Environment of Origin and Domestication Affect Seed Germination, Root Morphology, and Response to Water Deficit in Chile Pepper (*Capsicum annuum* L.)

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Hayes Graduate Research Forum 2022

Introduction

Global climate change threatens agriculture by increasing precipitation variability and drought (Masson-Delmotte et al., 2021). To mitigate these risks, we can improve crop tolerance to abiotic stresses, such as soil water deficit, through genetic improvement utilizing unique sources of tolerance (Takeda and Matsuoka, 2008). Landraces, or traditional varieties from crop centers of origin, often have adaptations to their specific environments and the associated abiotic stresses (Tanksley and McCouch, 1997; Acosta-Gallegos et al., 2007). Chile pepper (*Capsicum annuum* L.) landraces grow across a wide range of environments in their native Mexico and provide a unique opportunity to study drought adaptation. Previous work suggests that chile landraces from Mexico have adaptations that contribute to water deficit tolerance, specifically in above-ground traits such as plant biomass and architecture (McCoy et al., 2021). However, there is a lack of study on below ground adaptations to drought, likely due to the challenging nature of root experiments. Root traits, such as high root length or small diameter, play an important role in water deficit tolerance by improving the efficiency of water uptake (Comas et al., 2013).

In the present study, we explore seed germination, early-stage root growth and their responses to osmotic stress in a selection of chile pepper landraces from diverse environments of origin throughout Mexico. Additionally, we evaluate differences across levels of domestication by comparing wild-like landraces collected from forest environments alongside landraces collected from backyards.

Methods

To accomplish our objectives, we carried out two experiments: a growth chamber experiment to evaluate germination, followed by a greenhouse experiment to evaluate early-stage root growth. In the first experiment, seed from twelve accessions (individual landrace entries) were grown in a randomized complete block design in growth chambers (Table 1). Accessions were chosen based on the environment of origin and level of domestication. We made selections based on the precipitation of the wettest quarter (BIO16), which generally coincides with the most active growing season for pepper. Additionally, we included two levels of domestication: two accessions from unmanaged, forest systems and 10 accessions from semi-cultivated, backyard systems. We applied a water stress treatment using polyethylene glycol (PEG) to simulate osmotic stress (two levels: untreated water and 20% PEG solution), which has been shown to be a sufficient concentration to simulate the effects of drought (Bernau, 2018). We monitored seed germination for 14 days, and germination percent is reported.

Table 1. Accessions used in an osmotic stress germination experiment on chile pepper landraces, separated by level of domestication.

Accession	Landrace	BIO16 (mm)[1]
	Backyard[2]	
Ca0181	Chile Paradito	544
Ca0256	Chilgole	575
Ca0435	Miraparriba	935
Ca0437	Miraparriba	935
Ca0446	Miraparriba	829
Ca0448	Pico de Paloma	533
Ca0449	Pico de Paloma	533
Ca0454	Achilito de Monte	621
Ca0456	Huacle Roja	621
Ca0457	Huacle Amarillo	621
	Forest	

Ca0303	Chile de Monte	530
Ca0436	Tipinchile	829

[1]Bioclim variable = Precipitation of the wettest quarter.

[2]Levels of domestication refer to cultivation systems that accessions were collected from. Backyard = semicultivated system, Forest = unmanaged, wild system.

A second experiment studied the effect of greenhouse water deficit on root growth using a subset of the original twelve accessions (Table 2). We grew nine accessions in a randomized complete block design and subjected them to two levels of irrigation: well-watered (WW) and water deficit (WD) conditions (70 and 30% of field capacity, respectively). Five weeks after transplant, plants were destructively harvested, and soil was carefully cleaned from the root system. Roots were scanned using Winrhizo software (Regent Instruments Inc.) and total root length (RL), average root diameter (RD), root biomass (RB), specific root length (SRL; total root length/root biomass), and root to shoot ratio (R:S) are reported.

Table 2. Accessions used in a water deficit experiment on early-stage root growth in chile pepper landraces, separated by level of domestication.

Accession	**Landrace**	**BIO16 (mm)[1]**
	Backyard[2]	
Ca0181	Chile Paradito	544
Ca0256	Chilgole	575
Ca0435	Miraparriba	935
Ca0446	Miraparriba	829
Ca0454	Achilito de Monte	621
Ca0456	Huacle Roja	621
Ca0457	Huacle Amarillo	621
	Forest	
Ca0303	Chile de Monte	530
Ca0436	Tipinchile	829

[1]Bioclim variable = Precipitation of the wettest quarter.

[2]Levels of domestication refer to cultivation systems that accessions were collected from. Backyard = semicultivated system, Forest = unmanaged, wild system.

All data were analyzed in R (v. 4.1.2). We performed analysis of variance (ANOVA) using linear mixed models to identify the effects of water deficit on all accessions. ANOVA assumptions were checked, and data was log transformed for RL, RB, SRL, and R:S due to the heteroscedastic nature of the data. To elucidate the relationship of measured traits with environment of origin, we conducted multiple linear regression analysis using stress treatment, mean annual precipitation (BIO12), precipitation seasonality (BIO15), total available soil water content (TASW) and their interactions as the predictor variables. Finally, to determine the effects of domestication on traits, we performed *a priori* contrasts between grouped forest and backyard accessions. Due to the variable nature of root data, analyses involving root traits were considered significant at $P < 0.1$.

Results

An ANOVA of all traits revealed a significant effect irrigation treatment on RL ($P< 0.05$), as well as significant interactions between accession and treatment for percent germinated ($P< 0.05$), RD ($P< 0.05$), and SRL ($P < 0.1$; Table 3). A significant reduction was observed for RL between WW and WD treatments (Table 4). Interactions reveal differential responses to osmotic stress and soil water deficit (Figure 1). The effect of the PEG treatment generally increased in accessions as the precipitation of the originating environment increased. The trends are less clear in RL and RD; however, response to water deficit appears to have contrasting effects depending on the accession.

Table 3. Analysis of variance for six traits in two experiments evaluating the effects of osmotic stress on seed germination and root growth in chile pepper. Data were analyzed in R (version 4.1.2) using a linear mixed model. Source was considered significant if $P < 0.05$ or $P < 0.1$ in root traits.

Trait	Source	DF	F Value	P[a]
Percent Germinated	Accession	11	15.6749	***
	Peg	1	76.1863	***
	Accession by Irrigation	11	6.7596	***
Root Weight[b]	Accession	8	1.2188	NS
	Irrigation	1	2.4823	NS
	Accession by Irrigation	8	1.7038	NS
Root Length[b]	Accession	8	0.8528	NS
	Irrigation	1	4.4974	*
	Accession by Irrigation	8	1.0834	NS
Root Diameter	Accession	8	1.2289	NS
	Irrigation	1	2.8131	NS
	Accession by Irrigation	8	2.4726	*
Specific Root Length[b]	Accession	8	2.1039	^
	Irrigation	1	3.1346	^
	Accesion by Irrigation	8	2.0426	^
Root to Shoot Ratio[b]	Accession	8	0.255	NS
	Irrigation	1	0.2919	NS
	Accesion by Irrigation	8	0.5906	NS

[a]^, *, **, ***specify significant differences at P values of 0.1, 0.05, 0.01, and 0.001 respectively.

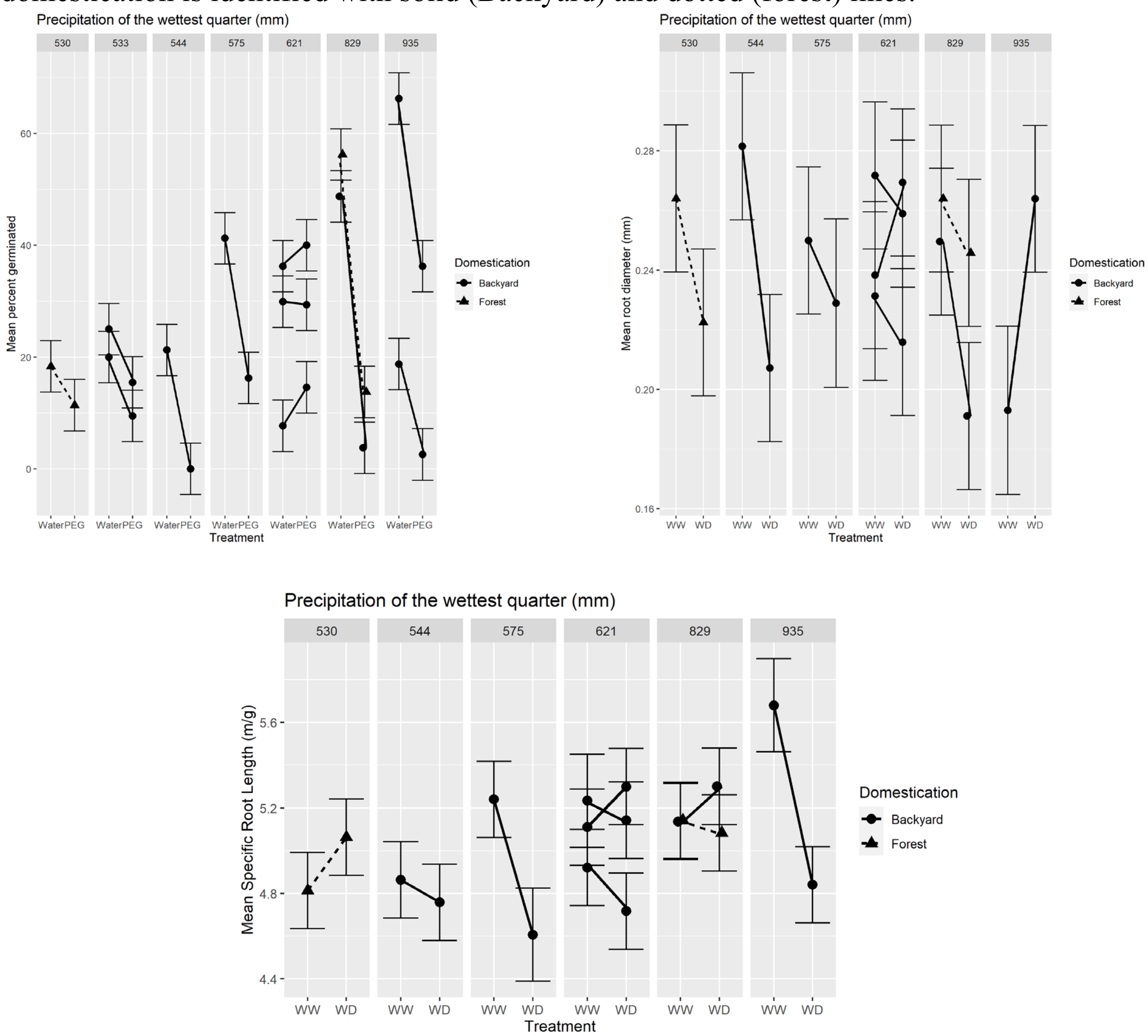
[b]Log transformation perfomed to account for heterscedastic data distribution.

Table 4. Mean separation for total root length from a greenhouse water deficit experiment on chile pepper.

Irrigation	Root Length[a]	SE[b]
Well-Watered	6.17 a	0.214
Water Deficit	5.54 b	0.208

[a]Different letters indicate significant differences (P = 0.05) between levels of irrigation.
[b]Indicates standard error of the mean.

Figure 1. Interaction plots from two osmoitic stress experiments on chile pepper showing mean percent germinated, root diameter, and specific root length. Osomit stress treatment is shown along the x-axis. Accessions are organized by precipitation of the wettest quarter and level of domestication is identified with solid (Backyard) and dotted (forest) lines.



Regression analysis with environmental parameters from the environment of origin indicate a significant relationship between germination percent, stress treatment, annual precipitation, and precipitation seasonality (Table 5). Root diameter and weight also had significant relationships with stress treatment and total available soil water of the environment of origin. Interestingly, these significant relationships are with the interaction of treatment and environmental variable. This suggests that the exact nature of the relationship depends on the treatment applied. For example, root diameter has a significant relationship with the interaction of treatment and TASW. Investigating this relationship further, we see that under WW conditions, there is a significantly positive relationship between root diameter and TASW, but under water deficit, the relationship is negative (Fig 2).

Table 5. Multivariate regression analysis in two experiments evaluating the effects of osmotic stress on seed germination and root growth in chile pepper. Data were analyzed in R (version 4.1.2) using a linear mixed model. Source was considered significant if P < 0.05 or P< 0.1 in root traits. Only traits with significiant relationships are shown.

Trait[a]	**Predictors**	**Beta**[b]	**SE**[c]	**P**[d]
Percent Germinated (0.393)	Treatment	-257.80104	138.18054	^
	Total Available Soil Water	-3.73346	1.47885	*
	Annual Mean Precipitation	-0.02847	0.01733	NS
	Precipitation Seasonality	-0.38119	0.21256	^
	Treatment by Total Available Soil Water	2.97291	2.09141	NS
	Treatment by Annual Mean Precipitation	0.05598	0.0245	*
	Treatment by Precipitation Seasonality	0.73695	0.3006	*
Root Biomass[e] (0.294)	Treatment	-21.550	12.34	^
	Total Available Soil Water	-0.21	0.134	NS
	Annual Mean Precipitation	-0.002	0.002	NS
	Precipitation Seasonality	-0.012	0.019	NS

	Treatment by Total Available Soil Water	0.416	0.184	*
	Treatment by Annual Mean Precipitation	0	0.002	NS
	Treatment by Precipitation Seasonality	0.021	0.026	NS
Root Length (0.215)	Treatment	-6440.664	4622.5479	NS
	Total Available Soil Water	-47.674	50.2814	NS
	Annual Mean Precipitation	-0.4157	0.6432	NS
	Precipitation Seasonality	-4.6123	6.9536	NS
	Treatment by Total Available Soil Water	119.7409	69.1121	^
	Treatment by Annual Mean Precipitation	0.1782	0.8849	NS
	Treatment by Precipitation Seasonality	10.323	9.8921	NS
Root Diameter (-0.011)	Treatment	-0.681	4.05E-01	NS
	Total Available Soil Water	-7.32E-01	4.10E-03	NS
	Annual Mean Precipitation	-3.66E-05	5.66E-05	NS
	Precipitation Seasonality	-7.03E-04	6.09E-04	NS
	Treatment by Total Available Soil Water	1.29E-02	6.06E-03	*
	Treatment by Annual Mean Precipitation	2.31E-05	7.76E-05	NS
	Treatment by Precipitation Seasonality	8.54E-04	8.67E-04	NS

[a]Measured trait with adjusted R^2 values in parenthesis

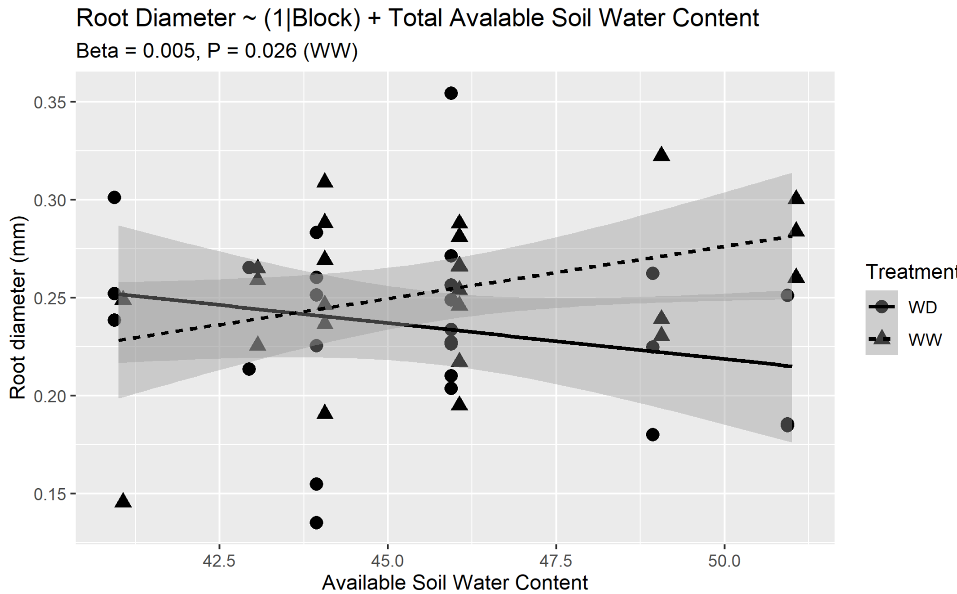
[b]Beta estimate indicates the change in trait value relative to one unit change of the predictor.

[c]Indicates standard error of the mean.

[d]^, *, **, ***specify significant relationship at P values of 0.1, 0.05, 0.01, and 0.001 respectively.

[e]Log transformation perfomed to account for heterscedastic data distribution.

Figure 2. A regression analysis in a greenhouse experiment on chile pepper demonstrating the significant relationship of root diameter to the interaction of water deficit treatment and total available soil water content of the environment of origin.



Finally, *a priori* contrasts revealed significant differences between forest and backyard accessions for germination percent, RB, RL, RD, and SRL (Table 6). Results revealed that stress treatment did not affect the significant differences between groups. Forest accessions have lower percent germination, RL, RD, and SRL, and higher RB, and R:S, compared to their backyard counterparts.

Table 6. Results of *a priori* group contrasts on six traits from two osmotic stress experiments on chile pepper (*Capsicum* sp.). WW = well-watered, WD = water deficit.

	Forest:Backyard			
Trait	WW		WD	
	t value	P[a]	t value	P
Percent Germinated	-20.537	*** <	-11.128	*** <
Root Biomass	13.471	*** >	13.59	*** >
Root Length	-22.382	*** <	-21.017	*** <
Root Diameter	-13.561	*** <	-13.103	*** <
Specific Root Length	-55.167	*** <	-53.625	*** <
Root to Shoot Ratio	15.646	*** >	13.77	*** >

[a]*, **, ***specify significant differences at P values of 0.05, 0.01, and 0.001 respectively. Greater than/less than symbols indicate the direction of the contrast vis-à-vis the order of the groups in the heading.

Discussion

Results provide evidence for drought adaptations in seed germination and root traits that could indicate tolerance to soil water deficit. For example, we observed a significant reduction in root diameter under water deficit in accession Ca0181, which originates from a low precipitation environment and could be exhibiting an adaptive response through improved water uptake efficiency often associated with a smaller root diameter. In addition to individual accession responses, we identified significant relationships with environmental parameters such as annual precipitation, precipitation seasonality, and total available soil water provide evidence for environmental adaptations in seed germination and root traits. Specifically, under well-watered conditions, root diameter was significantly smaller in accessions collected from environments with lower soil water content, suggesting an adaptation. Phenotypic plasticity may also be present, as demonstrated by the reduction in root diameter under water deficit conditions. Additionally, root traits appear to be significantly influenced by domestication, despite water treatment. Smaller root diameter and high root to shoot ratios in forest accessions suggest possible adaptations that improve water uptake in less domesticated accessions.

Chile root morphology and architecture is a relatively under studied subject. This work provides insight into chile root growth under water deficit and identifies accessions with possible tolerance. It also provides evidence for adaptations to drought suggesting that environmental parameters and level of domestication are effective ways of identifying tolerant germplasm. Results will be useful in continued studies on adaptations and may have direct implications in breeding for water deficit tolerance.

Acknowledgements

The author would like to acknowledge Katie McNamara, Dr. Kristin Mercer, and Dr. Leah McHale for there contributions to data collection and analysis for this project.

References

Acosta-Gallegos, J.A., J.D. Kelly, and P. Gepts. 2007. Prebreeding in common bean and use of genetic diversity from wild germplasm. Crop Sci. 47(SUPPL. DEC.). doi: 10.2135/cropsci2007.04.0008IPBS.

Bernau, V. 2018. Exploring local adaptation and drought tolerance in chile peppers (Capsicum spp.) of southern mexico.

Comas, L.H., S.R. Becker, V.M. V. Cruz, P.F. Byrne, and D.A. Dierig. 2013. Root traits contributing to plant productivity under drought. Front. Plant Sci. 4: 442. doi: 10.3389/fpls.2013.00442.

Masson-Delmotte, V., P. Zhai, A. Pirani, S. Connors, C. Péan, et al. 2021. Climate change 2021: the physical science basis. Contribution of working group I to the sixth assessment report of the intergovenmental panel on climate change.

McCoy, J.E., L. McHale, M. Kantar, L. Jardón-Barbolla, and K.L. Mercer. 2021. Environment of origin and domestication affect morphological, physiological, and agronomic response to water deficit in chile pepper (Capsicum sp.). bioRxiv: 2021.11.16.468826. doi: 10.1101/2021.11.16.468826.

Takeda, S., and M. Matsuoka. 2008. Genetic approaches to crop improvement: responding to environmental and population changes. Nat. Rev. Genet. 9(6): 444–457. doi: 10.1038/nrg2342.

Tanksley, S.D., and S.R. McCouch. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277(5329): 1063–6. doi: 10.1126/SCIENCE.277.5329.1063.