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To: Arizona Grain Research and Promotion Council

Subject: Final Report

Grant Project Title: Drought tolerance in barley: discovery of a possible mechanism involving root tip characteristics.

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Aim of this study.

The main goal of this research was to determine the underlying mechanisms of drought tolerance in cereal crops. To accomplish this goal, the University of Arizona research team studied barley and wheat root traits (specifically root tip border cells and root tip mucilage) that could promote mycorrhizal colonization and consequently improve absorption of water.

Results.

1. The first part of this investigation showed that drought-tolerant barley varieties produce higher mucilage area than conventional barley varieties. This research was published and Grain Council support was acknowledged (Carter et al. 2019).

2. In a more representative study, the root tip characteristics of interest were measured, such as border cell numbers (tables 1 and 2) and root tip mucilage production (tables 3 and 4) from all 70 barley and wheat lines.

3. Roots samples were taken from all 70 barley and wheat lines grown at the Maricopa Agriculture Research Station and screened for the presence of mycorrhizae (Fig. 1). Mycorrhizal colonization rates were quantified (tables 5 and 6).

4. Statistical analyses indicated that border cell numbers from high input barley and low input wheat lines have a positive linear relationship to mycorrhizal colonization (Fig. 2 and 3, respectively) which is confirmed by their respective correlation coefficients. Conversely, border cells from low input barley and high input wheat did not present a positive relationship to mycorrhizae (data not shown). In addition, mucilage production revealed weak linear negative relationships to mycorrhizal colonization in both barley and wheat lines (data not shown).

Discussion.

Root tip characteristics. The number of barley border cells and mucilage area differed from what it was found in the previous study. This could be due to the low number of observations ($n \geq 3$) in this study, or seeds coming from a different lot, or that it exists a high variability in barley seeds. Similar to barley, it was observed a high degree of variation in wheat. In addition, this variation could be due to the low number of observations and to the observed nature of the root structure of germinating barley and wheat seeds. This is, barley and wheat present more than one root when germinate, and each of these roots has a different border cell number and mucilage production. We hypothesize that this characteristic could be the cause of variation altering the results.

Mycorrhizal colonization. It was observed a low colonization rate in most barley and wheat lines under study. Root samples were taken early (February) in the growing season of these crops, which may suggest that mycorrhizae were not fully developed. For future studies, it is required to take samples at different times and also to implement additional screening methods to obtain more representative results.

Root traits-Mycorrhizae Relationship. The positive correlation between border cells of high input barley or low input wheat lines and mycorrhizal colonization (fig. 2) supports previous observations suggesting that border cells may promote the establishment of mycorrhizae (Discussed in Carter et al. 2019). This evidence suggests that root border cells could play a role in the mechanism responsible for drought tolerance in low input lines. However, it is interesting that low input barley or high input wheat lines are not associated with mycorrhizae. It could be that different soil conditions played a role in this result. Contrary to previous studies, results from the four groups of barley and wheat indicate that mucilage production is not involved in promoting mycorrhizal colonization, yet more studies are necessary to make a definitive conclusion.

Conclusions.

In order to improve the absorption of water in barley or wheat, it would be valuable to consider lines that produce high border cell numbers in drought tolerance breeding programs. Also, it would be of interest to reduce the variation of the results by studying other cereal crops with more robust root systems, such as corn. Overall, the observed positive correlation between border cells and mycorrhizae suggest root border cells may be a low cost and simple means to screen for enhanced drought tolerance in cereal crops.

Reference: Carter AY, Ottman M, Curlango-Rivera G, Huskey DA, D'Agostini BA, Hawes MA. 2019. Drought-tolerant barley: II Root tip characteristics in emerging roots. *Agronomy* 9(5): 220. doi:10.3390/agronomy9050220

TABLE 1. Number of root tip border cells produced by high and low input barley lines.

Barley Line	Border Cells ± SD	Homogeneous Group
High Input		
Chico	95 ± 23	acd
Nebula	150 ± 64	ace
Cochise	155 ± 56	acf
Baretta	225 ± 76	acf
Barcott OB22	233 ± 103	acdg
Barcott OW22	260 ± 107	acfgh
Kopious	383 ± 173	fgi
Low Input		
OB16	53 ± 40	a
OB18	74 ± 24	ab
OB20	84 ± 37	ac
OB11	91 ± 38	ac
Solar OW25	99 ± 23	ac
Solum OB21	103 ± 85	acd
Solum OW21	104 ± 68	acd
OB1	157 ± 58	ace
OB4	196 ± 75	acf
OB7	197 ± 66	acf
OB15	198 ± 71	acf
OB3	204 ± 47	acf
OB5	204 ± 46	acf
OB13	225 ± 22	acf
Solar OB25	238 ± 139	acfgh
OB8	258 ± 29	acfgh
OB6	*277 ± 148	bcfgh
OB19	288 ± 44	bcfgh
OB10	300 ± 115	cfgh
OB17	309 ± 205	defgh
OB2	333 ± 172	efgi
OB12	454 ± 94	hi
OB9	458 ± 268	gi
OB14	*533 ± 197	i

Values represent the mean ± standard deviation of at least two replicate samples from three independent seedlings for each plant line (n≥6). Cell numbers with the same homogeneous group are not significantly different according to Tukey test. *Data not normal according to Shapiro-Wilk normality test (p<0.05).

TABLE 2. Number of root tip border cells produced by high and low input wheat lines.

Wheat Lines	Border Cells ± SD	Homogeneous Groups
High Input		
Phoenix	35 ± 23	a
Maestrale	66 ± 44	ac
Powell	87 ± 59	ac
ASC-120	97 ± 21	ac
WB-Mohave	80 ± 37	ac
Kronos	106 ± 46	ac
Saragolla	118 ± 51	ac
Platinum	120 ± 40	ac
ASC-129	124 ± 80	ac
ASC-124	136 ± 44	ac
Desert King	141 ± 98	ac
ASC-121	144 ± 53	ac
Yecora Rojo OB24	151 ± 98	acd
Yecora Rojo OW24	*153 ± 37	acd
Topper	*159 ± 196	acd
Alberto	161 ± 69	acd
Miwok	*169 ± 147	acd
ASC-123	175 ± 77	acd
WB-Mead	*197 ± 181	ace
Westmore HP LCD	248 ± 90	bce
Tiburon	347 ± 281	de
ASC-122	391 ± 277	e
Low Input		
0W18	61 ± 26	ab
0W14	82 ± 17	ac
0W10	89 ± 82	ac
0W12	90 ± 23	ac
OW19	94 ± 26	ac
OW1	104 ± 49	ac
0W16	106 ± 50	ac
OW9	112 ± 25	ac
OW3	123 ± 93	ac
0W8	123 ± 36	ac
0W6	125 ± 84	ac
OW7	134 ± 60	ac
Xeric OB23	140 ± 83	ac
OW13	155 ± 69	acd
OW5	157 ± 78	acd
0W2	*158 ± 33	acd
Xeric OW23	159 ± 60	acd
OW11	161 ± 36	acd
OW20	170 ± 128	acd
0W4	171 ± 64	acd
OW17	186 ± 127	acd
OW15	256 ± 120	ce

Values represent the mean ± standard deviation of at least two replicate samples from three independent seedlings for each plant line (n≥6). Cell numbers with the same homogeneous group are not significantly different according to Tukey test. *Data not normal according to Shapiro-Wilk normality test (p<0.05).

Table 3. Root tip mucilage area produced by high and low input barley lines.

Barley Line	Mucilage Area (mm^2) ± SD	Homogeneous group
High Input		
Cochise	0.34±0.16	ab
Barcott OB22	0.36±0.18	ab
Kopious	0.43±0.11	ab
Nebula	0.44±0.15	ab
Baretta	0.47±0.06	ab
Barcott OW22	0.54±0.28	ac
Chico	0.56±0.23	ac
Low Input		
OB11	0.25±0.05	a
OB16	0.26±0.1	ab
Solar OB25	*0.29±0.09	ab
OB4	0.30±0.06	ab
OB7	0.30±0.18	ab
OB15	0.36±0.05	ab
OB3	0.38±0.08	ab
OB10	0.39±0.16	ab
OB19	0.39±0.2	ab
OB1	0.42±0.17	ab
OB13	0.44±0.15	ab
OB6	0.45±0.18	ab
OB8	0.47±0.35	ab
Solum OW21	0.48±0.06	ab
Solar OW25	0.48±0.01	ab
Solum OB21	0.53±0.2	ac
OB9	0.54±0.22	ac
OB5	0.58±0.14	ac
OB18	0.58±0.28	ac
OB20	0.68±0.36	ac
OB14	0.78±0.11	ac
OB12	0.91±0.04	ac
OB2	0.93±0.24	bc
OB17	1.18±0.61	c

Values represent the mean ± standard deviation of one root sample from three independent seedlings for each plant line (n=3). Mucilage areas with the same homogeneous group are not significantly different according to Tukey test. *Data not normal according to Shapiro-Wilk normality test (p<0.05).

TABLE 4. Root tip mucilage area produced by high and low input wheat lines.

Wheat Lines	Mucilage Area (mm^2) ± SD	Homogeneous Groups
High Input		
WB-Mohave	0.30±0.09	a
Phoenix	0.31±0.16	a
ASC-121	0.32±0.05	a
ASC-120	0.35± 0.04	ab
Maestrale	0.35±0.13	ab
Miwok	0.43± 0.20	ab
Topper	0.44±0.25	ab
Kronos	0.46±0.08	ab
WB-Mead	0.49±0.34	abc
ASC-123	0.50± 0.19	abc
Yecora Rojo OW24	0.54± 0.09	abc
Alberto	0.61±0.29	abc
Saragolla	0.72±0.22	abc
Westmore HP LCD	0.75±0.15	abc
Powell	0.81±0.53	abc
ASC-124	0.81±0.51	abc
Yecora Rojo OB24	0.89±0.15	ad
ASC-129	0.94±0.11	ad
Platinum	1.08±0.33	ad
Desert King	1.08±0.66	ad
Tiburon	1.52±0.75	cd
ASC-122	1.84±0.71	d
Low Input		
OW7	0.23± 0.07	a
0W10	0.24±0.06	a
0W6	0.30± 0.13	a
OW11	0.37±0.19	ab
Xeric OW23	0.45±0.13	ab
OW1	0.51±0.09	abc
OW9	0.51±0.07	abc
0W2	0.59± 0.14	abc
OW20	0.6±0.06	abc
OW17	0.62±0.17	abc
OW3	1.01±0.21	ad
0W4	1.49±0.33	cd
0W18	0.66± 0.27	abc
0W12	0.67±0.26	abc
OW13	0.7±0.04	abc
0W14	0.77±0.25	abc
OW19	0.8±0.25	abc
Xeric OB23	0.82±0.64	ad
OW5	0.84±0.18	ad
0W8	0.84±0.34	ad
0W16	1.04±0.45	ad
OW15	1.36±0.44	bd

Values represent the mean ± standard deviation of one root sample from three independent seedlings for each plant line (n=3). Mucilage areas with the same homogeneous group are not significantly different according to Tukey test. *Data not normal according to Shapiro-Wilk normality test (p<0.05).

Figure 1. Trypan blue staining of mycorrhizal structures in a root sample of drought-tolerant Barley (OW21) ‘Solum.’ Magnification 200x.

Table 5. Mycorrhizal colonization rates of high and low input barley lines.

Barley Line	Number of roots without mycorrhizal colonization	Number of roots with mycorrhizal colonization	n	Mycorrhizal colonization %
High input				
Nebula	10	0	10	0
Barcott OW22	9	1	10	10
Chico	9	1	10	10
Barcott OB22	5	1	6	17
Baretta	8	2	10	20
Kopious	7	3	10	30
Cochise	6	4	10	40
Low input				
OB18	10	0	10	0
OB19	10	0	10	0
OB2	9	1	10	10
OB3	9	1	10	10
OB7	9	1	10	10
OB8	9	1	10	10
OB12	9	1	10	10
OB13	9	1	10	10
OB5	8	2	10	20
OB6	8	2	10	20
OB10	8	2	10	20
OB11	8	2	10	20
OB17	8	2	10	20
OB20	8	2	10	20
Solum OB21	8	2	10	20
OB4	7	3	10	30
OB14	7	3	10	30
OB16	7	3	10	30
Solar OB25	7	3	10	30
Solum OW21	7	3	10	30
OB1	6	4	10	40
OB9	5	5	10	50
OB15	5	5	10	50
Solar OW25	5	5	10	50

Values reflect results of ten root replicate samples from one independent plant for each plant line (n=10).

TABLE 6. Mycorrhizal colonization rates of high and low input wheat lines.

Wheat Line	Roots without Mycorrhizae	Roots with Mycorrhizae	n	Mycorrhizal Colonization %
High Input				
ASC-124	10	0	10	0
Saragolla	10	0	10	0
OW24 Yecora Rojo	9	1	10	10
OB24 Yecora Rojo	9	1	10	10
ASC-120	9	1	10	10
ASC-122	9	1	10	10
ASC-123	9	1	10	10
Platinum	8	2	10	20
WB-Mohave	8	2	10	20
Desert King	8	2	10	20
Miwok	8	2	10	20
ASC-121	8	2	10	20
Phoenix	7	3	10	30
Topper	7	3	10	30
Powell	7	3	10	30
WB-Mead	7	3	10	30
Maestrale	7	3	10	30
Alberto	6	4	10	40
Kronos	6	4	10	40
Westmore HP LCD	6	4	10	40
ASC-129	6	4	10	40
Tiburon	4	6	10	60
Low Input				
Xeric OW23	10	0	10	0
0W14	9	1	10	10
OW19	9	1	10	10
OW3	8	2	10	20
OW1	7	3	10	30
0W2	7	3	10	30
0W4	7	3	10	30
0W8	7	3	10	30
0W12	7	3	10	30
0W16	7	3	10	30
OW5	6	4	10	40
0W6	6	4	10	40
OW9	6	4	10	40
0W10	6	4	10	40
OW11	6	4	10	40
OW17	6	4	10	40
OW20	6	4	10	40
OW7	5	5	10	50
Xeric OB23	5	5	10	50
OW13	4	6	10	60
OW15	4	6	10	60
0W18	3	7	10	70

Values reflect results of ten root replicate samples from one independent plant for each plant line (n=10).

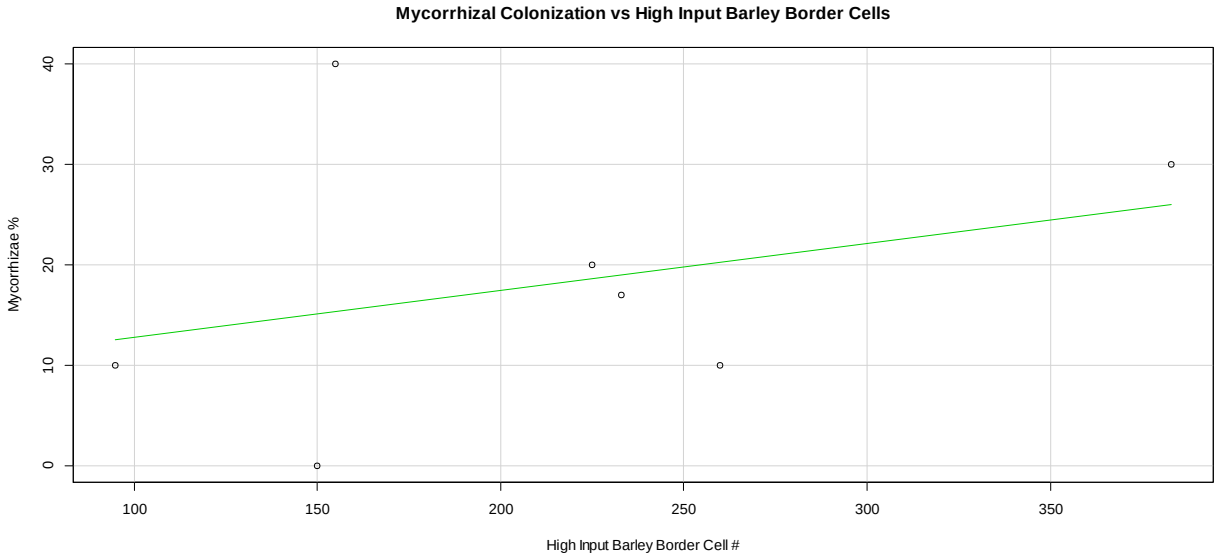

Figure 2. Positive linear relationship between mycorrhizal colonization and high input barley border cells. Pearson's correlation coefficient $r = 0.33$, p-value = 0.4757.

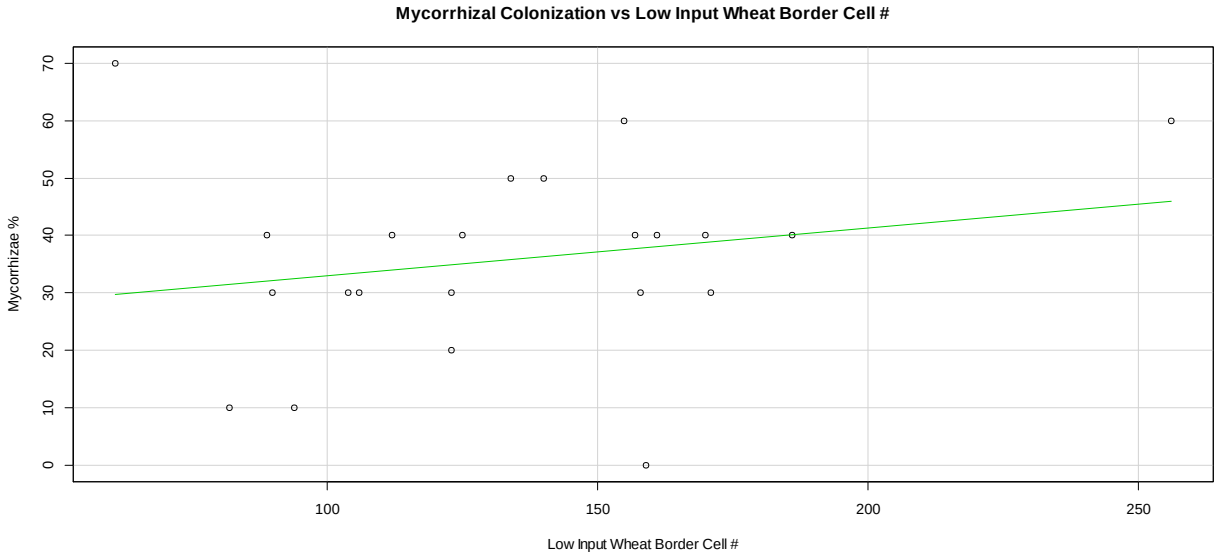

Figure 3. Positive linear relationship between mycorrhizal colonization and low input wheat border cells. Pearson's correlation coefficient $r = 0.21$, p-value = 0.3394.