

Supporting Information

Metabolite Profiling of Barley Grain Subjected to Induced Drought Stress – Responses of Free Amino Acids in Differently Adapted Cultivars

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Table S1: Plant Breeders of the Investigated Barley Cultivars

	Plant breeder
Argentinian Mutant 6519	Cerveceria y Malteria Quilmes, Argentina
Mackay	Department of Employment, Economic Development and Innovation, Australia (DEEDI)
Palmella Blue (Clho 3609)	Ethiopian Landrace
IPZ 24727	Bavarian State Research Center for Agriculture (LfL), Germany
Marnie	Saatzucht Josef Breun GmbH & Co. KG, Herzogenaurach Germany
Iron	Saatzucht Josef Breun GmbH & Co. KG, Herzogenaurach Germany
Barke	Saatzucht Josef Breun GmbH & Co. KG, Herzogenaurach Germany
Steina	Saatzucht Josef Breun GmbH & Co. KG, Herzogenaurach Germany
Streif	Saatzucht Streng-Engelen GmbH & Co.KG, Uffenheim, Germany
Emperor	University of Guelph, Canada
Polygena	Saatzucht Hadmersleben GmbH, Hadmersleben, Germany

Table S2: *P*-Values of Identified Metabolites from Each Single Cultivar Found to be Statistically Significant for the Factor Growing Conditions/Location Across Three Consecutive Seasons

Metabolite	Arg. Mutant 6519	Mackay	Palmella Blue	IPZ 24727	Mamie	Iron	Barke	Steina	Streif	Emperor	Polygena
	<i>p</i> - value				<i>p</i> - value						
<i>metabolites with decreasing levels</i>											
mannitol	<0.001 (a,b)	<0.001	0.0076	<0.001 (b)	<0.001	0.002	<0.001 (a)	<0.001	<0.001	<0.001 (b)	<0.001
glutamic acid	_ ^x	<0.001 (a,b)	<0.001 (a)	0.0068 ^{xx}	-	-	-	-	-	0.0022 (a,b)	-
2-glycero-phosphoric acid	-	-	-	-	-	-	-	-	-	0.0026 (a) ^{xx}	-
<i>metabolites with increasing levels</i>											
glucose	<0.001 (a,b) ^{xx}	<0.001	0.0021 (a)	-	<0.001	<0.001	<0.001	0.0032	<0.001 (a)	<0.001 (b)	<0.001
fructose	-	0.0014	0.0076 (a)	<0.001 (a,b)	0.0016	<0.001 (a,b)	<0.001	-	<0.001	<0.001	<0.001
raffinose	-	<0.001	0.002	0.0093	-	-	<0.001 (a,b)	-	0.0044	-	-
citric acid	0.0072 (a,b)	<0.001	-	0.0041	0.0025	<0.001	<0.001	-	0.006	<0.001 (a)	0.0095
iso-citric acid	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001 (a,b)	<0.001	<0.001	<0.001 (a,b)	<0.001
malic acid	<0.001	-	-	-	-	-	-	-	-	-	-
succinic acid	0.0081 (a)	<0.001	<0.001 (a)	0.0014	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
threonic acid	-	0.0041 (a)	<0.001	<0.001 (a,b)	<0.001 (b)	<0.001	<0.001	-	<0.001 (b)	<0.001	<0.001 (a)
GABA	-	0.0083 ^{xx}	-	-	<0.001 (a)	0.0021	<0.001	-	-	<0.001	-
glycine	<0.001	-	-	-	<0.001	<0.001 (a)	0.0096	-	<0.001	<0.001 (a,b)	-

a statistically significant for factor growing condition/location x season; b statistically significant for factor season; x not statistically significant for factor growing conditions/location;

xx Rain-out-shelter vs. C2: not consistent over all growing seasons

Table S3: Repeatabilities, Mean Recoveries (n = 3), Linearity, Response Factors and Limits of Quantitation Calculated for Free Amino Acids

Compounds	Repeatability RSD ¹ [%]	Mean Recovery [%]	R ²	Response Factor	LOQ ³ [mg/kg flour]
alanine	2.9	78	0.97	0.55	6.6
valine	3.7	89	0.97	0.61	7.3
leucine	1.8	89	0.97	0.65	7.8
proline	3.6	98	0.96	0.61	7.3
isoleucine	6.1	87	0.96	0.61	7.3
glycine	2.3	74	0.95	0.85	10
serine	1.8	103	0.94	0.50	6.0
threonine	3.8	95	0.95	0.55	6.6
aspartic acid	2.5	96	0.97	0.62	7.5
phenylalanine	2.8	91	0.97	0.53	6.3
glutamic acid	2.8	100	0.92	1.17	14
asparagine	3.2	94	0.93	0.81	9.8
glutamine	8.3	68	0.92	0.73	8.7
lysine	4.5	100	0.97	0.61	7.4
tyrosine	5.5	91	0.97	0.57	6.8
tryptophan	11.8	101	0.92	0.56	6.7

¹ RSD: relative standard deviation (n = 3)

² R: regression factor

³ LOQ: limit of quantitation

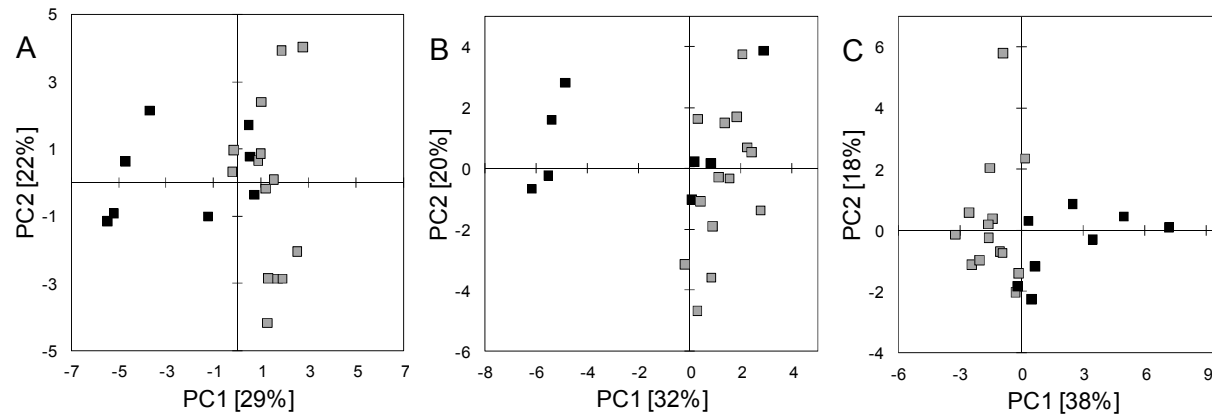


Figure S1

PCA based on agronomic parameters of the two groups of stress resistant/tolerant barley cultivars (black symbols) and of cultivars reported to be either stress susceptible or without specific reports on drought resistance/tolerance (grey symbols) grown under drought stress in a rain-out-shelter in A 2010, B 2011, C 2012. Two field replicates were analyzed in triplicate.

The underlying agronomic parameters comprise yield, grain size, straw weight, thousand kernel weight, kernels per ears, number of ears, relative chlorophyll content of leaves (single-photon avalanche diode measurements at different time points during plant growth), protein content, and malt extract (Reichenberger, G., PhD-thesis, Technische Universität München, in preparation).

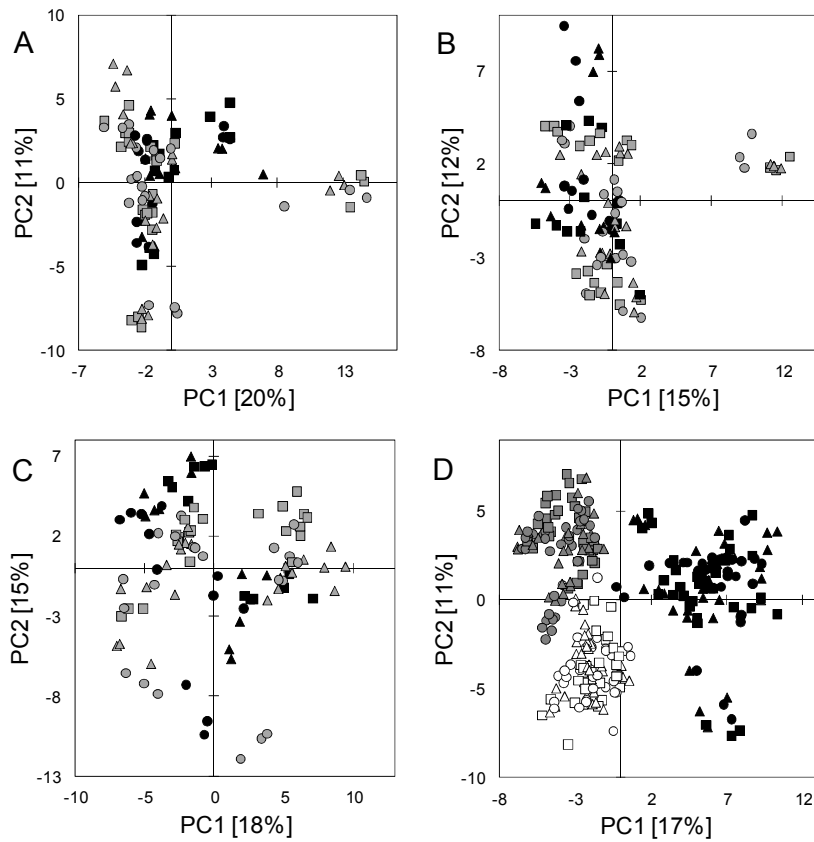
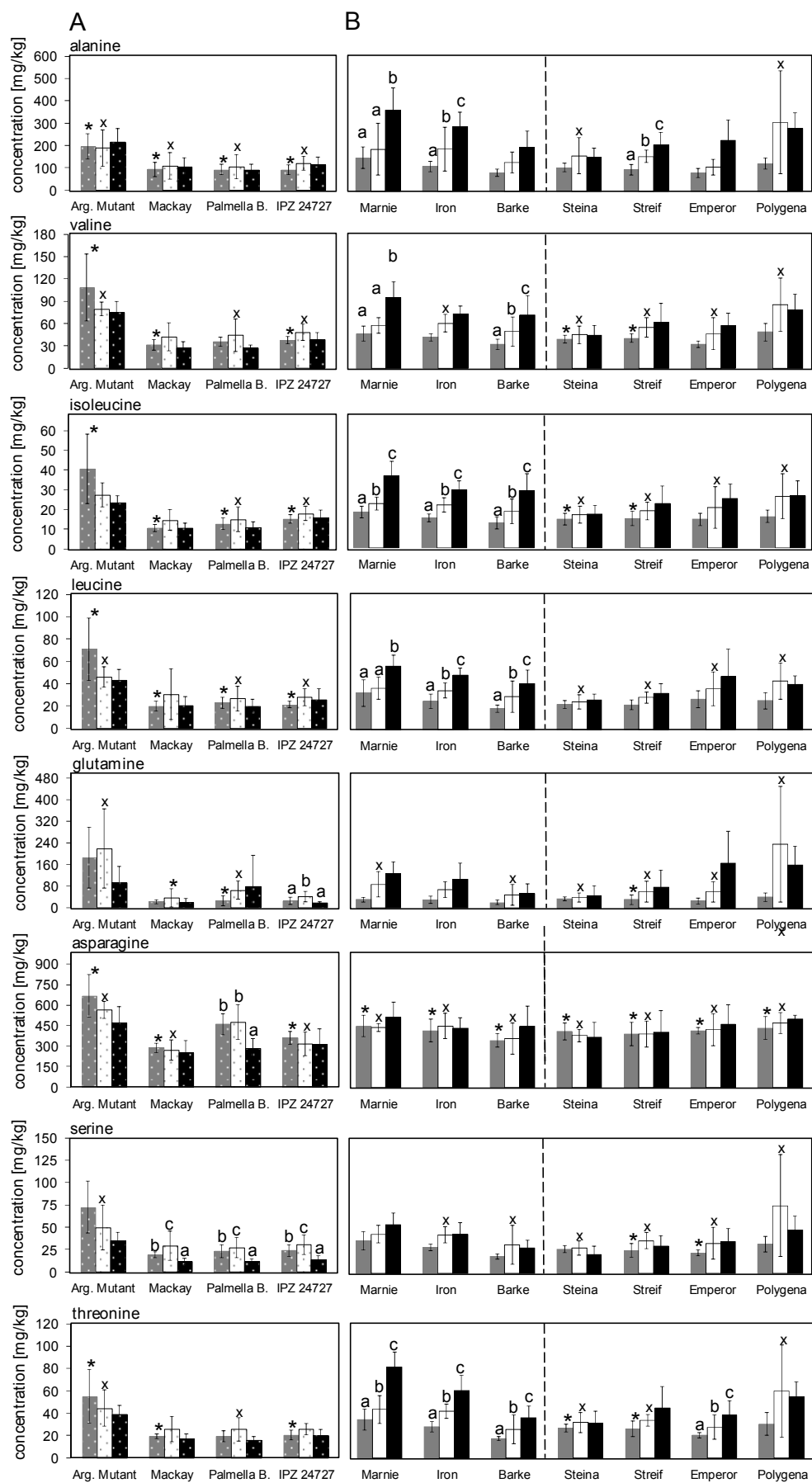


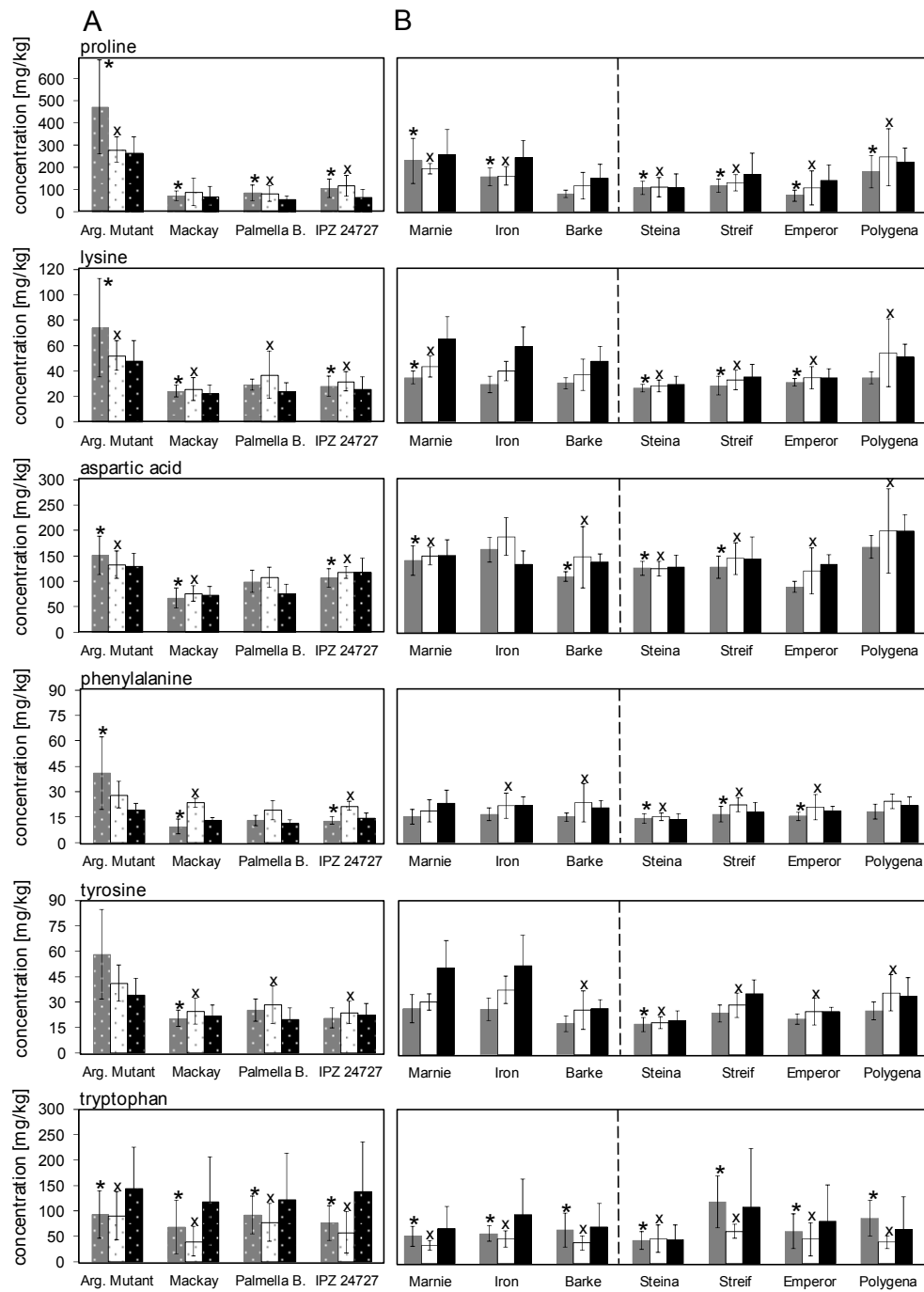
Figure S2

PCA of GC/MS metabolite profiling data of combined lipophilic fractions 1-2 from stress resistant/tolerant barley cultivars (A-C, black symbols) and from cultivars reported to be either stress susceptible or without specific reports on drought resistance/tolerance (A-C, grey symbols) grown under drought stress in a rain-out-shelter (squares) and under natural weather conditions at control location 1 (circles) and control location 2 (triangles) in A 2010, B 2011, C 2012 and in D 2010 (black symbols), 2011 (white symbols), 2012 (grey symbols). One field replicate was analyzed in triplicate.

Supplementary Figure S3 – Part 1



Supplementary Figure S3 – Part 2



x rain-out-shelter vs. C2 * rain-out-shelter vs. C1: not consistent across all growing seasons

Figure S3

Concentrations of proteinogenic, free amino acids. Dotted bars indicate A stress tolerant/resistant cultivars and plain bars indicate B cultivars reported to be either stress susceptible or without specific reports on drought resistance/tolerance. Small letters display statistical significance for the factor growing conditions/location across three consecutive seasons by ANOVA ($p < 0.01$) and Tukey's test ($p < 0.01$). Two field replicates were analyzed each in triplicate. Grey bars: control location 2; white bars: control location 1; black bars: rain-out-shelter