

Effects of cultivation and landrace on germination in Mexican wild and cultivated chile pepper

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“The approach to the problems of farming must be made from the field, not from the laboratory...In this the observant farmer and labourer, who have spent their lives in close contact with nature, can be of greatest help to the investigator.”

Sir Albert Howard, *An Agricultural Testament* (1940)

I. Introduction: A Landrace Against Time

Throughout the world, the impacts of climate change on food production are far-reaching, potentially devastating and in many places already underway, with altered precipitation regimes, shifting dynamics of pests and pathogens, and increasingly frequent extreme weather events all among the anticipated and ongoing effects (Parry 2004; Schmidhuber and Tubiello 2007). In light of these changes, the identification of crops with environmentally-adaptive traits is of paramount importance, though the ability of farmers and scientists to identify and conserve these genetic resources reflects, in many ways, a race against time. Crop genetic diversity is eroding at an alarming rate, not only through continuous processes of globalization and industrialization in agriculture, but also as a result of climate change itself, with 37% of the world's species "committed to extinction" by the year 2050, according to mid-range climate-warming scenarios (Thomas et al. 2004; FAO 2011). In the face of climate change, locally-adapted crop varieties, also known as "landraces," offer one important wellspring of adaptive genetic resources, often possessing specific traits for disease and pest resistance, nutritional quality and environmental tolerance. And yet this vital gene pool remains under constant siege: genetic erosion of landraces in "centers of diversity" — the evolutionary homes of domesticated crops and their wild progenitors — continues to unfold at the hand of both human and climate-mediated forces. To prevent the extinction of this genetic material, conservation programs must be grounded in understandings not simply of the biological responses of landraces to climate change, but also of the cultural, historical, and biogeographical factors that ultimately inform these responses—a view shared by many advocates of in-situ, or on-farm, conservation programs (Frankel 1974; Altieri and Merrick 1987; Mercer and Perales 2010). It is through this interdisciplinary lens that

the study that follows examines the drought responses of diverse *Capsicum annuum sp* landraces, including 26 different accessions across 10 distinct landraces from Oaxaca, Mexico, analyzing the effects of each accession’s domestication level and cultivation type on different seed characteristics related to drought tolerance.

II. Literature review

A. Adaptive plasticity and evolution: The biological basis for in-situ conservation

As the climate continues to change, agricultural drought events — typically characterized by their intensity, length, and timing relative to plant development (Dracup et al 1980) — will continue to unfold in both frequency and severity. Predicting drought events is no easy task, requiring an assessment of social, economic and infrastructural indicators collected at both the national and sub-national levels. Such assessments have indicated that Central America, Northwest of South America, Central and South Asia, and almost all of Africa are among the most vulnerable regions to drought, and within these geographic ranges, countries with a “high dependence on subsistence agriculture and primary sector activities” are particularly vulnerable to its social consequences, which include food insecurity, famine, human conflicts and widespread mortality (Carrão 2016). In Oaxaca and elsewhere, variable and drier growing seasons and less reliable precipitation will bear the greatest consequences for smallholder farmers — and especially those growing in rain-fed systems — and will necessitate the adoption of various climate adaptive strategies, which may include changing cultural practices, increasing the resilience of agro-ecosystems, or improving seed varieties. Unfortunately, the successful adoption of such strategies is fraught by complexity: The ability of farmers to adapt to climate

change is contingent not only on the farming experience, educational level, farm size and even gender of individual smallholders, but also on their access to technology, complementary inputs, extension services and climate change information (Mercer et al 2012; Tambo and Aboudlaye 2012; Madina and Barjolle 2018). Certain widely-touted strategies like transgenic adaptation may be inappropriate in certain places, where the lived realities of farmers and the unique assets of agricultural communities — including the vast agro-biodiversity of traditional landrace varieties— may be more compatible with alternative adaptation strategies like participatory breeding programs and on-farm experimentation with landraces (Mercer et al 2012). Of course, the success of these strategies is ultimately contingent on the genetic conservation of traditional landraces, which can occur either in-situ (on-farm) or ex-situ (in seed banks).

Though both strategies are equally important in preserving agro-biodiversity, in-situ conservation, in particular, offers several distinct advantages in the maintenance of landrace populations. Defined as “the maintenance of variable populations in their natural or farming environment [and] within the community of which they form a part,” in-situ conservation situates the farm itself at the site for conservation, thereby allowing continuous processes of evolution to unfold across different spatial scales, environmental gradients, and cultivation regimes (Brush 2000: 29). This, in turn, allows landrace varieties to maintain local divergences, special adaptations to marginal or stressful environments, and ultimately heightened genetic diversity and allelic richness between and within populations (29). This genetic variation serves as the basis for species survival through two co-occurring processes: evolution and plasticity.

As one possible biological response to rapid climate change, phenotypic plasticity refers to the expression of a range of phenotypes that a single genotype can express when experiencing different environments (Pigliucci 2001). While phenotypic changes do not require genetic

change, the capacity for plasticity can be genetically-controlled and is thus heritable (Scheiner and Lyman 1989). Phenotypic plasticity can be documented for any number of functional traits in plants — leaf mass, root architecture, and seed size among them — and is mediated on both the genetic and molecular levels through epigenetics and signaling cascades (Nicotra 2010). It should be noted that phenotypic plasticity is distinct from evolution, which refers to changes in allele frequency within a population that shift its mean phenotype over time. Essential to both evolution and plasticity, however, is genetic variation, which refers to the differences in allelic makeup both between and within populations, often observed as genetic polymorphisms (the presence of multiple alleles at one loci) and differences in resulting phenotypes. In a context of rapid climate change, phenotypic plasticity could serve as a buffer against devastating extinction events, affording otherwise vulnerable populations a better chance for adaptive evolution to new conditions (Nicotra 2010; Mercer and Perales 2010).

Of course, both phenotypic plasticity and evolution are only as valuable as their adaptive or non-adaptive potential, which reflects the costs and tradeoffs of new phenotypes to plant fitness — i.e. how closely these novel traits reflect a crop's "phenotypic optimum" in the new environment (Ghalambor 2007). Whether it be moisture stress or extreme temperatures, novel environmental conditions provide the catalyst for "cryptic genetic variation," or "the phenotypic and genotypic variance that is 'hidden' or unexpressed under normal environmental conditions" (Ghalambor 2007: 400), to rear its sometimes adaptive, sometimes ugly head, inducing phenotypes that are either beneficial or deleterious within these new extremes. Such stress also reveals the canalized nature of individual traits, which describes the capacity of a trait to remain the same, regardless of environmental variability (400). And although the precise genetic basis of phenotypic plasticity remains a mystery, and the time-scales required for

adaptive evolution an even greater mystery still (Shaw and Etterson 2012), it is generally agreed that the persistence of adaptive plasticity in a population is linked to the environmental variation and types of ancestral selection that have shaped its gene pool (Ghalambor 2007: 400).

For many advocates of *in situ* conservation, it is this historically-wrought dimension of landraces that is so important, encompassing an accumulation of human-mediated selection pressures like the nutritional needs, cultural preferences, and cultivation systems of the local farming communities that shaped and stewarded them, in addition to the effects of natural selection (Altieri and Merrick 1987). In fact, implicit in the very definition of a landrace – which describes a “dynamic population of cultivated plants possessing historical origin and distinct identity, and lacking formal crop improvement, while often being genetically diverse, locally adapted and associated with traditional farming systems” — is the profound role of humans in shaping them (Villa et al 2005).

Unfortunately, much of the information necessary for designing appropriate in-situ conservation programs is incomplete, due on the one hand to limited funding for research, and on the other to the sheer vastness of all the relevant fields, which include not only the life history traits, habitat requirements, eco-geography and genetic structure of different landraces, but also the political-economic and social factors that impact them (Rotach 2005: 538). The practicality and appropriateness of in-situ conservation programs to the farmer-stewards of landraces is highly important, albeit poorly understood. Moreover, there exists paucity a of research cataloguing the impacts of different on-farm management practices on specific genes and genetic combinations (FAO 1996). This speaks to a need for research about landraces that “takes a sufficiently ecological and evolutionary perspective, placing genetic variation within its appropriate environmental and management context” (Mercer and Perales 2010: 489).

With respect to chile peppers, these gaps in knowledge leave us asking a number of important questions: How do common cultivation systems that currently house traditional landraces — from the large, highly technified plantations of Oaxaca’s Central Valley to the small, rainfed backyard gardens of the region’s coasts and sierras — differentially impact the genetics and characteristics of the landraces grown there? How are farmers unintentionally altering the chile pepper gene pool, including genetic diversity and the plasticity and persistence of drought tolerance within these landraces, across different management regimes?

As the world’s second-highest most produced vegetable today (Madhavi-Redi et al 2016), *Capsicum annuum* represents an important crop species throughout the world, its significance rooted not only in its high nutritional and medicinal properties, economic value, and ubiquity across global cuisines, but also in the vast diversity of its gene pool. In fact, among all 5 domesticated and 32 wild species within the *Capsicum* genus, *Capsicum annuum* contains the most genetic diversity, which studies have shown cluster along distinct cultural lines (Aguilar Mendelez et al 2009). And yet chile peppers — whose formidable pungency can fend off even a bear — are by no means immune to the vagaries of climate, with moisture stress in particular accounting for up to 70% of yield loss in peppers (Madhavi-Redi et al 2016).

That being said, the literature abounds with studies documenting drought-adaptive phenotypes across different chile pepper genotypes, including traits related to root architecture (Kulkarni and Phalke 2009); germination and seedling characteristics (Balasankar 2017); and countless other morphological and physiological adaptations (Madhavi-Redi et al 2016). And yet many of these studies tend to isolate varieties from their unique historical and geographical contexts, focusing exclusively on the biological responses of cultivars, without accounting for how that biological response may be related to ecological context or past evolution. This

disconnect limits the ability of farmers and scientists to identify other genotypes for conservation, as well as best practices to preserve their local adaptations.

Fortunately, a growing body of research has begun mapping individual genotypes and adaptive phenotypes of chile peppers along their evolutionary lines, with an emphasis on domestication. Full genome sequencing and Quantitative Trait Loci (QTL) analyses have enabled researchers to co-locate adaptive phenotypes and better understand how genetic variation within the chile pepper germplasm relates to historical selection. Qin et al (2014), for example, have identified putative loci for stress and defense response, growth and development, protein synthesis, disease and pest resistance, and seed traits like dormancy, which may have all experienced a common, human-mediated “selection sweep” (Qin et al 2014: 5138). Other studies affirm the importance of wild and less cultivated chile pepper types to genetic diversity, with Pacheco Olvero et al (2012) demonstrating that wild chile types exhibit the most genetic variation (3.62 alleles per locus), followed closely by landraces (3.37) and finally by hybrids (3.08). Similar studies also indicate that cultivation leads to the loss of genetic variation, but that there is more to these changes than meets the eye. By comparing a broad and diverse range of cultivated, let-standing and wild types, González-Jara et al (2011) found not simply a reduction in genetic variation of up to 50% in cultivated chile peppers compared to their wild and let-standing counterparts, but also the presence of unique genes or “private alleles” exclusive to certain cultivated types — which they cite as “evidence that traditional managed habitats may be relevant reservoirs of genetic variation, particularly when native [wild] populations are declining” (González-Jara et al 2011: 9). And although studies have shown that the structure of genetic variation can be related to the cultural uses of chile pepper varieties (Zhang et al 2016), a

degree of inconsistency across landrace studies suggests an important layer of sociocultural complexity that makes the mapping of these populations particularly challenging.

Specifically, two landrace varieties may be closely related but have a different name and geographic origin (Zhang et al 2016), while others may have the same name but exhibit vastly different phenotypes (Fukuoka 2005). Such within-landrace variation may be traced back to individual farms, as seeds are selected by the same farmer over multiple generations and may be strongly influenced by their preferences and perceptions (Cleveland and Soleri 2000).

Differences between communities may also shape variation. For example, Zimmerer and Douches (1991) demonstrated that genetic variation within landraces was greatest between “micro-regions,” or “fields of an area whose inhabitants share a local cultural identity and ties to the same outlet for marketing and production,” with patterning a product of the ecological conditions of a micro-region, as well as social and cultural factors like the scale and direction of seed exchange and the cultivation patterns of local farmers (Zimmerer and Douches 1991: 187). This complex patterning of genetic variation in landraces speaks to the importance of examining the past and present particularities of a place — the cultivation systems, sociocultural dynamics and ecological conditions that might have shaped landraces there — in a balanced and holistic way. The paper that follows therefore examines drought responses of chile pepper seeds through the lenses of their historical selection, including their domestication and cultivation in Oaxaca, Mexico.

B. Domestication and Cultivation: Historical selection as a window into adaptive capacity

Whether a chile pepper is eaten fresh or dry, spicy or mild, red or green all reflect the many ways peppers have been shaped by humans for millennia. Anthropological analyses of pepper

remains from Mexico indicate that wild chile harvesting began around 8,000 years ago, and their cultivation and eventual domestication around 6,000 years ago (Perry and Flannery 2007). While multiple lines of evidence point to several possible centers of domestication and diversification for chile peppers, including northeastern and central-eastern Mexico (Kraft et al 2014), some areas are fertile ground for the discovery of unique, climate-adapted genotypes. The state of Oaxaca, in particular, encompasses great cultural diversity, serving as home to a variety of traditional landraces, wild types, and even feral genotypes found along roadsides and fencerows (Kraft et al 2012; 2014). Ranging from the small, bright-red, formidably pungent pods of the forest-dwelling *chile piquin* to the large, sweet, multi-colored fruits of the Dulce landrace, the vast array of phenotypes in Oaxaca speaks to the powerful ways that domestication and diversification have and will continue to impact the chile pepper gene pool.

Specifically, domestication refers to the “sustained, multi-generational, mutualistic relationship” between two species (Zeder 2014)— a process that is at once ongoing, dynamic and described in terms of several distinct stages. These stages include the onset of domestication, i.e. the formation of phenotypically distinct cultivated and wild populations; followed by diversification, or the evolution of crops as they expand from their geographic range, adapt to new environments, undergo conscious selection according to local preferences, and are subject to modern breeding for agronomic traits like yield and uniformity (Meyer and Perugannan 2013). While diversification traits are selected through conscious, human-mediated processes like seed saving for a particular phenotype, domestication traits are often unconsciously selected for by humans and may even be maladaptive in certain contexts, evidenced by the poor survivability of many domesticated crops outside of their cultivated environments (Meyer et al 2012).

Chile peppers are no exception, exhibiting symptoms of this so-called “domestication syndrome” — or a suite of traits that distinguishes cultivated species from their wild progenitors— that includes changes in the reproductive strategy of a crop, its seed and fruit size, branch morphology, and even secondary metabolites (Meyer et al 2012). Domestication traits in chile peppers are most apparent in fruit characteristics, including increases in fruit size, changes in fruit position from upright to pendent, and changes from deciduous to non-deciduous fruits, which eases the harvest of chile pepper fruits while simultaneously limiting their predation by birds, the primary dispersal agent for wild types (Harrison 1991; Pickersgill 2016). But domestication is also apparent in chile pepper seeds, whose small but powerfully pungent exteriors offer a window into the evolutionary ecology and adaptive capacity of chile pepper landraces.

Indeed, the seed and seedling stages are critically important in the life history of all plants, exhibiting a large degree of phenotypic variation in physiological and morphological traits (Khan et al 2012). Cochrane et al (2014) synthesize the ecological and evolutionary functionality of these traits as demonstrated across the literature, writing that “seed mass influences seedling survival; seed dispersal is a mechanism for dealing with environmental variability and uncertainty; seed dormancy spreads the risk of temporal variance in germination success over multiple seasons; and germination timing influences the number of seeds that become seedlings and their subsequent survival” (Cochrane et al 2014: 13). These traits bear practical importance to farmers as well, influencing seed germination and seedling establishment and thus the livelihood of smallholders and commercial farmers alike. As a growing body of QTL research demonstrates, these seed characteristics can even be correlated with other important phenotypes related plant to development and environmental tolerance; for example,

Khan et al (2012) demonstrated that QTL's for root growth in tomato co-located with seed size and seed weight, suggesting that these traits might be linked by a common genetic or mechanistic basis (Khan et. al 2012).

The effects of domestication, in particular, on the functional traits of chile seeds are documented throughout the literature and include increased seed size, decreased seed dormancy, and increased germination (Luna-Ruiz et al 2018), with faster germination serving as “the only trait consistently distinguishing wild from cultivated *C. annuum*” (Pickersgill 2016). Researchers who have noted variation in germination timing in other species consider evolved and plastic responses in seeds to be types of “bet-hedging strategies,” which may involve tradeoffs between different functional traits like seed mass and dormancy (Donohue et al 2005; Simons and Johnston 2006). Unfortunately, the exact selection forces and biological mechanisms behind many of these tradeoffs are unclear (Milla et al 2015), though in chile peppers, insights from a growing body of research into the capsaicin biosynthetic pathway may offer fertile ground to begin connecting these dots (Luna-Ruiz et al 2018).

Capsaicin — the secondary metabolite responsible for pungency in peppers — serves a number of adaptive functions, at once protecting seeds from fungal, microbial and invertebrate predators (Levey et al 2006), while being linked to abiotic stress tolerance, evidenced by the ability of more pungent cultivars to maintain higher yields under water stress relative their less pungent counterparts (Phimchan and Techawongstien 2012; Khan et al 2014). Variation in capsaicin and thus pungency in peppers may result from variation in natural selection pressures that occur across different spatial and temporal scales. For example, capsaicin production can increase at higher elevations, which tend to be more arid (Tewksberry et al 2006), as well as with increasing foraging pressure from hemipteran insects (Tewksberry et al 2008). Reductions in

capsaicin may change production of other secondary compounds such as lignin, which may reflect a functional tradeoff between chemical defenses and physical defenses along a shared biosynthetic pathway (Levey et al 2006; Tewksberry et al 2008). And yet the pungency of a population also hinges on selection by humans, including intentional selection for a particular landrace “ideotype” based on regional preferences and cultural uses (Kraft et al 2010; Castellón-Martínez et al 2012), as well as unintentional selection by virtue of cultivation itself.

As Luna-Ruiz et al (2018) write, “the very act of moving plants from natural habitats into culturally-managed habitats such as milpas alters the mix of selection pressures, leading to increased adaptation to cultivation and to actual physical protection from pests and predators by cultural managers” (Luna-Ruiz 2018: 4). In these environments, common practices such as fertilization, irrigation, pest control, weed management, and harvesting — which together result in changes to soil fertility, disturbance regimes, and trophic interactions — can drive the evolution of individual crop traits and integrated phenotypes (Milla et al 2015), as well as the evolution of new ecological relationships, such as a shift from herbivory to pestilence (Bernal and Medina 2018). Linking both trait profiles and ecological relationships to the conditions that created them can help elucidate the effects of both domestication and cultivation on different species (Milla et al 2015). For chile peppers, the importance of the milpa — the agro-ecosystem where they were first domesticated and where they continue to evolve — cannot be understated.

As a type of shifting-cultivation system found throughout Mesoamerica, a “milpa” refers to a polyculture of locally adapted varieties of maize, squash, beans, tomatoes, herbs and chile peppers. This agro-ecosystem serves not only as the foundation of food security in many smallholder farming communities, but also as a living, breathing artifact— a reflection of

thousands of years of co-evolution (Benitez 2018), which refers to the process whereby “a trait of one (or more) species evolves in response to a trait (or suite of traits in several other species)” (Ghersa 1994: 85). Indeed, almost every important agricultural crop we know and love today, from sunflowers to maize, potatoes to squash, was domesticated within the context of a “traditional agro-ecosystem,” evolving and co-evolving within systems characterized by their use of locally available resources, high degrees of agro-biodiversity, and common properties of resilience, productivity, and tolerance in the absence of conventional inputs like fertilizers and pesticides (Altieri and Merrick 1988). And yet industrialization in agriculture has threatened not only the existence of these agro-ecosystems, but also the systems of traditional knowledge that underpin them (Kleiche-drax 2016).

In Oaxaca, spatio-temporal data about smallholder farming reveals that traditional agro-ecosystems have declined dramatically in recent years, with a reduction in the average land availability for milpa cultivation linked to the expansion of capital-intensive production systems and the fragmentation of smallholder farms in the region (Robson 2011; Bermeo et al 2014). Here, the erosion of traditional knowledge has coincided with phenomena such as increases in off-farm employment and rural-to-urban migration, as well as the intensification of agriculture through shorter fallow periods and the integration of synthetic inputs on-farm (Pérez-García and Castillo 2016). Additionally, changes in land-use throughout Oaxaca are profound, with deforestation accounting for the loss of over half a million hectares of forested areas during the last 20 years and subsequent declines in biodiversity and environmental services (Velázquez et al 2003). Today, milpa cultivation exists alongside a variety of other cultivation types throughout the region, including plantations, described by Toledo et al. as “areas of monoculture whose production is directed mainly to the markets,” as well as managed and artificial forests and home

gardens (Toledo et al 2003). Like milpas, these backyard systems are characterized by a high degree of spatio-structural complexity; inter- and intra-specific genetic diversity; and the maintenance of landrace populations through cultural practices such as seed exchange (Aguilar-Støen 2009; Galluzi 2010). Additionally, attitudes towards management of landraces within these systems may vary based on farmer age, farm size, farm location, output market, personal preferences, and even gender (Birol et al 2008), with women increasingly viewed important actors in the conservation of agro-biodiversity and in the dissemination of traditional knowledge (Eyssartier et al 2018). But how, exactly, do these distinct differences between cultivation systems and dramatic agricultural, social and ecological changes to the Oaxacan landscape impact the diversity of chile pepper landraces within the region?

Changing social and ecological conditions and the resulting genetic erosion of landrace diversity have been widely documented, though the effects of specific cultivation regimes and individual practices on the co-evolution of traits and ecological relationships is less clear. Moreover, a tendency to conflate domestication — or the morphogenetic impacts of human selection — with cultivation, the human-mediated selective forces that *drive* domestication, permeates the literature (Harris 2015). This makes it difficult to examine the interactions between domestication and cultivation and thus the agricultural, ecological and biological mechanisms underlying these interactions.

That said, the study that follows aims to disentangle the effects of cultivation from those of domestication, examining the drought responses of 26 accessions from across 10 distinct landraces in terms of their cultivation intensity and domestication level. Specifically, this study aims to:

1. **Determine the effects of domestication level on drought tolerance by measuring germination under droughty and wet conditions in landraces from milpa and backyard cultivation systems; and**
2. **Determine the effects of cultivation system on drought tolerance by measuring germination under droughty and wet conditions for the subset of landraces cultivated in multiple systems.**

III. Hypothesis

We hypothesize that moisture reduction will stress all chile pepper seeds, but that this stress will affect landraces differently according to their evolutionary, cultural and biogeographical backgrounds. With respect to domestication, the more highly domesticated landraces — namely Chile de agua, Dulce and Costeño rojo— will exhibit greater germination percentages (num_germ) and faster germination rates (germ_rate) than less domesticated types. These highly domesticated populations will exhibit more non-adaptive responses to drought, such as the inability to withhold germination by “sensing” drought conditions, than their less domesticated counterparts, which may withhold or stagger germination. Similarly, accessions from the more cultivated plantation systems will exhibit greater and faster germination than their less cultivated milpa and backyard counterparts, but to varying degrees for individual landraces.

IV. Materials and Methods

A. Germplasm selection: Germplasm for this experiment was collected throughout the states of Oaxaca and Yucatan in Southern Mexico, which both represent possible centers of diversity and domestication for chile peppers. Twenty-six accessions from 10 different landraces were selected based on domestication level, previous growing environment, and geographic range (Table 1). Landraces were selected from across a domestication gradient (wild to highly improved) and from four cultivation systems, listed from least to most intensively managed: forested, backyard, milpa, and plantation (Table 3). We calculated a domestication gradient by scoring chile peppers according to various phenotypic indicators of domestication, with larger fruit size, seed size and seed mass, as well as pendant fruit position and non-deciduous peduncle correlating to a higher domestication score. Values for each trait were found throughout the literature. These values were added together for all the landraces, and each landrace was assigned a domestication level based on this trait-based domestication score (Tables 2). To glean possible variation resulting from eco-typic effects, at least two landraces from every ecozone was selected. These zones include Costa Chica, Costa Central, Isthmus, Central valleys, Sierra Sur, and Yucatan (Table 4). Seeds of each accession had been produced under uniform conditions in the greenhouse with randomized maternal plants in order to reduce environmental maternal effects on seed germination.

B. Experimental Design: We designed this experiment to measure seed germination and response to drought in a diverse collection of chile pepper accessions. We used a Randomized Complete Block Design in which each of the four blocks had randomized Petris dishes (i.e.,

experimental units) for each combination of accession (26 levels) and drought treatment (droughty and control) for a total of 52 Petri dishes. We simulated the drought stress treatment with a 20% solution of polyethylene-glycol (PEG) 8000, a popular compound used in drought simulations due to its unique molecular weight, which is small enough to influence osmotic potential, but large enough to remain unabsorbed by plants. A no PEG treatment was used as a control.

C. Procedures: Preliminary work indicated the importance of precision and sanitation during the plating process. All experimental units consisted of a constant ratio of sand, seeds and water: three tablespoons of sand to 20 mL of water or PEG solution, and 10 seeds per plate, with exceptions due to limited availability of seeds or human error. Exceptions include petri numbers 16 and 29 from block 2; numbers 4, 6, 11, 18, 21, 27, 35 and 48 from block 3; and numbers 10, 13, 28, 30, 36, and 40 from block 4. To ensure moisture retention in each plate, each petri dish was sealed with petroleum jelly applied to the lip of every plate, followed by a parafilm seal. In order to prevent contamination of petri dishes by various seed- and airborne pathogens, a number of sanitation procedures were employed. Plastic petri dishes were cleaned according to a four-sink method: washing with soap and rinsing with water, followed by soaking for two minutes in a 10% bleach solution and surface sterilization with rubbing alcohol. All other materials (sand media, distilled water, beakers and test-tubes, etc.) were autoclaved. Prior to plating, all seeds were surface sterilized with a (0.825%) sodium hypochlorite solution and rinsed thoroughly with sterile water. Sterilized seeds were plated beneath a laminar flow hood so as to reduce airborne contamination. Germination chamber settings were controlled for temperature and light,

maintaining a constant temperature of 28 degrees C and days and nights simulated by 12 hours of dark alternated with 12 hours of light.

D. Measurements and Data Collection: Plating of each block was staggered and occurred from March 21-25. Germination data was taken for a total of 14 days for all petri dishes, with data collected two times per day during the first week, and once per day during the second week. Seeds were deemed germinated when the radicle had protruded from the seed coat. Raw data included initial counts (init_count); daily counts of germination from hour 0 (germ_0) to hour 360 (germ_360); final counts of total germinated and ungerminated seeds (num_germ and num_ungerm).

E. TZ Test: After data collection was complete for germination, a TZ test was employed to glean measurements of total viability and dormancy. TZ testing unfolded according to protocols outlined in the Association of Official Seed Analyst's *Handbook on Seed Testing* (2000). All ungerminated seeds were imbibed for 24 hours at 30 degrees C. Seeds were partially split along their embryonic axes, then returned to the germinator to soak in a 0.01% tetrazolium solution for another 24 hours, this time at 35 degrees C. Seeds that stained red were considered viable, while those that were white or mottled were considered dead. Data was collected for number of seeds germinated after initial imbibition (num_germ_24) and the number of stained seeds (num_red).

F. Response variables and statistical analysis:

To minimize the effects of cultivation and thus isolate the effects of domestication within our model testing objective one, separate analyses were conducted for accessions from milpa

systems and backyard systems. The milpa comparison group included 6 distinct landraces from across five domestication levels (Chile de Agua – 1; Costeño Rojo – 2; Taviche – 3, Tusta – 4, and Payaso – 5), while the backyard comparison group included four distinct landraces across four domestication levels (Dulce – 2, Tusta – 4, Paradito – 5, and Piquin – 6) Accessions were selected from across 4 cultivation types: plantations, milpas, backyards, and forests. Three sets of pairwise comparisons (Plantation-Milpa; Milpa-Backyard; and Backyard-Forst) examined the differential impact of each growing environment on individual landraces.

We analyzed germination as a proportion of germinated to non-germinated seeds for all sampled accessions. Germination proportion is an indicator of germinability— or the “cumulative percentage of seeds that have germinated by the end of an experiment” (Rana and Santana 2006)— and is a widely recognized index of germination capacity. Although 400 seeds is generally considered the minimum for a representative sample for a good determination of germination, smaller quantities are acceptable for species for which it is not realistic to acquire such quantities (Ranal and Garcia de Santana 2006).

Although we were not able to perform statistical analyses on these other metrics, we plotted germination curves to identify temporal patterns in germination, including delays caused by dormancy (Table 11) (Ranal and Santana 2006; McNair et al 2012; Baskins and Baskins 2014). We also calculated measurements of this temporality including time of first germination (t_0) and time of last germination (t_g), as well as measurements of central tendency, including the mean, median, and germination proportion at 50% time (T_{50}).

Using generalized linear models and analyzing the data in SAS (Proc Glimmix), we constructed two models. In the first, we aimed to better understand how landraces that vary in domestication level differed in germination and response to PEG. Specifically, we used

ANOVA to discern the effects of PEG level, landrace, and the interaction of the two as fixed effects, while including the random effect of block. Our model statement as entered into SAS read: `model germ_prop= PEG landrace PEG*landrace`. We ran this model twice, once for the accessions that came from the milpa cultivation system, and once for those from backyards. To address objective two, we used a second model to discern how the cultivation system from which accessions were collected affected germination. To do so, we subsetted the data and compared germination of seeds from two landraces in each of two cultivation systems for their germination response to PEG (SAS model statement: `model germ_prop= PEG landrace cultivation PEG*landrace PEG*cultivation PEG*landrace*cultivation;`). In each model, we included PEG level, cultivation, and their interaction as fixed effects, with block as a random effect.

For the final analysis to address objective two, we compared “bird pepper” or wild-like chile pepper from different cultivation systems using four separate t-tests to discern the effects of the combination of cultivation system and drought.

V. Results

A. Analysis of germination percentage across a domestication gradient

Our first analysis of domestication defied expectations as we found no gradient in germination that paralleled the domestication gradient (Table 6.1). Within the milpa system, the six landraces, which together reflected five domestication levels, exhibited significant differences in germination ($P < .0001$) (Table 5.1). Mean separation revealed that these landraces clustered into three distinct groups (Table 6.1). Germination percentage proved highest in Costeño Rojo (88.9%), Tusta (87.4%), and Payaso (84.6%), which had significantly higher germination than Chile de Agua (57.6%) and Taviche (39.2%). Dulce (72.6%) had intermediate germination and was not distinguishable from the high germinators or the lower Chile de Agua, though it had significantly higher germination than Taviche.

By contrast, analyses of landraces from the backyard cultivation system, which included four distinct landraces, each representing a different domestication level, revealed no significant effect of landrace on germination percentage ($P = 0.1058$; Table 5.2). Trends in germination percentage for the backyard group appeared unrelated to domestication level, with the most domesticated and least domesticated landraces within this comparison group, Dulce and Piquin, respectively, exhibiting near identical germination percentage (Table 6.2).

In terms of PEG effects, both analyses of domestication level demonstrated that water stress (i.e., higher PEG levels) tends to decrease germination for landraces, albeit to different degrees depending on the cultivation system of origin. Analysis within the milpa system indicated a non-significant trend towards decreased germination under the PEG treatment ($P = .0976$; Table 5.1), with PEG reducing germination by around seven percentage points (75.3%

vs. 68.1%; Table 7.1). By contrast, PEG significantly decreased germination in the backyard comparison group ($P=.0085$; Table 5.2) by 25 percentage points (86.6% vs. 61.9%; Table 7.2). This trend towards decreased germination in response to water stress was expected.

The GxE (or landrace x PEG) interaction, which demonstrates the different ways in which the landraces responded to the PEG treatment, was insignificant across both the milpa and backyard comparison groups ($P=.2964$ and $P=.6675$, respectively; Table 5). Nevertheless, it is interesting to note that, in the backyard system, Paradito trended to having a greater response to PEG than other landraces (Figure 1.1). Similarly, in the milpa system, there appears to be some interesting (though not significant) variation in the strength and direction of PEG responses (Figure 1.2).

B. Analysis of difference in germination percentage between cultivation systems

When we compared accessions from different cultivation systems, we found some interesting results. The analysis of data from seeds of both plantation- and milpa-cultivated Chile de Agua and Costeño Rojo landraces showed non-significant effects of the cultivation system from which they were originally collected (Table 8.1). However, we did observe a significant interaction between PEG and landrace ($P=.0487$; Table 8.1), with PEG significantly reducing germination in one landrace (Chile de Agua), but not the other (Costeño Rojo) (Table 10).

The analysis of backyard- and milpa-cultivated Tusta and Dulce landraces revealed a different pattern. Aside from the expected and significant reduction in germination with PEG application, we found a significant landrace x cultivation effect ($P=.0325$) (Table 8.2), indicating that the effects of cultivation on germination percentage was largely contingent on the landrace in question (Table 10.2). Although further mean separation was inconclusive (all lsmeans were

deemed equal), we found that Tusta landraces collected from milpas trended to having higher germination than those collected from backyards (14% difference); by contrast, the backyard Dulces trended to having higher germination than those from the milpas (12% difference) (Table 10.2). The overall effect of PEG was also significant, with PEG reducing germination (Table 8).

In the final set of pairwise comparisons, we compared accessions of bird peppers (wild types) that had been cultivated under backyard and forest systems. Analysis revealed no significant effects of cultivation on germination. The overall effect of PEG proved significant at $P=.0025$ (Table 9), although forest peppers were more significantly affected ($P=.0414$; Table 9), while backyard peppers only trended towards reduced germination ($P=.0831$; Table 9).

C. Temporal patterns in germination

In addition to the ANOVA results outlined above, we constructed germination curves separately for each landrace to informally assess germination of seeds from each system assayed with and without PEG (see Figures 3-11). Temporal variables extracted from these figures are indicated in Table 11. Though not analyzed via ANOVA or regression, trends in these curves and temporal indices clarify the ways in which PEG slows germination. The effects of PEG on different metrics of germination timing appear to be dependent on cultivation and landrace, with a large degree of variation between individual landraces, and within these landraces, variation among cultivation systems. For example, the germination curves of the Taviche and Costeño Rojo landraces (Figures 8 and 3) are fairly consistent, regardless of treatment, suggesting minimal effects of cultivation system and PEG on temporal patterns in germination. By contrast, separation between the curves of the Chile de Agua, Dulce, Tusta, Payaso, Paradito, and bird pepper landraces indicate that the PEG and cultivation treatments had a larger effect on the germination timing of these landraces (Figures 4, 5, 6, 9, 10 and 11).

D. TZ Results

The results of the TZ testing (Figure 12) indicate possible differences in dormancy among landraces, though this data was not analyzed via ANOVA and thus be interpreted with caution. While a majority of seeds from most landraces exhibited a degree of non-deep dormancy, germinating after 24 hours of imbibition at 30 degrees C (i.e. after the PEG solution had washed off), a handful exhibited deeper levels of dormancy, staining red after soaking in the TZ solution. The fact that a larger proportion of the remaining ungerminated Chile de Monte and Paradito seeds stained red suggests that these landraces may retain dormancy more than other genotypes.

VI. Discussion

In this work, we observed several expected effects: Namely, water stress reduced germination across the board, and different landraces expressed variation in germination levels. Contrary to our expectations, we found that neither the domestication gradient nor the cultivation system had consistent or expected effects on seed germination, suggesting that other sources of variation such as environmental factors may play a larger role in shaping the germination behavior of chile pepper seeds. Studies with sampling done to account for these other sources may be illuminating.

Lacking a domestication effect, could environment be important?

The results of objective 1 suggest that the perceived domestication level of individual landraces might be a poor predictor of germination, despite a decent body of literature that observes increased germination among domesticates relative their wild counterparts (Rojas-Aréchiga, et al 2001). Although our analysis of milpa-cultivated accessions observed significant differences in the germination of individual landraces (which fell into three distinct groupings of high, moderate, and low germination), there was decent variability of domestication levels within these groupings. Such variability suggests that any effects of domestication itself were not as salient as other sources of variation. This was evidenced by insignificant differences between the more domesticated landraces (Costenjo Rojo, Dulce) and the less domesticated landraces (Payaso, Tusta). Moreover, the most domesticated landrace, Chile de Agua, ended up in the low germination group, defying the expectation that it would exhibit the highest germination. Within the analysis of seeds from backyard systems, germination proportion again appeared unrelated to the domestication level of individual landraces, with non-significant differences observed between the most domesticated (Dulce) and least domesticated (Piquin) type.

This incongruence between the domestication gradient and germination gradient contradicts the initial hypothesis that the more domesticated landraces would display greater germination, while the less domesticated landraces would display lower germination, with domestication level exhibiting no clear and consistent effect across the sampled accessions. These results parallel those of Maas and Usongo (2007), who observed germination to be greatest among “semi-domesticated” types compared to their “cultivated” counterparts, but who also noted that their results may have been skewed by the conflation of domestication and cultivation within their experimental design (Maas and Usongo 2007). Thus, on the one hand, the variation observed within our experiment may be grounded in the flawed nature of our domestication gradient, which could be improved through incorporation of genetic analyses. On the other hand, the insignificance of domestication might suggest the importance of other effects, including regional eco-typic effects, on germination.

That said, we calculated our domestication index based on a suite of “domestication traits” observed throughout the literature (Aguilar-Rincón et al 2010; Pickersgill 2016), though a wide variety of other typologies exist. For example, while some studies rank domestication exclusively along a spectrum of human-plant relationships (Vodouhè et al 2011), others use genetic markers (Leff 2016) or trait-based indices (Maas and Usago 2007). The failure of our domestication gradient to describe variation in germination may speak to the importance of a more integrated approach, such as that of Dempewolf et al (2008), who ranked domestication as a product of myriad morphogenetic and cultural indices: phenotypic differentiation, history and extent of cultivation, and genetic shifts among them.

Of course, the effects of the environmental differences among collection locations on landrace germination might have also served as an unaccounted-for source of variation, with the

effects of location possibly overriding that of domestication. Many studies have investigated the impacts of macro-, meso- and micro-scale ecological gradients on genetic variation in landraces, specifically the development of distinct “eco-types” adapted to different environmental conditions (Zimmerer and Douches 1991; Zhang et al 2016). Given that the analyses grouped together landraces from dramatically different bioclimatic zones — from the drier, hotter climates of the Yucatan Peninsula to the wetter, more-humid climate of Oaxaca’s western coast— it seems plausible that the “landrace effects” observed might actually be a reflection of ecotypic effects. Thus, any effects of environment on germination characteristics may have swamped out variation based on domestication level. Of course, it should be noted that region can be confounded with landrace, as some landraces are only found within one region and relatively fewer are found across eco-regions. Future investigations of domestication effects could account for these regional effects by sampling landraces grown in different regions.

A closer examination of the direction of selective forces in these regions may provide expectations for the strength of these eco-typic effects relative to those of domestication. For example, while the ancestral selection of landraces in regions with frequent precipitation (Costa Chica, Sierra Sur, Costa Central) may not have necessitated the evolution of bet-hedging strategies such as variation in germination timing, natural selection in drier and more drought-prone areas (Isthmus, Yucatan, Central Valley) might, conversely, encourage the evolution of such strategies, as evidenced throughout the literature by studies on dormancy under desert conditions (Lewandrowski 2018) and the adaptivity of variation in germination timing across geographic regions (Donahue 2005).

Other aspects of the environment such as altitude may also select upon crop populations, possibly contributing to ecotypic effects on functional traits. For example, studies have shown

that the environmental changes that accompany changes in elevation can result in local adaptation of crop landraces and that such adaptation can be asymmetrical (Mercer et al. 2008). Altitudinal effects might explain why the germination of the Chile de Agua landrace (collected at elevations ranging from 1488 to 1685 ft above sea level) and the Taviche landrace (collected at 1461 ft) was significantly lower than that of other landraces, collected for the most part at elevations below 1000 ft. Relative humidity in the germination chamber could have served as one unaccounted-for source of variation, differentially impacting these low- and high-elevation landraces given that relative humidity generally declines at higher altitudes. This logic does not, however, explain why the Tusta accessions grown at the same high altitude germinated more robustly than their counterparts from the Costa Central, though an examination of cultivation may illuminate this difference (Appendix 1). The occurrence of such within- and between-landrace inconsistencies (Appendix 2) suggests that the ability of an accession to express ecotypic variation hinges on the interplay of myriad factors— domestication, cultivation, and biogeography among them.

Evolutionary agroecological importance of germination characteristics in diverse cultivation systems

Our second set of analyses sought to discern the effects of cultivation on landraces through comparisons of individual growing systems that shared a pair of landraces, disentangling these cultivation effects from those of domestication. Unfortunately, variation in our data made it difficult to discern the effects of cultivation, providing little support for our hypothesis that seeds from accessions collected from more intensively cultivated systems would have increased germination. Nevertheless, we found a significant interaction between landrace and cultivation,

which suggested that the effects of cultivation may be landrace-dependent and speaks to the importance of examining cultivation effects on a landrace by landrace basis. Specifically, the direction of the effects of cultivation appear to differ across landraces, which interestingly may be related to domestication level of a particular landrace. We observed that greater intensity of cultivation lead to increased germination for the less-domesticated Tusta and bird pepper landraces, but to decreased germination for the more domesticated Dulce and Chile de Agua landraces. However, we would need data from more comparisons to understand this better. Certain strategies might help to bring these cultivation effects to the forefront in future studies, including a larger sampling of accessions within each region, a greater sampling of cultivation systems within each domestication level, and a greater sampling of domestication levels from each cultivation system.

Additionally, a shift in thinking about how we define the cultivation system variable might also be necessary in these studies. Our ranking of cultivation system could benefit from the integration of data about specific cultivation practices, field-level differences, and information about the sociocultural backgrounds of individual farmers — factors that are all important to landrace selection and diversity (Zhang et al 1999; Soleri and Cleveland 2000; Fukuoka 2005). These variables may have served as a significant source of unexplained within-cultivation variation in this experiment, given the possibility that the broad-brush categories of “milpa,” “backyard” and “plantation” cultivation type might have grouped together farms with different growing practices and farmers with different preferences and perceptions. For example, our analysis of plantation-grown Chile de Agua grouped together “open fields,” “technified plantations with irrigation and plastic soil coverage” and “highly technified organic greenhouse” beneath the over-arching umbrella of “plantations,” which might have eclipsed the subtle, albeit

important differences between these individual farming systems. In future studies, a better strategy might be to quantify accessions based on qualitative indices of cultivation intensity — perhaps along a spectrum that accounts for practices like irrigation and fertilizer use, farm attributes like its size and rotation scheme, landscape-level indicators of agro-ecological diversity, farmer perceptions of the health of their agro-ecosystems, and demographic information about individual farmers. Of course, these indices might ultimately lead us to the same groupings.

Additionally, more information about cultivation systems would be helpful in determining what would constitute adaptive seed behavior in a given cultivation context. What is adaptive in the context of a large, irrigated plantation might not necessarily be adaptive in a rainfed backyard cultivation system. Moreover, specific cultivation practices like the propagation methods employed in each system are important, especially with respect to germination. While a farmer who direct seeds crops in a rainfed system may be dramatically affected by a drought event, a farmer who transplants his or her crops in the same system may be less affected, given that they are better able to control the amount of moisture allocated to the vulnerable seedlings and even manipulate the drought tolerance of their crops through transplanting (Leskovar 1998). Seed lots that germinate rapidly and more uniformly, such as the Costeño Rojo landrace, might excel in the former, and seed populations that retain a degree of dormancy, such as the Paradito landrace, might be more suited to the latter. In each case, then, their dormancy and germination characteristics might be adaptive; however, it is tough to know from the data we have. Future studies may benefit from embracing a strategy of trait-based ecology, which correlates different “functional trait profiles” to their adaptive contexts, such as “resource-aquisitive” trait profiles to nutrient-rich environments and “resource-conservative” trait profiles to nutrient poor

environments (Milla et al 2015). Doing so for chile pepper seeds would require a more comprehensive understanding of the functionality of different seed traits such as seed mass, seed hardness, capsaicin concentration, pathogen resistance and germination timing within different cultivation contexts, described in terms of their propagation strategies, irrigation practices, agro-ecological conditions, and the vulnerability of each to climate stress. (Appendix 3-4).

VII. Conclusion

In conclusion, the domestication and cultivation levels of the sampled accessions did not accurately predict their germination characteristics, which exhibited no clear and consistent pattern across both sets of analysis. Nevertheless, significant differences were observed between landraces, possibly speaking to the interplay of domestication and eco-typic effects. We also noted a significant interaction between landrace and cultivation that suggested that the direction of cultivation effects may depend on the particular landrace and perhaps its domestication level. In this way, the study demonstrated the need for a larger sampling of accessions across regions, cultivation systems, and domestication levels, as well as the integration of site-specific data about cultivation practices in any future examination.

VIII. Appendix

1. The unique cultivation context of Tusta may serve to explain its capacity to maintain high germination. Tusta is established in temporary conditions in hillside lands, with slopes ranging from 3 to 30% and with production processes that make use of fire-fallow rotations, traditional technologies and minimal use of agrochemicals (Aguilar-Rincón et al 2010: 79), which contrasts with the more uniform conditions and industrialized practices of Chile de Agua (23) and Taviche (68) cultivation systems. Could the heterogeneity of Tusta cultivation systems translate to reduced sensitivity to altitudinal effects?
2. Interestingly, the results of the experiment at once support and challenge the idea that variation in germination timing might be linked to location. In support of this notion, both the backyard-cultivated Paradito and Dulce landraces from the hot, dry Yucatan appear to have exhibited delayed and reduced germination under the PEG treatment indicating that they are able to sense drought and respond by not germinating (Figures 10 and 5, respectively; Table 11). Yet, domestication may still play a role, since the more domesticated Dulce from that region exhibited a less plastic response than Paradito (Figure 2). The interplay of domestication, cultivation, and ecotypic effects may be illuminated by a more intentional sampling of populations based on region.
3. **Personal observations**
 - i. **Contamination:** Throughout the data collection process, contamination within petri dishes was noted, with a marked degree of contamination observed for the Chile de Agua and Taviche landraces. Contamination was also observed for the Dulce and Costeño Rojo

landraces, albeit to a lesser degree and occurring later on in during the experimental window.

ii. Seed hardness: At the end of the experimental window, a number of seeds (largely from the Chile de Agua landrace) exhibited a sort of “squishiness,” which might suggest greater permeability of their seed coats to water or death. Consequently, the squishiness of these seeds made any meaningful examination of their embryos impossible during TZ testing, as these embryos tended to be liquefied and thus indistinguishable. Interestingly enough, the interior of these seeds tended to stain a deep red in the TZ solution, which might suggest that they had been colonized by some sort of bacterial or fungal pathogen.

4. Personal observations throughout the experiment support the idea that cultivation may influence other seed traits. For example, differences in the incidence of contamination (though not explicitly tested for) were noted throughout the experiment, with the Chile de Agua and Taviche landraces proving most sensitive to contamination and the seeds of other landraces like Tusta proving less sensitive. These differences in contamination might be grounded in differences in capsaicin — the anti-fungal compound responsible for pungency — with Vera-Guzmán et al (2011) observing higher concentrations of capsaicin in Tusta, Piquin and Paradito (51.4, 116, 142) and lower concentrations in Chile de Agua and Costno Rojo (4.9 and 14.9 respectively). These differences in capsaicin might be a product of altered selection pressures resulting from domestication and cultivation, including decreased foraging pressure from hemipteran insects and protection from abiotic stress (Tewksberry 2008) (Khan et al 2014) (Ruiz et al 2018), which could explain why the more domesticated, more cultivated landraces tended towards higher incidence of contamination in our experiment. Theoretically, reduced

synthesis of capsaicin (a chemical defense) would also lead to greater synthesis of lignin (a physical defense) due to tradeoffs at the molecular level along a shared biosynthetic pathway (D.J Levey et al). And yet the same seeds that experienced the least contamination tended to hold up better throughout the experiment, while those that experienced the most contamination (Chile de Agua) tended to be “squishier” — i.e. more permeable and tender, with liquefied embryos, implying less lignin in their seed coats (See personal observations; page). Because of the importance of capsaicin and lignin as chemical and physical defenses against myriad biotic and abiotic stresses, future studies would benefit from examining the relative production of these compounds not only in response to immediate stresses that mimic climate change, but also in relation to their evolutionary selection history. For although studies have examined the evolutionary ecology of pungency in wild chile peppers (Tewksbury et al 2008), a lack of research comparing wild types to domesticated and semi-domesticated varieties from a range of cultivation systems leaves us asking how the degree of domestication, the history of recent cultivation, and biogeographical origin of a chile accession impacts the chemical constituents of its seed coat. Moreover, correlating patterns of variation in the composition of seed coats with patterns of variation in other life history attributes such as germination timing could elucidate the evolutionary and ecological relationship between important adaptive phenotypes. How has selection — both by nature and by man — influenced the co-evolution of seed traits?

VIII. Tables

Table 1. Sampled germplasm

Includes environmental and cultivation data for all of the sampled accessions.

<u>Accession</u>	<u>Landrace</u>	<u>Cultivation</u>	<u>Notes</u>	<u>Region</u>	<u>Village</u>	<u>Elevation</u>
188	Tusta	Milpa		Sierra sur	Santa Lucia Miahuatlan	
185	Tusta	Milpa	Local commerce between communities	Sierra sur	San Pablo Coatalan, Los Coatalanes	
187	Tusta	Milpa		Sierra sur	San Baltazar Loxicha	
93	Tusta	Milpa		Costa central		
179	Tusta	Backyard		Sierra sur	San Pablo Coatalan, Los Coatalanes	1461
106	Tusta	Backyard		Costa central	Los Reyes	222
84	Costeño rojo	Plantation		Costa chica	Guadalupe Victoria, Pinotepa Nacional	189
77	Costeño rojo	Plantation		Costa chica	Anil	155
165	Costeño rojo	Plantation		Costa central		
98	Costeño rojo	Milpa	Very complex polyculture	Costa chica	Lagatero, Pinotepa Nacional	20
204	Paradito	Backyard	Traspatio casco urbano	Yucatan	Mani	36
217	Payaso	Milpa	Irrigation channels	Isthmus	Rancho llano, tehuantepec	
126	Chile de Monte	Forest	Deciduous tropical forest	Isthmus	Saachilac	20
173	Chile de Agua	Plantation	Open field	Central valley	Santa Cruz Nexilla, Sola de Vega	1461
132	Chile de Agua	Plantation	Highly technified organic greenhouse	Central valley	Coatecas Altas, Ejutla de Crespo	1533
135	Chile de Agua	Plantation	Technified, with irrigation, plastic soil coverage	Central valley	La Labor, San Dionisio Ocotlan	1548
189	Chile de Agua	Plantation	Open field	Central valley	Paraje Coatequillas, Ejutla de Crespo	
151	Chile de Agua	Plantation	Technified, with irrigation, plastic soil coverage	Central valley	Paraje Coatequillas, Ejutla de Crespo	1488
143	Chile de Agua	Milpa		Central valley	La Lobera, Santa Ines del Monte	1685
215	Chile de Agua	Milpa		Central valley	Abasolo	1579
186	Taviche	Milpa	Local commerce between communities	Sierra sur	San Pablo Coatalan, Los Coatalanes	1461
213	Dulce	Milpa		Yucatan	Cansahcab, dzibtzantun	8
211	Dulce	Backyard		Yucatan		

Table 2. Calibrating the domestication gradient

Lists phenotypic indicators of domestication for chile peppers as described throughout the literature, including fruit size, seed size and seed mass, as well as pedant fruit position and non-deciduous peduncle (Pickersgill 2016). Values for each landrace were gathered throughout the literature and summed for all the landraces. Each landrace was assigned a domestication level from 1-6 based on this trait-based domestication score, with level 1 indicating most domesticated and level 6 indicating least domesticated types.

<u>Landrace</u>	<u>Fruit size</u>	<u>Seed size*</u>	<u>Fruit position**</u>	<u>Trait-based Domestication score:</u>	<u>Domestication level</u>
Chile de agua	15 cm (length) 6 cm (diameter)	3	3	21	1
Dulce	6.6 (length) 6.5 cm (diameter)	3	3	12.6	2
Costenjo rojo	6 cm (length) 2 cm (diameter)	3	3	12	2
Taviche	4.5 cm length	2	3	9.5	3
Tusta	2.8 cm (length) 1.6 cm (diameter)	1	3	7	4
Paradito	3 cm length	1	0	4	5
Payaso	1.5 cm length	1	0	2.5	5
Chile de monte	5-7mm diameter (ovalados)	1	0	2	6
Piquin	5-7mm diameter (ovalados)	1	0	2	6

* large = 3, medium = 2, small = 1 ** upright = 0, pedant = 3

Table 3. Accessions ordered by domestication level and cultivation intensity

Depicts analysis criteria for Objectives 1 and 2. Landraces and accessions are listed across a domestication gradient and as pairwise comparisons of growing environment.

<u>1)</u> Domestication level	Growing environment/ accession number				
	Landrace	Forest	Backyard	Milpa	Plantation
1	Chile de Agua			143, 215	173, 151, 135, 132
2	Costeño Rojo			98	84,77,165
2	Dulce		211	213	
3	Taviche			186	
4	Tusta		179, 106,	185, 93	
5	Payaso			217	
5	Paradito		204		
6	Bird peppers	126	88		

Table 4. Eco-region of origin

At least two landraces from every ecozone were selected. These zones include Costa Chica, Costa Central, Isthmus, Central valleys, Sierra Sur, and Yucatan.

<u>Costa chica</u>	<u>Costa central</u>	<u>Isthmus</u>	<u>Sierra sur</u>	<u>Central valleys</u>	<u>Yucatan</u>
Costenjo Rojo	Costenjo Rojo	Payaso	Taviche	Chile de agua	Dulce
Piquin	Tusta	Chile de monte	Tusta	Tusta	Paradito

Table 5. ANOVA of landraces selected across a domestication gradient

ANOVA, generated in SAS GLIMMIX, identifying the factors affecting percent germination across the milpa and backyard comparison groups. The effects of Landrace on milpa-collected accessions proved significant in the first comparison group, while the effects of PEG on backyard-collected accessions proved significant in the second comparison group. Significance ($P < 0.05$) is indicated in bold.

1) ANOVA Milpa Group				
Effect	DF	Den DF	F Value	Pr > F
PEG	1	65	2.82	0.0976
landrace	5	65	15.88	<.0001
PEG*landrace	5	65	1.25	0.2964

2) ANOVA Backyard Group				
Effect	DF	Den DF	F Value	Pr > F
PEG	1	25	8.15	0.0085
landrace	3	25	2.26	0.1058
PEG*landrace	3	25	0.51	0.6775

Table 6. Germination of landraces selected across domestication gradient

LS-Means and standard errors for milpa- and backyard-collected accessions of landraces selected across a domestication gradient, with a “Domestication Level” of 1 indicating most domesticated and 6 indicating least domesticated. Mean separations use a Tukey-Kramer analysis and means sharing the same letter in their group designation do not differ significantly.

1) LS-means for landraces grown in the Milpa cultivation system				
Landrace	Germination Percentage	Standard Error	Group	Domestication level
Costenjo rojo	88.8636	4.3924	A	2
Tusta	87.3548	6.0690	A	4
Payaso	84.5833	6.0690	A	5
Dulce	72.5631	6.0690	B A	2
Chile de agua	57.6389	6.0690	B C	1
Taviche	39.2045	3.2439	C	3
2) LS-means for landraces from Backyard cultivation system				
Landrace	Germination Percentage	Standard Error	Group	Domestication level
Dulce	84.2361	7.6301	A	2
Piquin	81.6667	7.6301	A	6
Tusta	73.5417	12.4599	A	4
Paradito	57.5000	5.3953	A	5

Table 7. PEG effects in milpa and backyard cultivation systems

LS-Means for PEG effects on the milpa and backyard cultivated accessions. Accessions from backyard systems trended towards larger decreases in germination in the presence of PEG, while milpa-collected accessions experienced lower decreases in germination. Mean separations use a Tukey-Kramer analysis and means sharing the same letter in their group designation do not differ significantly.

1) LS-means for PEG Milpa		
PEG	Estimate	
n	75.3172	A
y	68.0855	A

2) LS-means for PEG Backyard		
PEG	Estimate	
n	86.6146	A
y	61.8576	B

Table 8. ANOVA of landraces grown in different cultivation systems

ANOVA, generated in SAS GLIMMIX, identifying the factors affecting percent germination for pairwise comparisons of different growing systems, including Milpa- and Plantation-collected accessions of the Chile de Agua and Costeño Rojo landraces (8.1) and Backyard- and Milpa-collected accessions of the Dulce and Tusta landraces. Significance ($P < 0.05$) is indicated in bold.

1) ANOVA for Milpa vs. Plantation cultivation				
Effect	Num DF	Den DF	F Value	Pr > F
PEG	1	73	7.40	0.0081
landrace	1	73	57.64	<.0001
cultivation	1	73	0.74	0.3927
PEG*landrace	1	73	4.02	0.0487
PEG*cultivation	1	73	1.22	0.2727
landrace*cultivation	1	73	0.71	0.4011
PEG*landrace*cultivation	1	73	0.01	0.9189
2) ANOVA for Backyard vs. Milpa cultivation				
Effect	Num DF	Den DF	F Value	Pr > F
PEG	1	53	4.96	0.0302
landrace	1	53	0.12	0.7255
cultivation	1	53	0.03	0.8544
PEG*landrace	1	53	0.65	0.4245
PEG*cultivation	1	53	0.72	0.4004
landrace*cultivation	1	53	4.82	0.0325
PEG*landrace*cultivation	1	53	0.13	0.7188

Table 9. Paired T-test of wild types from different growing environments

Paired T-test of bird pepper landraces grown in forest and backyard cultivation systems. Factors affecting percent germination include PEG and cultivation type. Significance ($P < 0.05$) is indicated in bold.

T-test for Forest and Backyard Cultivation			
Effect	Mean germination	Standard Error	P for diff
PEG	42.86	6.44	0.0025
No PEG	80	7.24	
Forest	53.75	8.22	0.4466
Backyard	71.67	11.38	
Forest PEG	37.5	7.5	0.0414
Forest No PEG	70	9.13	
Backyard PEG	50	11.55	0.0831
Backyard No PEG	93.33	6.67	

Table 10. LS-Means for significant PEG*Landrace and Landrace*Cultivation interactions

10.1 depicts LS-Means for the significant PEG*Landrace interaction observed for Plantation- and Milpa-collected accessions of Costeño rojo and Chile de Agua with PEG (y) and without PEG (n). 10.2 depicts LS-Means for the significant Landrace*Cultivation interaction observed for Backyard- and Milpa-collected accessions of the Dulce and Tusta landraces. Mean separations use a Tukey-Kramer analysis and means sharing the same letter in their group designation do not differ significantly.

1. PEG*landrace Least Squares Means				
PEG	landrace	lsmean	Standard Error	Group
n	Chile de agua	64.6273	4.8045	B
n	Costenjo rojo	90.4857	5.9607	A
y	Chile de agua	42.7636	4.7504	C
y	Costenjo rojo	87.1710	5.9607	A
2. Landrace*cultivation Least Squares Means				
landrace	cultivation	Estimate	Standard Error	Group
Dulce	Backyard	84.2361	6.9986	A
Dulce	Milpa	72.5631	6.9986	A
Tusta	Backyard	73.5417	4.9488	A
Tusta	Milpa	87.3548	3.4993	A

Table 11. Temporal patterns in germination

Temporal patterns in germination, including time of first germination (t_0), time of last germination (t_g), and germination proportion at 50% time (T_{50}).

Landrace	PEG	System	T50 (germ_180/mid)	T0 (hours)	Tg (hours)	
Taviche	n	milpa		30	70	300
Chile de monte	n	forest		45	70	300
Chile de agua	n	plantation		56.4	50	280
paradito	n	backyard		57.5	50	270
Chile de agua	n	milpa		61.3	50	270
tusta	n	backyard		65	70	330
piquin	n	backyard		70	50	270
Dulce	n	milpa		70.7	50	200
Dulce	n	backyard		80	50	270
tusta	n	milpa		81.8	50	330
Costeño rojo	n	plantation		82.6	40	330
Costeño rojo	n	milpa		82.9	50	260
payaso	n	milpa		88.6	70	250
Average			67.0615385	55.3846154	281.538462	
Dulce	y	backyard		18.9	70	340
Chile de agua	y	plantation		22.7	70	310
Chile de monte	y	forest		27.5	70	310
Taviche	y	milpa		34.1	70	330
Chile de agua	y	milpa		34.2	70	310
paradito	y	backyard		35	90	200
tusta	y	backyard		35.4	70	360
piquin	y	backyard		36.7	40	270
tusta	y	milpa		42.1	70	330
payaso	y	milpa		56.4	90	330
Dulce	y	milpa		66.7	70	290
Costeño rojo	y	plantation		74.3	50	330
Costeño rojo	y	milpa		80	50	220
Average			43.3846154	67.6923077	302.307692	

Table 12. Tusta Landrace Location Effects

Location effects on Tusta landrace grown in the Sierra Sur and Costa Central. See Appendix 1.

Tusta	Elevation	Mean germination proportion	n
Sierra sur	1461	89.49044586	31
Costa Central	200	69.93865031	16

IX. Figures

Figure 1.1 Milpa-collected accessions of landraces selected across a domestication gradient

Non-continuous norms of reaction for landrace x PEG treatments for landraces within the milpa comparison group. Only landrace was a significant source of variation ($P = <.0001$; Table 5.1). LS-Mean and Standard Error values derived from Table 6.

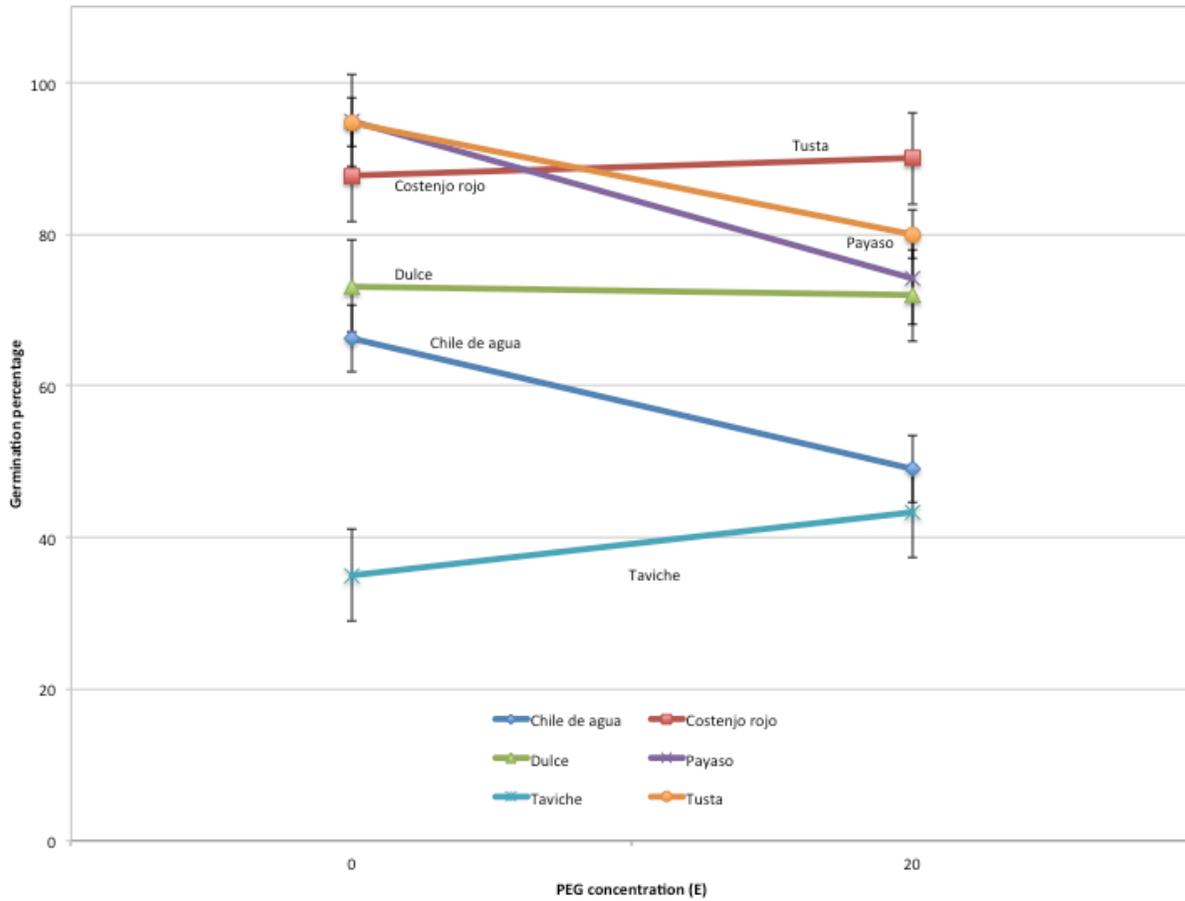


Figure 1.2 Backyard-collected accessions of landraces selected across a domestication gradient

Non-continuous norms of reaction for landrace x PEG treatments for landraces within the backyard comparison group. Only the effects of PEG were deemed significant ($P=0.0085$; Table 5.2). LS-Mean and Standard Error values derived from Table 6.

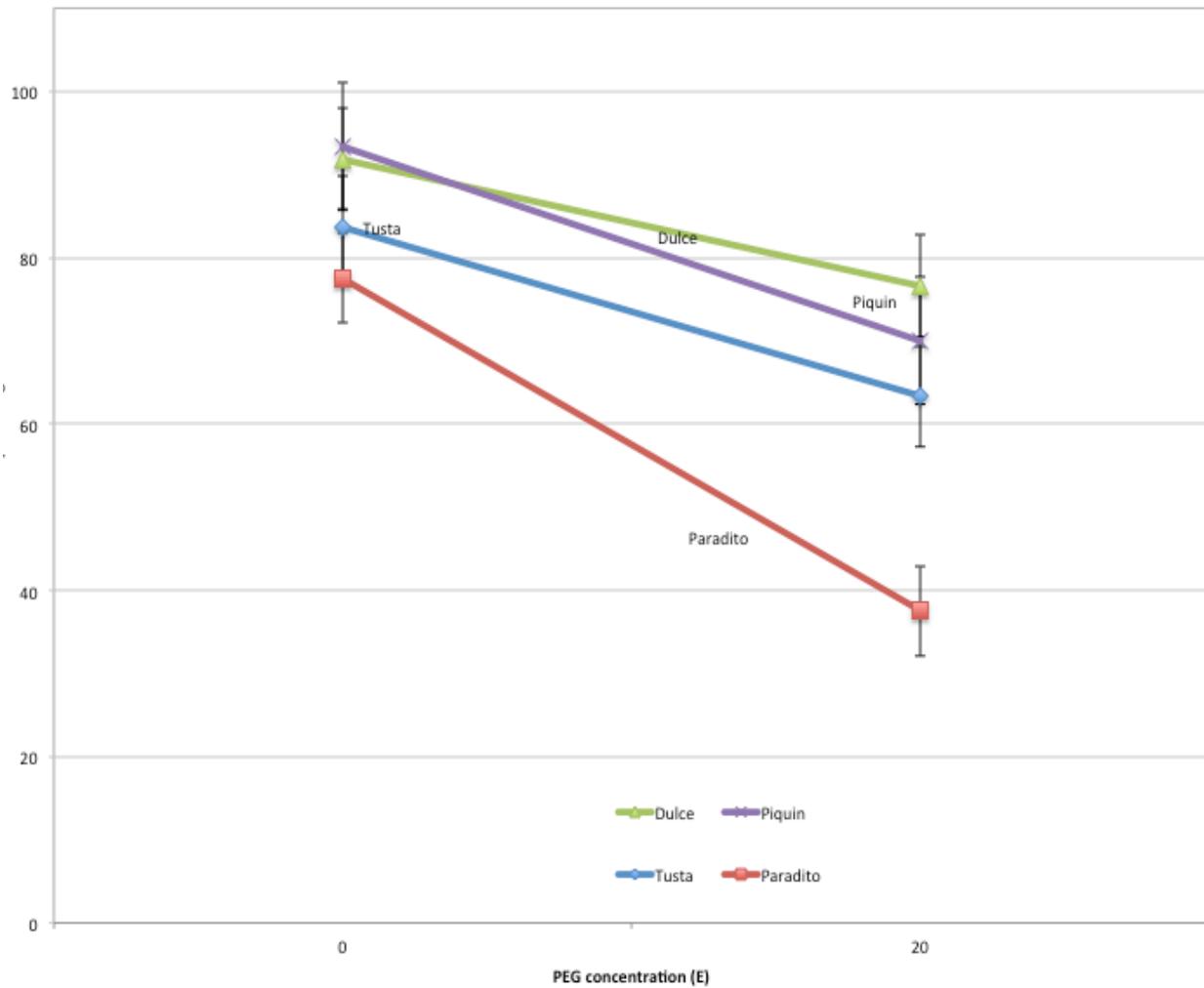


Figure 3. Costeño Rojo germination curves

Germination curves for the Costeño Rojo landrace, including average values for germ_proportion_0 through germ_proportion_360 from across 4 replications. Curves represent a milpa accession (no. 98) and plantation accession (no. 77, 84, 165) with and without PEG treatment.

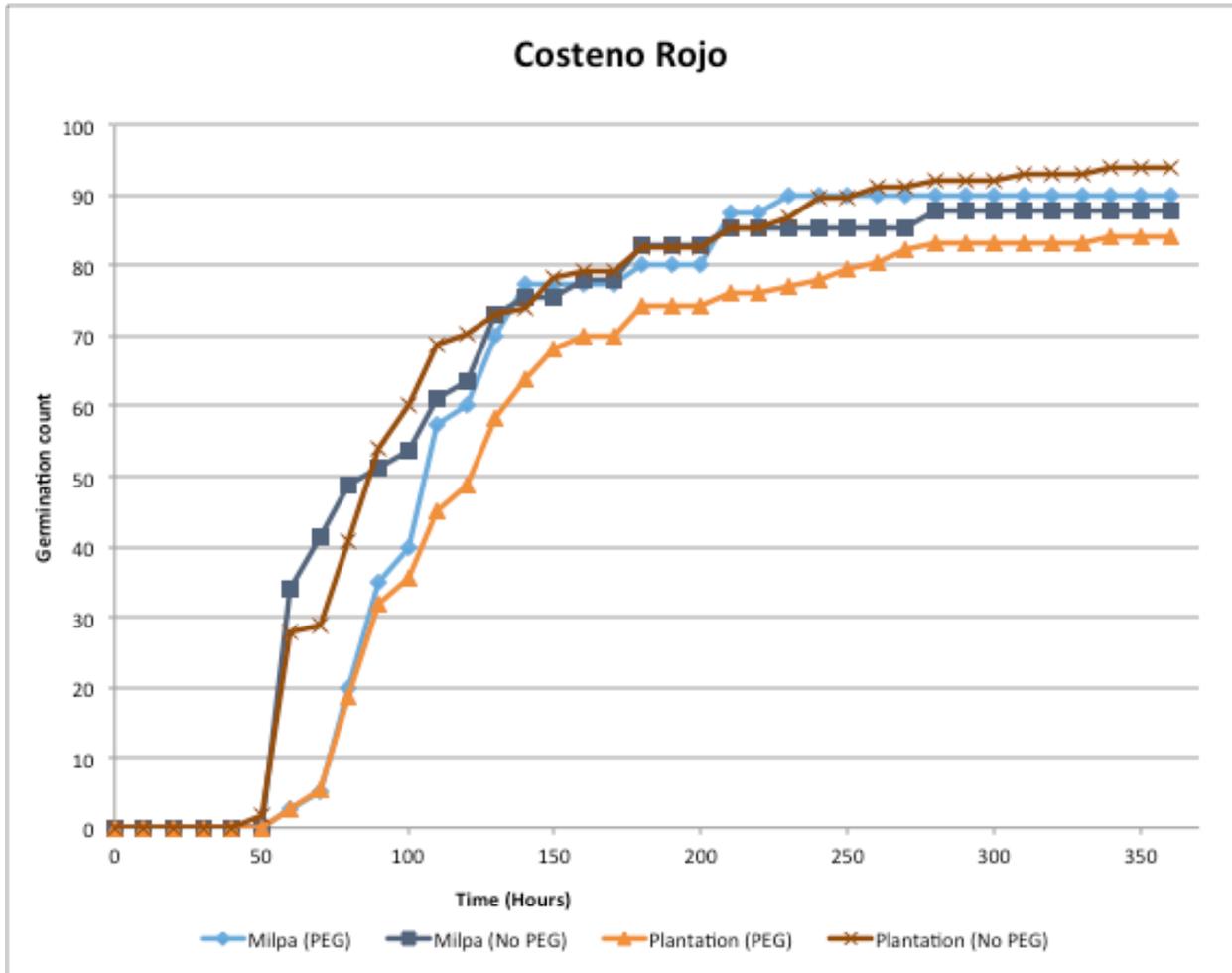


Figure 4. Chile de Agua germination curves

Germination curves for the Chile de Agua landrace, including average values for germ_proportion_0 through germ_proportion_360 from across 4 replications. Curves represent milpa accessions (no. 143, 215) and plantation accessions (no. 173, 151, 135, 132) with and without PEG treatment.

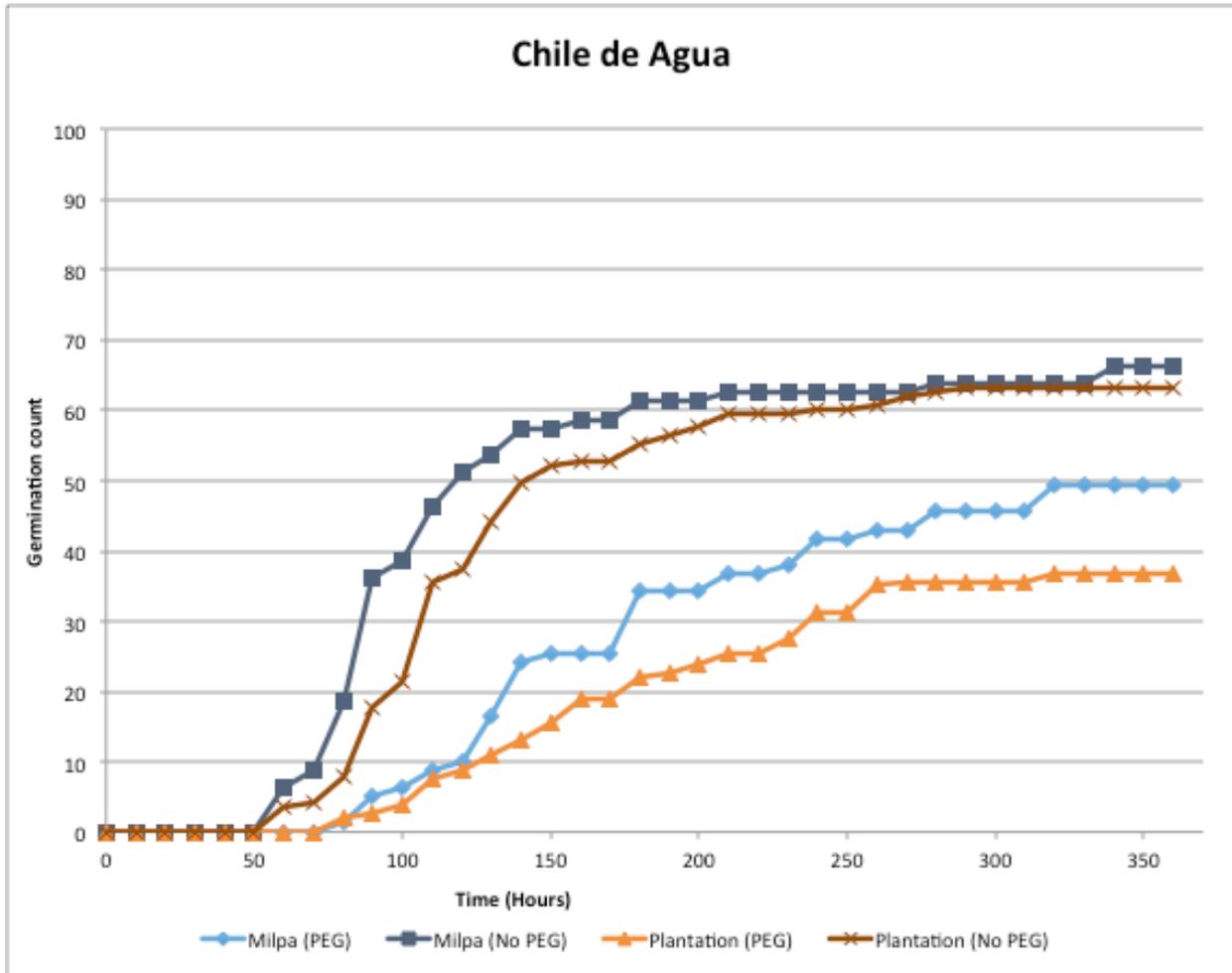


Figure 5. Dulce germination curves

Germination curves for the Dulce landrace, including average values for germ_proportion_0 through germ_proportion_360 from across 4 replications. Curves represent a milpa accession (no. 213) and backyard accession (no. 211) with and without PEG treatment.

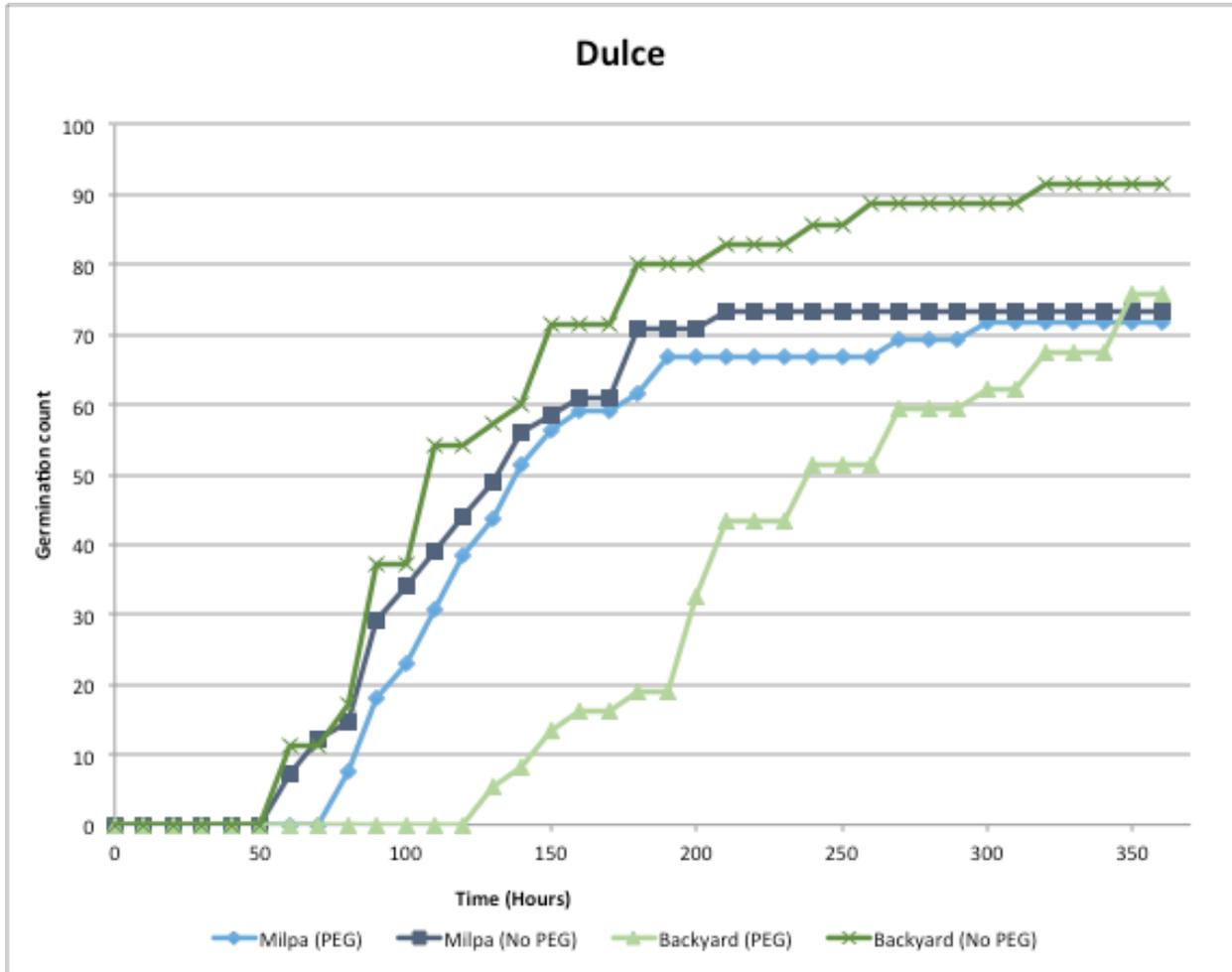


Figure 6. Tusta germination curves

Germination curves for the Tusta landrace, including average values for germ_proportion_0 through germ_proportion_360 from across 4 replications. Curves represent milpa accessions (no. 93, 185) and backyard accession (no. 179, 106) with and without PEG treatment.

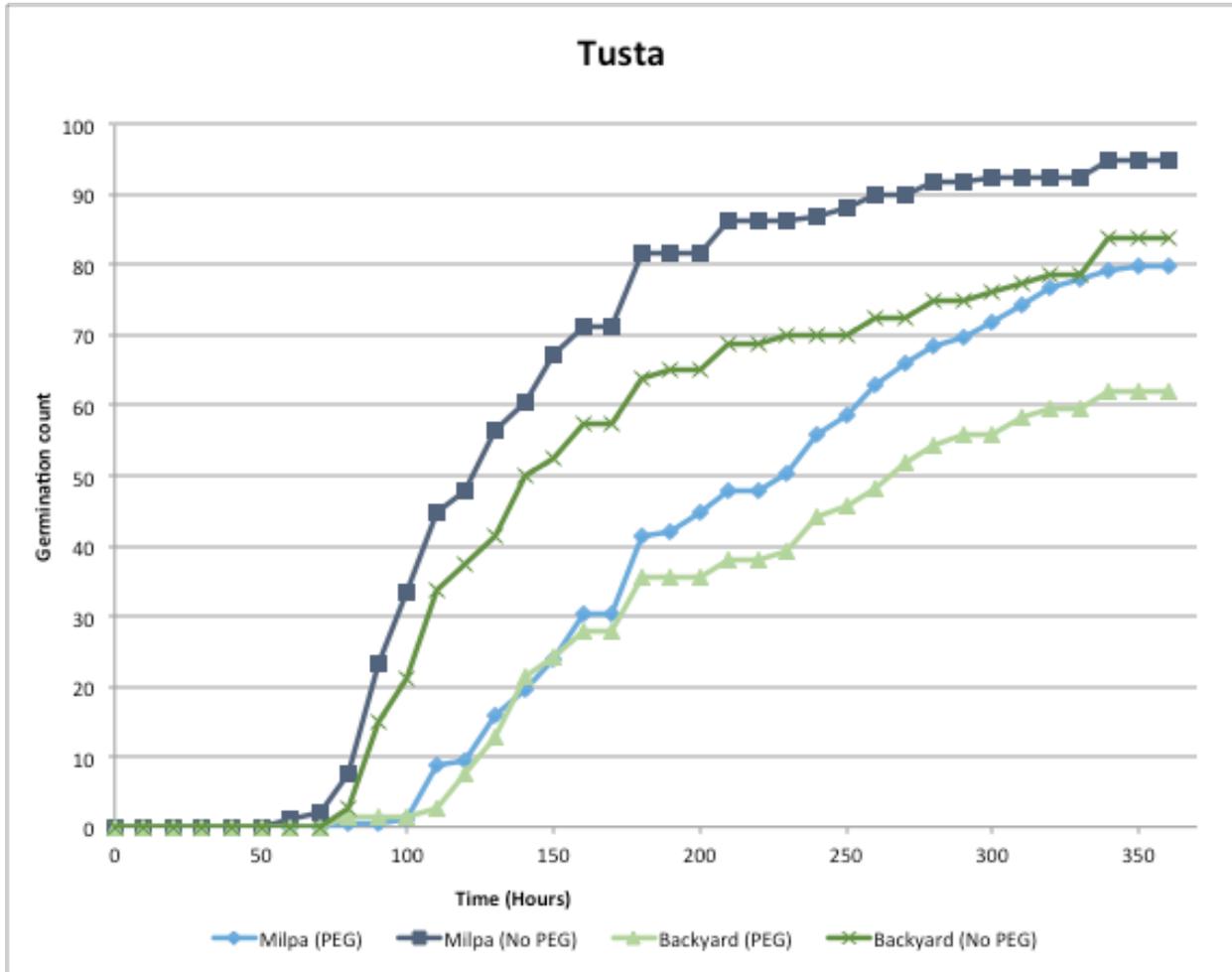


Figure 8. Taviche germination curves

Germination curves for the Taviche landrace, including average values for germ_proportion_0 through germ_proportion_360 from across 4 replications. Curve represents milpa accessions (no. 186) with and without PEG treatment.

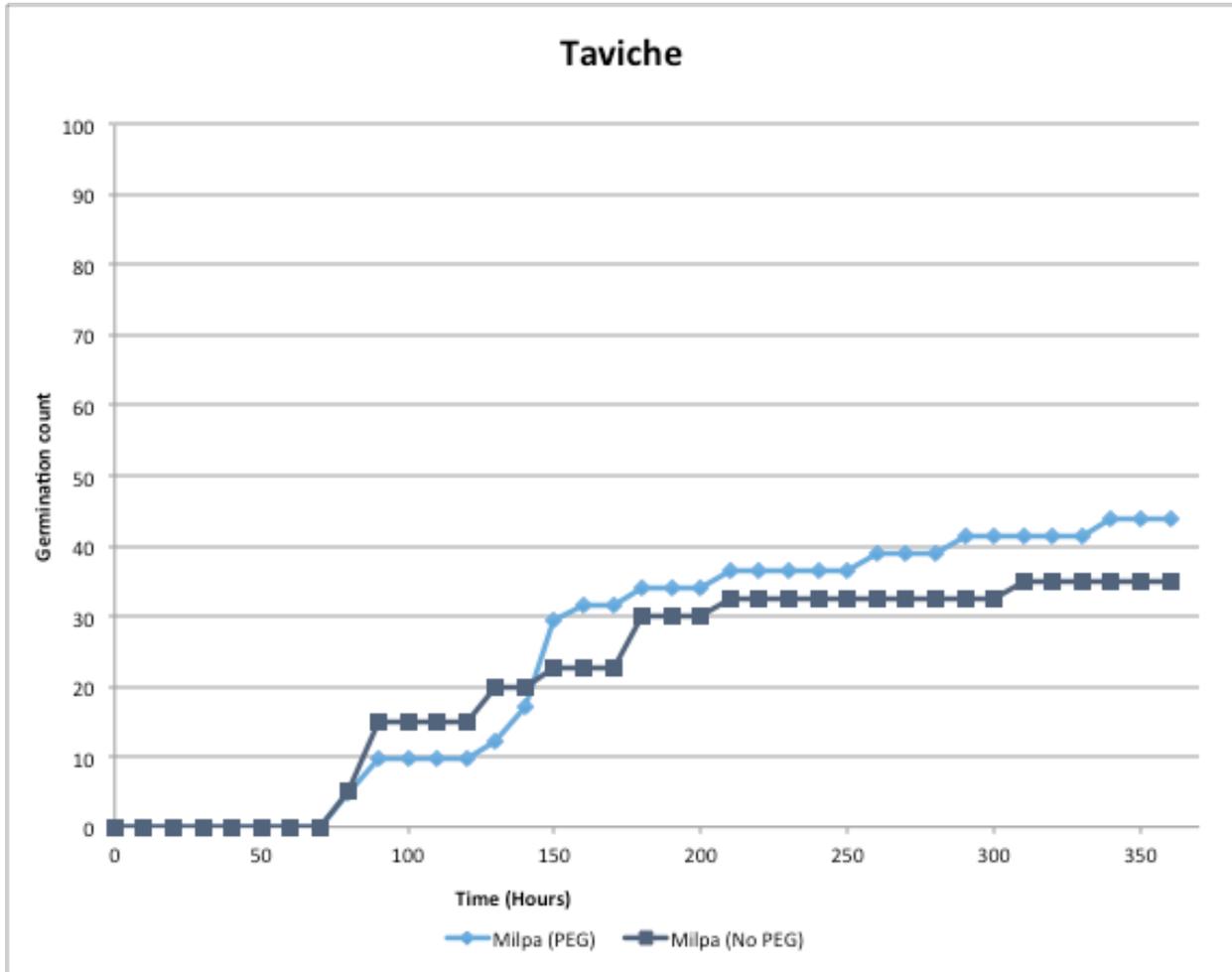


Figure 9. Payaso germination curves

Germination curves for the Payaso landrace, including average values for germ_proportion_0 through germ_proportion_360 from across 4 replications. Curve represents milpa accession (no. 217) with and without PEG treatment.

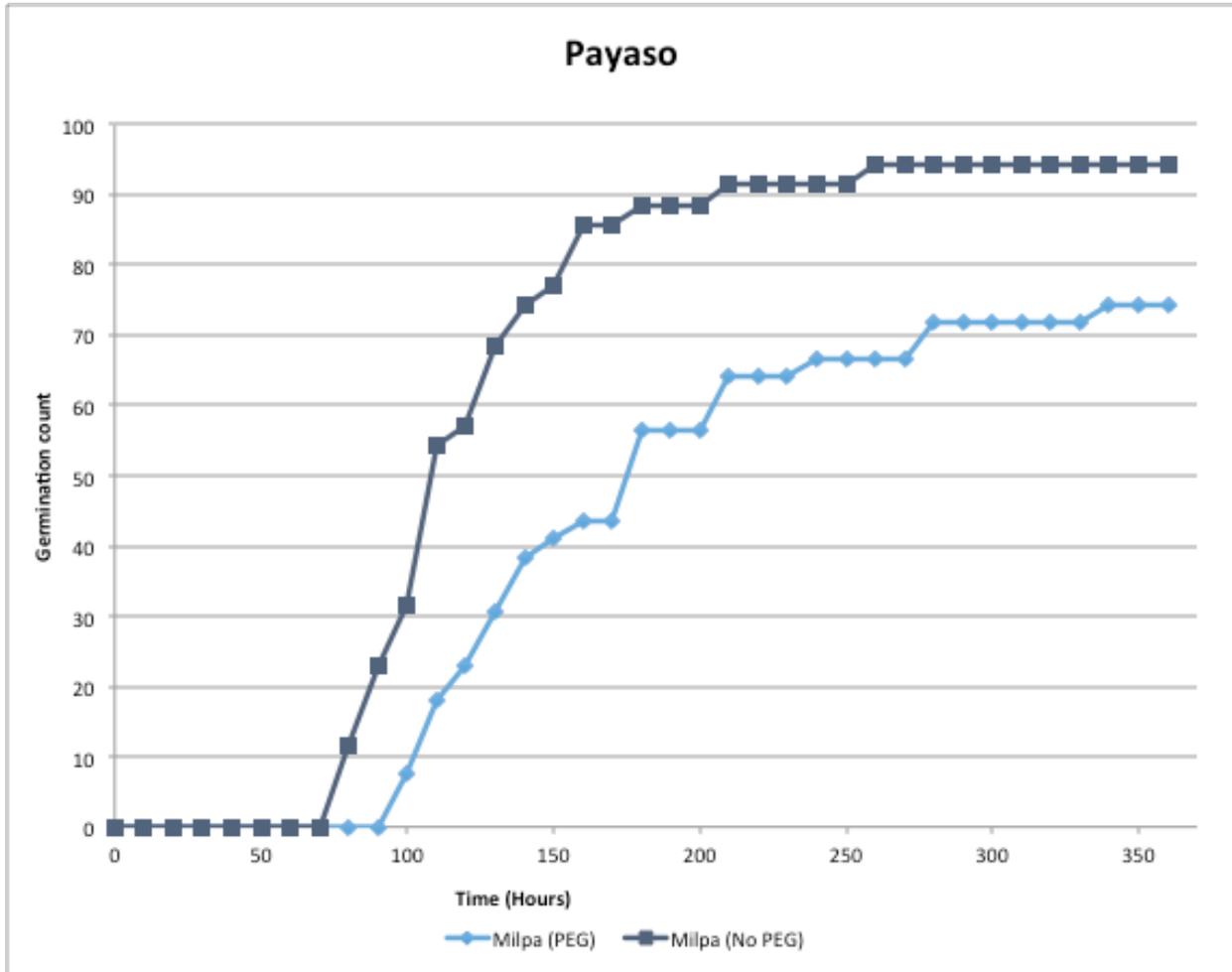


Figure 10. Paradito germination curves

Germination curves for the Paradito landrace, including average values for germ_proportion_0 through germ_proportion_360 from across 4 replications. Curve represents backyard accession (no. 204) with and without PEG treatment.

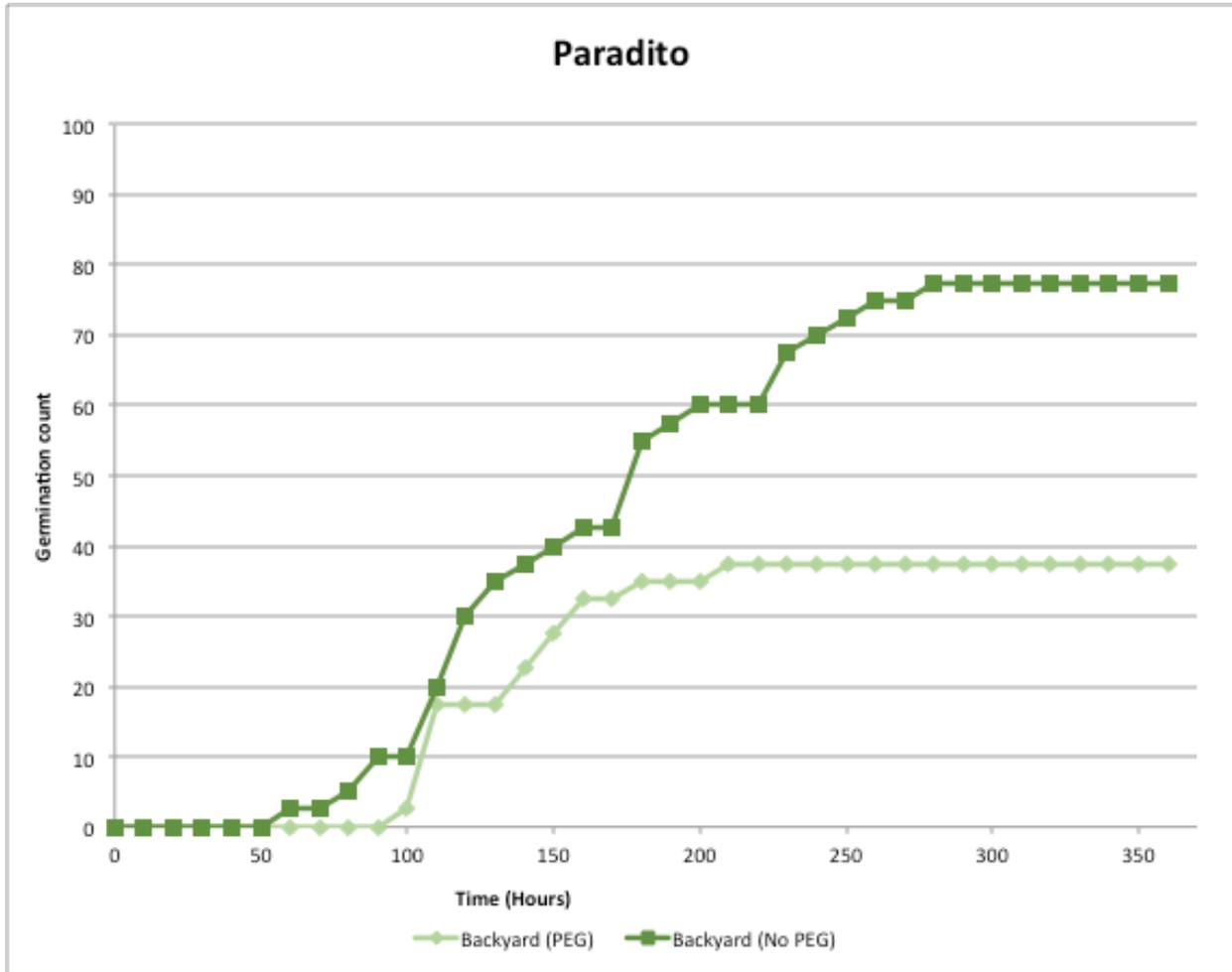
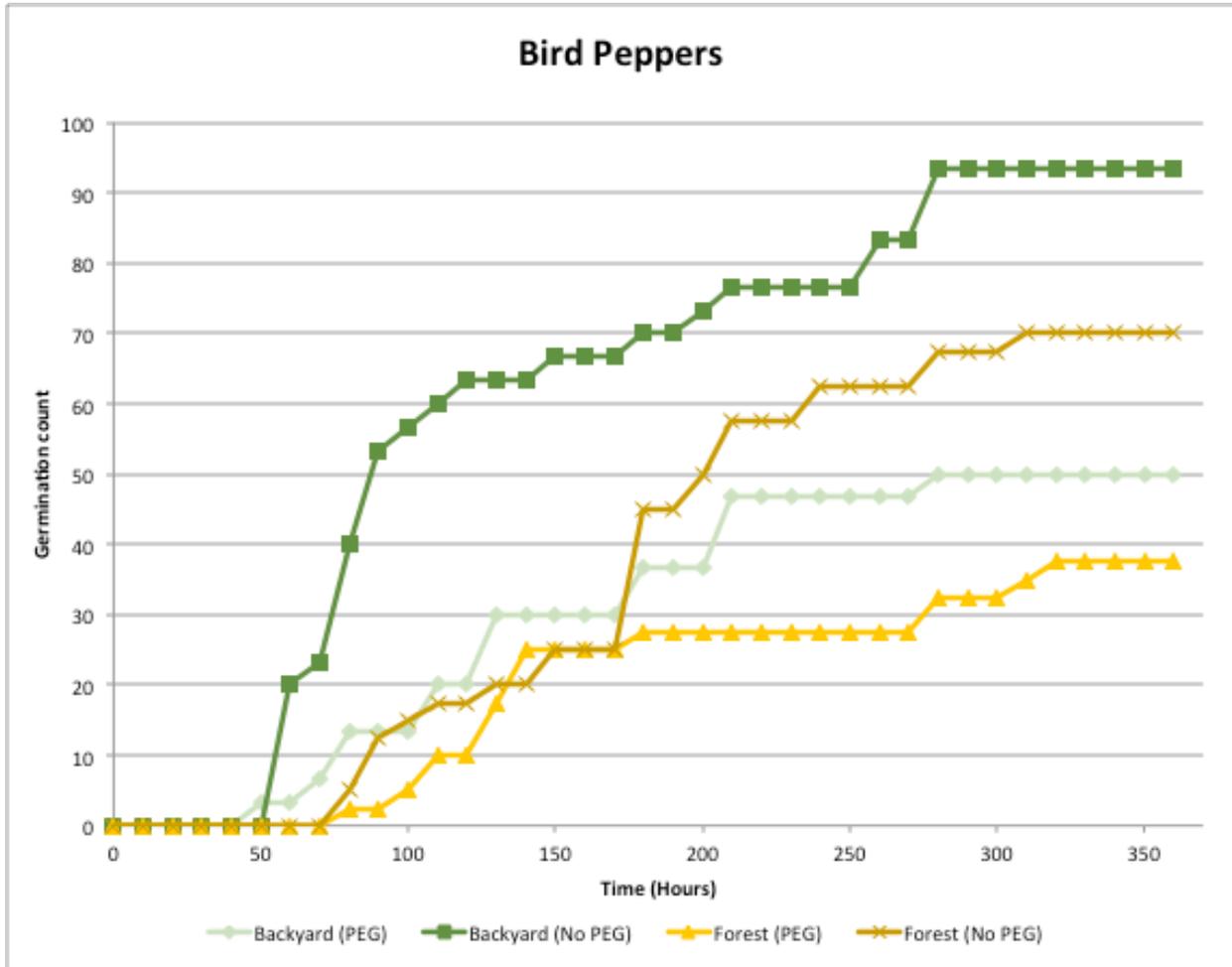


Figure 11. Wild type germination curves

Germination curves for the bird pepper landraces, including average values for germ_proportion_0 through germ_proportion_360 from across 4 replications. Curves represent forest accession (no. 126) and backyard accession (no. 88) with and without PEG treatment.



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