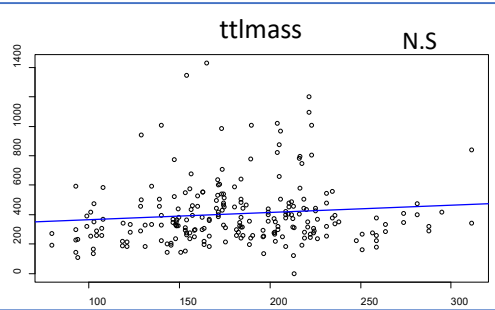
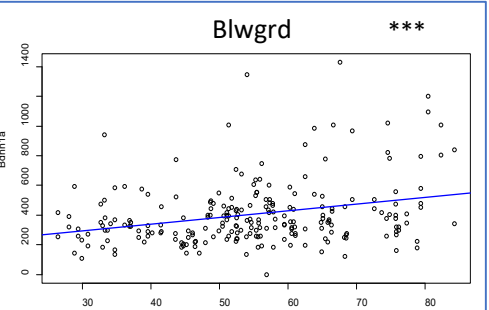
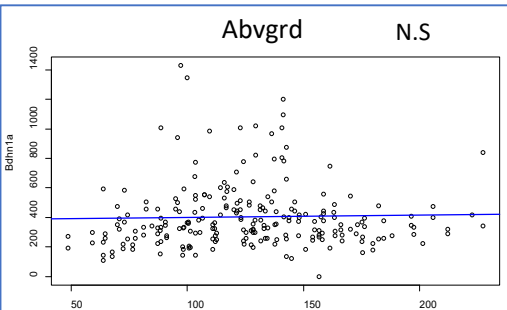
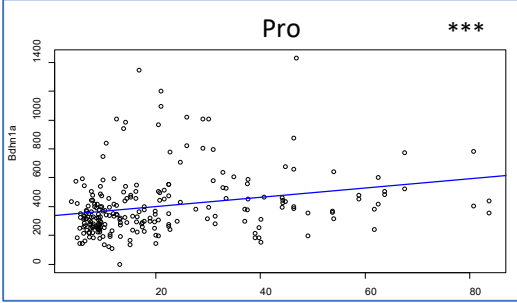
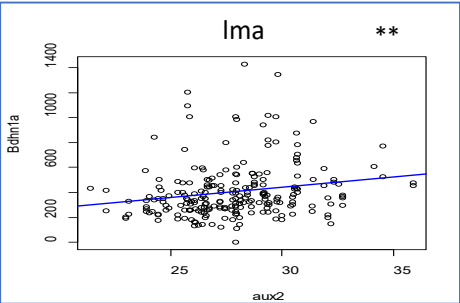
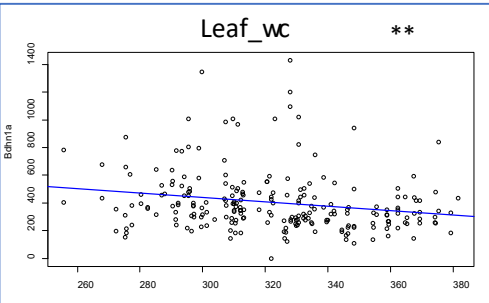
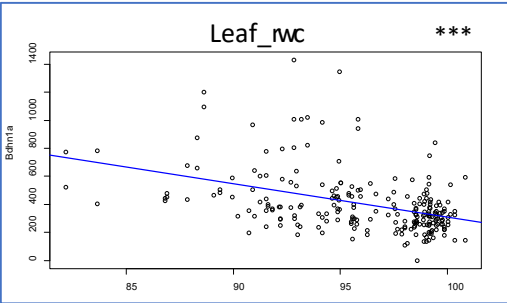


Fig.S11

Bdhn2 vs phenotypic traits

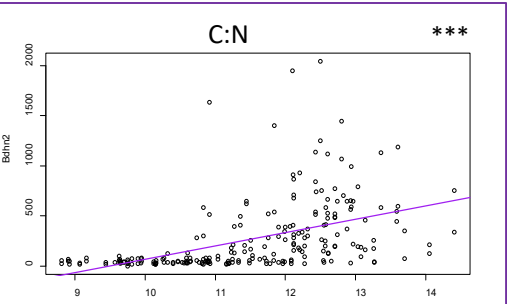
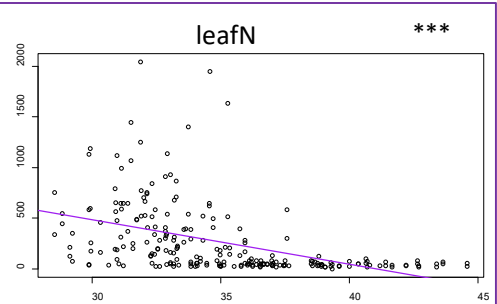
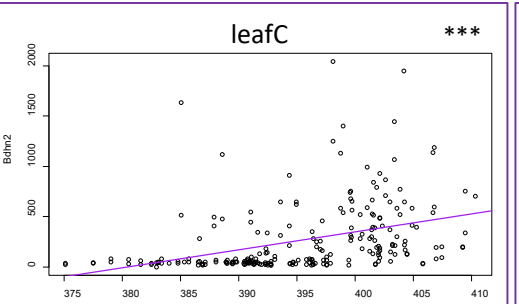
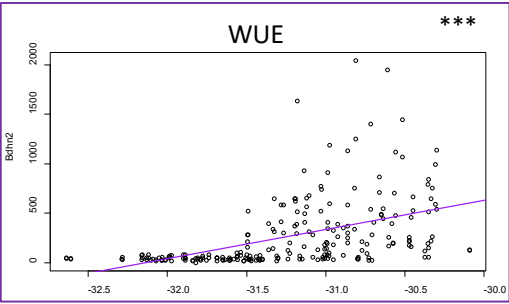
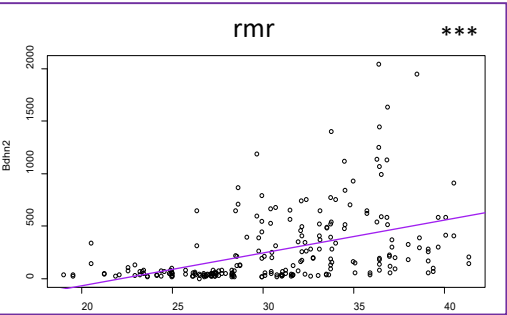
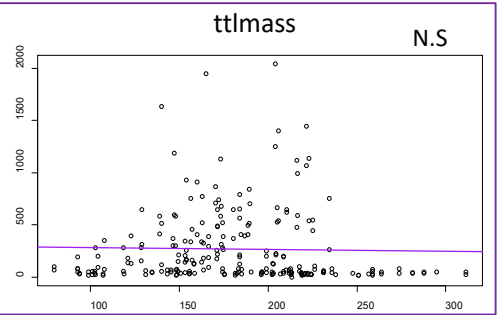
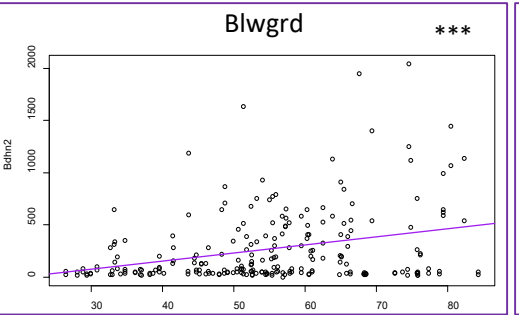
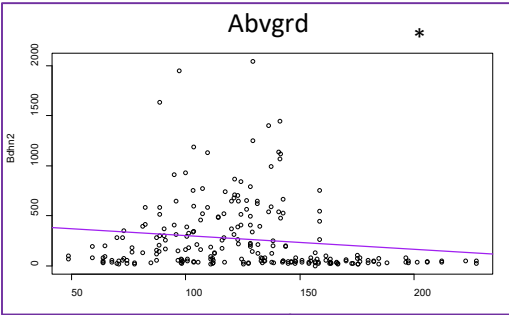
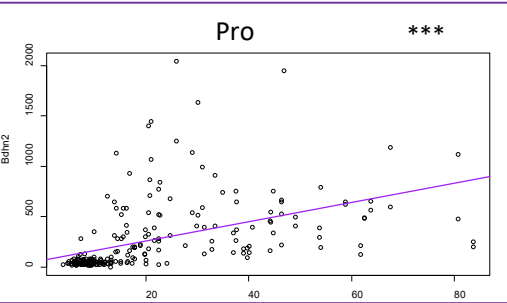
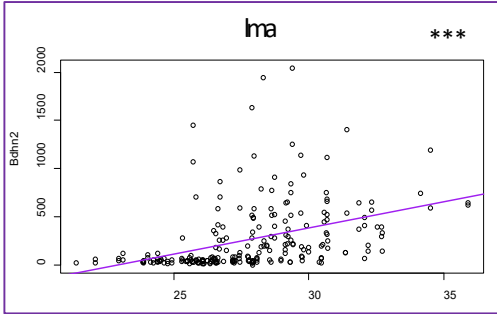
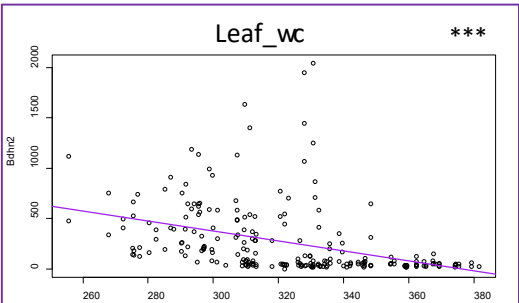
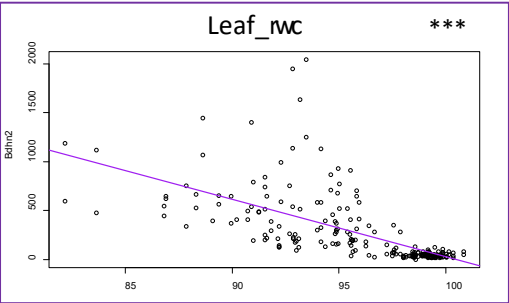


Fig.S11

Bdhn3vs phenotypic traits

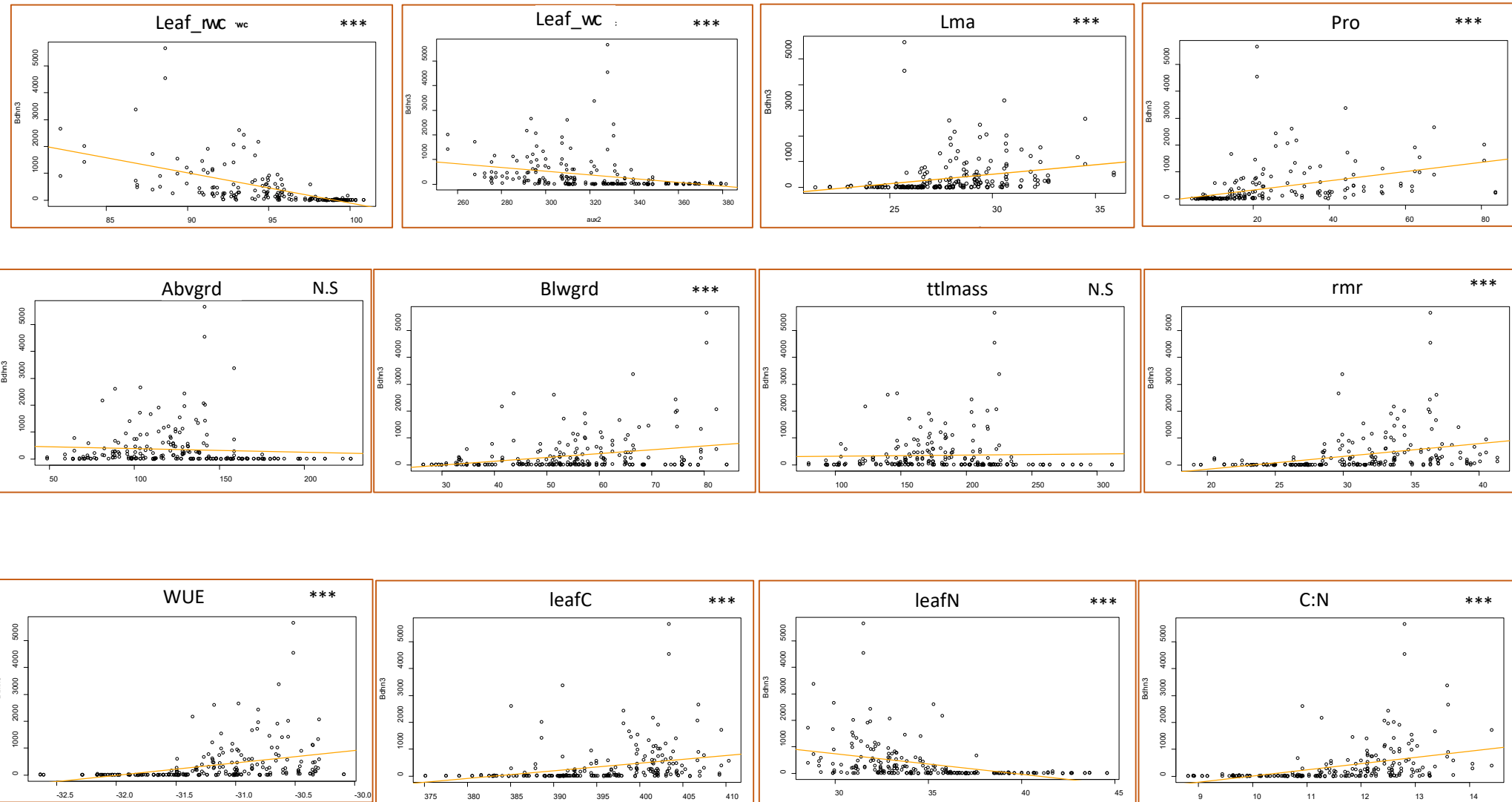


Fig.S11

Bdhn7vs phenotypic traits

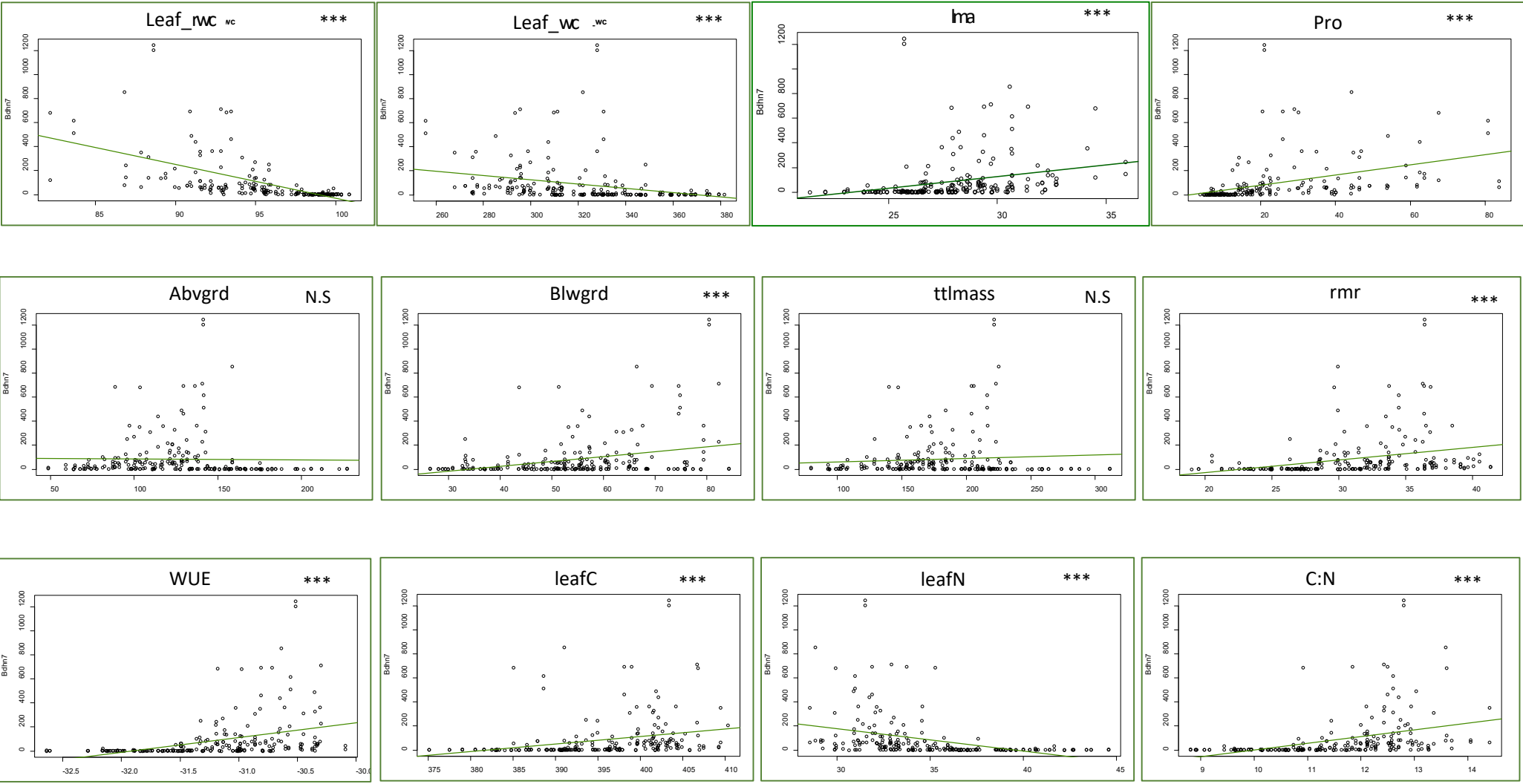


Fig.S12

