Increasing the productivity and market value of pulse crops for arid organic conditions

Final Report for the Organic Farming Research Foundation

1. Project Summary

In this project, we evaluated factors impacting productivity and product value in grain legume crops in semi-arid organic farms. Specifically, we focused on 1) characterization of yield and other agronomic traits in diverse grain legume crops grown in semi-arid organic environments, 2) selection of breeding materials based on yield and other characteristics in these same environments, and 3) understanding the genetics of unique seed types that provide a high value to organic farmers. To this end, we evaluated a total of 72 varieties or breeding lines representing four species at each of two locations in the interior of California. Our results show that tepary beans (Phaseolus acutifolius) vastly outyielding common beans (P. vulgaris) in these environments, while the one trialed lima bean (P. lunatus) was intermediate between these species in yield. Significant differences existed in yield between breeding lines, informing selection by growers and breeders. Our genetic results identified at least 11 previously unidentified mutations across five genes, leading to a broad array of seed colors found in legumes. Comparisons between different varieties show that the same genes have been repeatedly selected for seed type diversity between species and between diverse varieties within species. This understanding will be useful for breeders working to maximize market value. This funding helped the project team continue to advance breeding work to maximize crop productivity and product value for organic farmers in arid conditions. It also enabled the distribution of seeds and knowledge regarding the promising lines at events such as the California Organic Seed Summit and a booth at UC Davis's Picnic Day.

2. Introduction to Topic

Legumes are important staple crops and nutrient sources globally, particularly for the organic sector. Symbiotic relationships between legume roots and nitrogen-fixing Rhizobia bacteria allow these crops to remain productive in low fertility soils by creating useful forms on nitrogen from atmospheric nitrogen. This is particularly useful for the organic sector, which restricts the use of synthetic nitrogen fertilizers. All of these positive attributes have made legumes, like the common bean (*Phaseolus vulgaris*), an essential component of organic crop rotations. While common bean is the most consumed grain legume worldwide (Parker et al. 2023), climate change is increasing heat and aridity globally, necessitating the investigation of alternative legume crops with greater resilience as staple foods. One potential alternative is the

tepary bean (*P. acutifolius*). Native to the Sonoran Desert, they have been shown to have notable resilience to drought and heat which is relevant due to climate change (Moghaddam et. al., 2021). Lima beans (*P. lunatus*) are also known to inhabit broad ecogeographic conditions and display a variety of stress resistances (Parker and Gepts 2021). In this trial, we quantify the difference in yield and other traits between common bean and tepary bean when grown under arid, organic conditions.

Product value is also critically important for organic farmers. Specialty heirloom-type seed classes offer a high-value unique niche market opportunity for smaller scale organic growers. Restaurant managers who use heirloom cultivars are, on average, willing to pay US\$10.52/kg for organic, locally produced heirloom beans. Consumers at farmers markets showed willingness to pay an average of between \$13.20 and \$17.60/kg for the same products (Swegarden, Sheaffer, & Michaels, 2016). These high values allow smaller-scale organic growers to capitalize on their ability to grow a unique and high-quality product, and specialty seed colors are important in distinguishing these types in the market. To date, however, the genetic basis of the diverse seed color patterns in beans and other legume crops are not well understood, leaving breeders without the tools needed to breed for improved types in these classes.

In this study, we first grew and quantified the difference in yield and other traits between common bean and tepary bean when grown under semi-arid organic conditions at two locations. We then evaluated the genetic diversity and sequence variation at candidate genes that might underlie the high-value seed patterns preferred by organic growers and consumers. Our results shed light on grain legumes which are highly productive and have high market value for organic farmers in increasingly arid environments

3. Objectives Statement

<u>Objective 1.</u> Identification of tepary bean (*Phaseolus acutifolius*) and cowpea (*Vigna unguiculata*) varieties with yields greater than major commercial legume classes (pinto, black, and pink beans) when grown in arid organic environments.

Explanation and rationale: Tepary bean and cowpea are known to be resistant to heat and drought. In collaborative trials with organic farmers in arid environments, we screened 13 varieties of tepary bean, that are particularly productive and valuable to growers in these environments.

<u>Measurable successful outcome</u>: Specific cowpea and tepary bean varieties with statistically significantly higher yields relative to other major market classes, based on Tukey's test.

<u>Results summary and deviation from work plans:</u> All 13 tested tepary beans and the one tested lima bean ranked higher in yield than any of the 13 common beans evaluated, demonstrating their yield superiority in semi-arid organic conditions. The cowpea crop was entirely consumed in the pod in less than a week by ground squirrels, despite installation of fencing to exclude them. Cowpeas showed heavy pod set, but growers should be warned of squirrel preference for cowpeas over other legume crops.

<u>Objective 2.</u> Selection of common bean (*Phaseolus vulgaris*) breeding lineages with yields in arid organic environments greater than parents of similar seed types.

<u>Explanation and rationale</u>: Fifteen unreleased common bean breeding lines with desirable seed colors, disease resistance, and resistance to aridity-associated pod shattering will be tested for yield in organic conditions against their heirloom parent varieties.

<u>Measurable successful outcome</u>: Statistically significantly higher organic yields for specific breeding lineages relative to their heirloom parents, based on student's *t*-test.

<u>Results summary and deviation from work plans:</u> Significant differences were identified between pairs of common bean breeding lines within locations, but not between locations. Significant differences were identified between tepary beans between wand within locations.

<u>Objective 3.</u> Genetic characterization of three candidate genes (*t, bip^{ana}, and P^{hbw}*) associated with high-value seed color patterns in common bean, to identify mutations (with predicted loss- or gain-of-function effect) and/or differential gene expression.

<u>Explanation and rationale</u>: Many of the highest-value varieties of organically-grown bean have mutations in a small number of color-related genes, which have not yet been studied in detail. Our preliminary results have provided insight into the genetic regions responsible for these changes, which have yet to be confirmed. This genetic investigation will facilitate genetics-informed breeding of high-value, high-productivity beans.

<u>Measurable successful outcome</u>: Identification of specific genetic differences with significant PROVEAN score (indicating a loss or gain of gene function); and/or statistically significantly different gene expression level by *t*-test, with patterns confirmed by sequencing genetically diverse varieties.

<u>Results summary and deviation from work plans:</u> We identified 11 unique mutations across five seed major seed color genes. For three of these, we developed selectable markers that can be screened in breeding populations to identify types that have inherited. The only deviation from the work plan was identification of more genetic variants in more genes than predicted, in part by using better genetic sequencing methods.

<u>Objective 4.</u> Identification of homologous seed color genes in cowpea and tepary bean based on genomic synteny (similarities in gene order) and maximum sequence conservation.

<u>Explanation and rationale</u>: Cowpea, tepary bean, and common bean are species of the same plant family and display similar sets of variation in seed color patterns. The genes controlling seed color patterns in common bean are expected to have comparable homologous genes in the other species, which likely underlie desirable seed variation in those species.

<u>Measurable successful outcome</u>: Identification of genetic regions with high synteny and BLAST similarity to the common bean genes, as candidates for control of seed color in cowpea and tepary bean.

<u>Results summary and deviation from work plans:</u> Comparable genes in tepary bean and cowpea were identified for all targeted genes. At least one type of partly-colored cowpea and partly-colored lima bean were shown to have a deleted regulatory region also identified in common bean.

4. Materials and Methods

We evaluated 13 types of common bean (*Phaseolus vulgaris*), 13 types of tepary bean (*Phaseolus acutifolius*), one lima bean (*Phaseolus lunatus*), and 45 types of cowpea (*Vigna unguiculata*) at each of two certified organic farms. One location was at Rio del Rey Farm in San Diego County (33.277, -117.026), overseen by Mike Reeske, with the other at the Student Organic Farm at UC Davis in Yolo County (38.542, -121.767). Fields were planted in June and phenotypic data was collected throughout the season, including flowering date, time to maturity, plant stature, pod placement, canopy height, and yield. Germplasm included nine unreleased UC Davis common bean lines; nine tepary breeding lines from USDA-TARS (e.g., Porch et al. 2013, 2022, 2024); and other diverse materials from North American breeding programs or GRIN-NPGS (Table 1). *Phaseolus* plots consisted of 120 seeds planted into double rows, 6.1 m in length and 1.5 m in width (0.75 m per row), and were planted in randomized complete block designs. The 45 accessions of cowpea were grown in an augmented design (40 unreplicated, five

replicated controls) at each of two locations. Approximately 500 kg/ha of feather meal fertilizer was added before planting, and trials were irrigated with approximately 1.8 cm of water per week until 85 days after planting. The irrigation regime was intended to cause, at most, only very mild drought stress. Weeds were manually removed. Trials were cut 106 days after planting, threshed, and cleaned seeds were weighed.

For our seed color projects, whole-genome shotgun sequencing was conducted on a collection of 37 diverse accessions to identify genomic sequences related to seed color. Detailed methods on sequencing and genetic marker development can be found in Parker et al. (2023).

5. Project Results

In our multi-locations field tests, significant differences were found among the evaluated varieties ($P < 2 * 10^{-16}$, two-way anova), locations ($P < 2 * 10^{-16}$), and variety*location interaction ($P < 2 * 10^{-3}$). This indicates that some accessions are genetically higher-yielding than others in these environments, that the environments had different overall yields, and that varieties showed different responses to the two different environments. All tepary beans ranked higher in yield in Yolo County than all common beans (Fig. 1), with 193% higher yields on average. In San Diego County, the common bean accession Viva ranked higher in yield than three of 13 tepary beans; all other common beans ranked lower in yield than all tepary beans (Fig. 1). Tepary beans averaged 372% higher yields than common bean at this site. Based on multi-location data, the common bean accessions California Early Light Red Kidney (CELRK) and UCD Holstein 0701 did not differ significantly from the tepary bean landrace G40119; all other teparies significantly out-yielded all other common beans (Table 1). The one lima breeding line UCD E27 was somewhat intermediate in yield between the species, differing insignificantly from the seven lowest-yielding tepary bean lines, but significantly higher than all common beans. Teparies had shorter canopies (Fig. 2), higher levels of lodging (Fig. 3), and lower pod placement (Fig. 4) relative to common beans. Teparies also reached harvest maturity sooner, averaging 80 days to harvest in Yolo County, at which stage no variety of common or lima bean had reached harvestable maturity (Fig. 5).

These results showcase the value of tepary beans, including the new breeding materials, as a source of resistance to abiotic stresses imposed by semi-arid organic conditions. In contrast, common bean nearly collapsed in these environments. Our results highlight the value of tepary bean to the organic community. Nevertheless, tepary beans are known to lack diversity in seed color (e.g. absence of reds, purples, and various mottling patterns), lack large seed size, and are susceptible to bean common mosaic virus. Common bean and tepary bean can be hybridized, and interspecific hybridization could combine the beneficial characteristics from both species (Barrera et al. 2022). Future research could also involve testing the consumer preference between

common bean and tepary bean to better understand the market potential of tepary beans for diverse consumers.

Our sequencing of 37 varieties of diverse seed color (Fig. 6) identified mutations at five distinct seed color candidate genes. These genes are all predicted to encode members of a three-part protein complex, with some level of interchangeability within members of each subcomponent (Fig. 7). Within a single gene ('T' or 'Totally Colored'; with white patches when mutated), seven distinct mutations were identified (Fig. 8). The white patches found in accessions with the Anasazi pattern (Fig. 6 panels A2, B1, B2, B4) are highly temperature-sensitive, which is an issue for product stability for many market chains. Our results indicate that the two mutations found in types with this pattern may both be in the parts of their protein products that interact with the other. This protein-protein interaction may therefore be the cause of the sensitivity to temperature, and suggests that color stabilization could be achieved by introducing other mutations in the gene, such as that of Dapple Grey (Fig. 6 panel D4) which has the T gene deleted in its entirety. We were also able to develop genetic tests for three of these genes. These can be used by breeders to select the desirable mutations, most of which are recessive and therefore masked in many early generation progeny. This will enable the backcrossing (essentially a dilution) of the useful genes into types with high yield but otherwise unremarkable seeds, which would not fill a unique high-value niche for smaller organic growers.

We identified the equivalent genes for seed color in tepary bean and cowpea. Based on the results of our sequencing data, we identified genetic deletions in partly-colored lima bean and cowpea that strongly parallel a mutation in the same region as a partly-colored cowpea. This understanding could be readily used to develop genetic tests in these other species, and will inform future breeding and genetics work in these drought-tolerant legumes.

Entry	Species	Yield (kg/ha)*	Significance groups**
G40173A	acutifolius	2015	а
TARS-Tep 22	acutifolius	1942	a
TARS-Tep 101	acutifolius	1925	а
G40068	acutifolius	1811	ab
TARS-Tep 112	acutifolius	1810	ab
TARS-Tep 23	acutifolius	1781	ab
TARS-Tep 51	acutifolius	1721	abc
TARS-Tep 93	acutifolius	1614	abed
TARS-Tep 58A	acutifolius	1519	abed
PI 440786	acutifolius	1481	abed
TARS-Tep 97	acutifolius	1371	bed
UCD E27 LIMA	lunatus	1176	cd
TARS-Tep 100	acutifolius	1174	cd
G40119	acutifolius	1119	de
CELRK	vulgaris	581	ef
UCD Holstein 0701	vulgaris	574	ef
Viva	vulgaris	537	f
BXO100	vulgaris	513	f
Cal DRK	vulgaris	440	f
PT11-13	vulgaris	411	f
UCD 1004	vulgaris	382	f
UCD Jac Cat 0908	vulgaris	327	f
UCD Purple Star	vulgaris	259	f
UCD Pink 9634	vulgaris	244	f
Raven	vulgaris	187	f
UCD 1005	vulgaris	156	f
UCD Andino 0233	vulgaris	85	f

Table 1. Yield and significance groups of evaluated lines.

*Mean of all plots across both locations; **Fisher's LSD

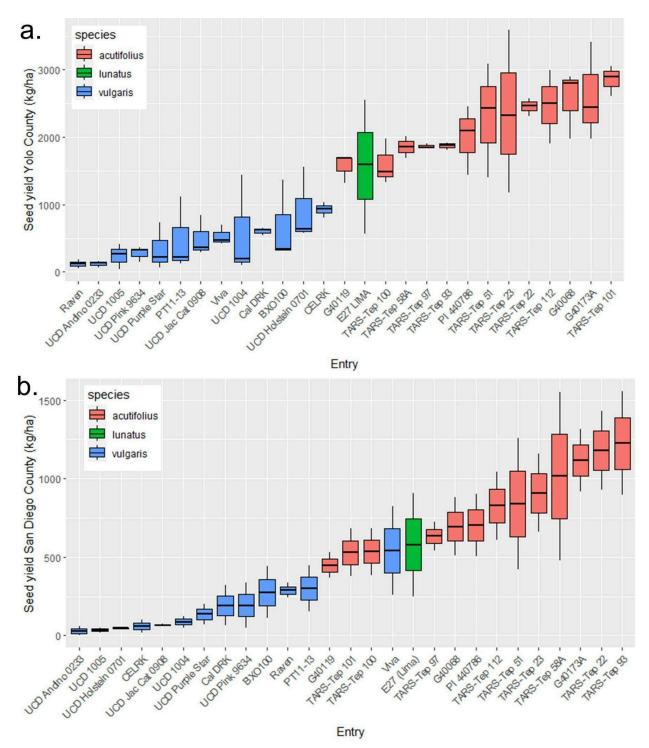


Fig. 1. Yield of common beans (*P. vulgaris*), lima bean (*P. lunatus*), and tepary bean (*P. acutifolius*) on semi-arid certified organic farms in a) Yolo County and b) eastern San Diego County. Yolo County tepary bean yields averaged 1423 kg/ha compared to 486 kg/ha for common bean, a 372% yield advantage for tepary bean. In San Diego County, yields averaged 819 kg/ha for tepary bean 174 kg/ha for common bean, a 192% advantage.

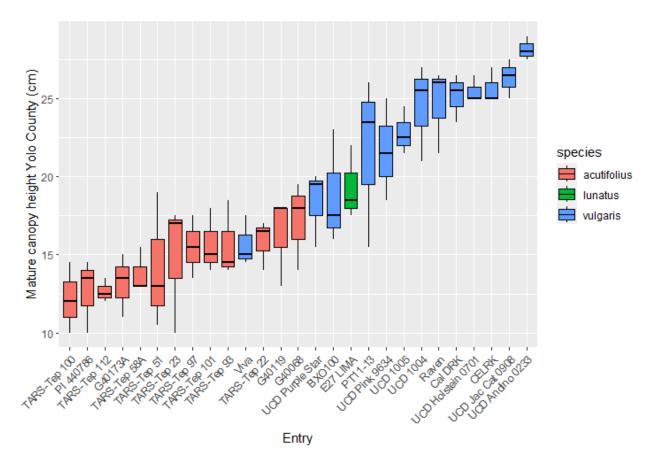


Fig. 2. Mature canopy height of common beans (*P. vulgaris*), lima bean (*P. lunatus*), and tepary bean (*P. acutifolius*) in Yolo County. Tepary beans averaged 15 cm in height, while common beans averaged 23 cm, and the one lima bean averaged 19 cm.

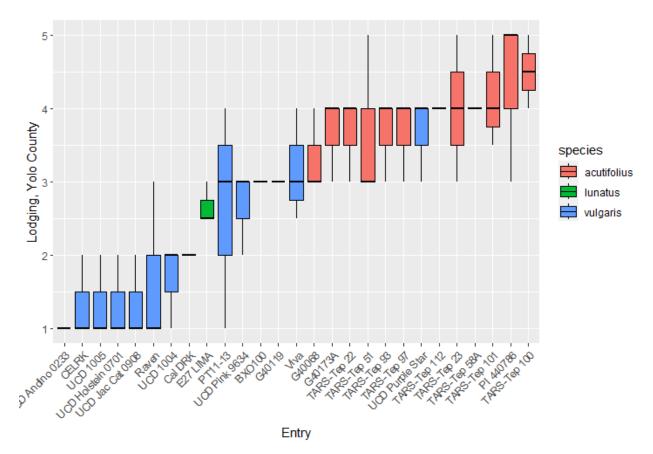


Fig. 3. Lodging score of common beans (*P. vulgaris*), lima bean (*P. lunatus*), and tepary bean (*P. acutifolius*) in Yolo County. Lodging was evaluated with a 1-5 scale: 1=no lodging; 5=completely prostrate). Tepary beans averaged 3.8, while common beans averaged 2.1, and the one lima bean averaged 2.7.

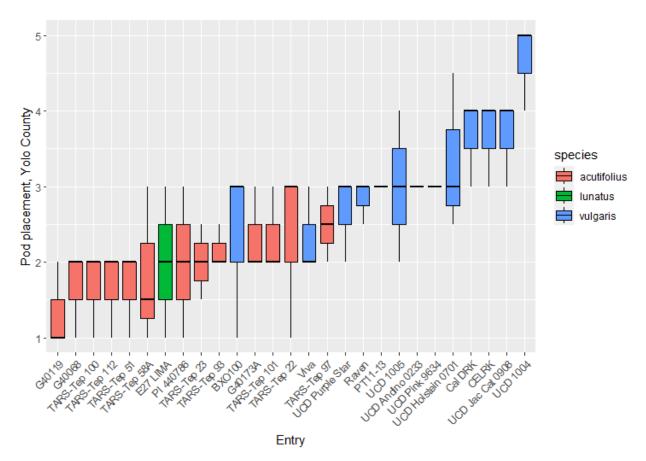


Fig. 4. Pod placement score of common beans (*P. vulgaris*), lima bean (*P. lunatus*), and tepary bean (*P. acutifolius*) in Yolo County. Pod placement was evaluated with a 1-5 scale: 1=on ground; 5=at top of canopy). Tepary beans and the one lima bean each averaged 2, while common beans averaged 3.1.

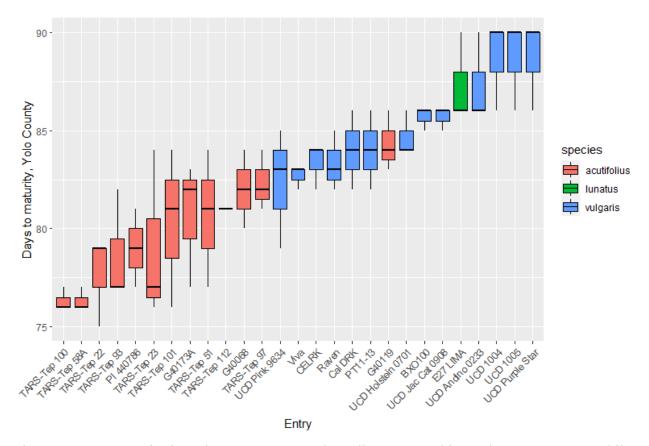


Fig. 5. Days to maturity in Yolo County. Tepary bean lines matured in 80 days on average, while common bean maturity time averaged 85 days and the one lima matured in 87 days on average.

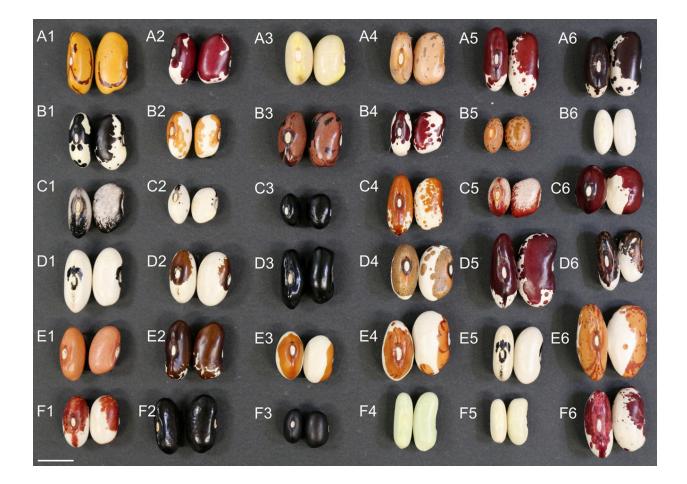


Fig. 6. Seed color of the diverse accessions used for whole genome sequencing. At least 11 distinct candidate mutations were identified among this panel. Among the 22 lines with partial white patching alone, seven independent mutations were identified in a single gene (T), which we found to be linked to a WD40 repeat factor transcription factor.

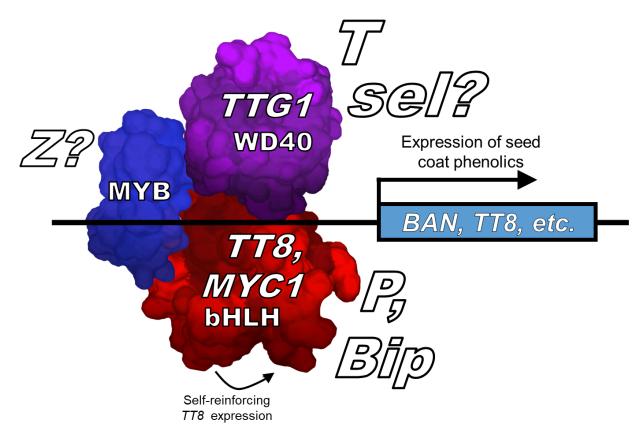


Fig. 7. Protein complexes formed of three components (WD40-repeats, bHLH, and MYBs) are known to influence seed color in plants. Some components can be "swapped out" interchangeably with several related proteins. We found mutations in five genes that encode members of this complex. Our results suggest that all the complex seed color patterning of common bean are attributable to members of this fundamental protein complex, which goes on to activate pigmentation pathways.

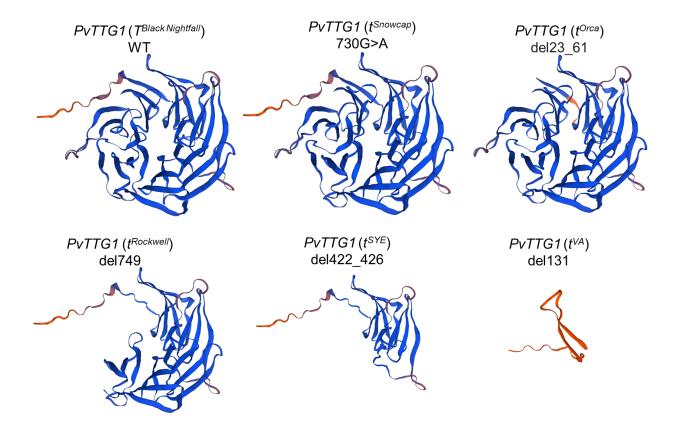


Fig. 8. Models of proteins encoded by non-mutant ("WT") and five mutated forms of the *T* ("*Total color*") gene in common bean, identified in this study. Each mutation is linked to white patches. Two additional mutations in this gene were identified (a full deletion and a rearrangement in regulatory sequence), as well as mutations in four other genes.

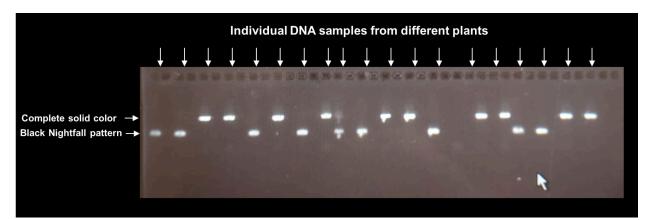


Fig. 9. An example of a genetic test to evaluate seed color genetics in beans. DNA was used to conduct a test using a process called PCR, and the output solution from each plant was loaded into distinct vertical columns. Types inheriting the partial loss-of-function gene copy (and the color pattern seen in Figure 6 panels C1 and C5) have a low fluorescent band pattern, while types

inheriting the full-color pattern have a higher band, and types with one copy of each gene variant have both bands.

Traditional Gene Name	Common bean gene	Explanation	Tepary equivalent	E score*	Cowpea equivalent	E score*
Sel (Sellatus)	Phvul.001G192200	WDR	Phacu.CVR.001G237300	0	Vigun01g176200	0
Z (Zonal)	Phvul.003G132100	PvTT2	Phacu.CVR.003G150400 Phacu.CVR.007G206200, Phacu.CVR.007G206800,	0	Vigun03g396500	0
P (Pigment)	Phvul.007G171333	PvTT8	Phacu.CVR.007G206900	0	Vigun07g110700	0
T (Totally colored)	Phvul.009G044700	PvTTG1	Phacu.CVR.009G093100	0	Vigun09g139900	0
Bip (Bipunctata)	Phvul.010G098500	PvMYC1	Phacu.CVR.010G120900	0	Vigun10g129300	0

Table 2. Seed color-related genes in common bean, tepary bean, and cowpea

*Expect score, based on BLAST of complete nucleotide sequence.

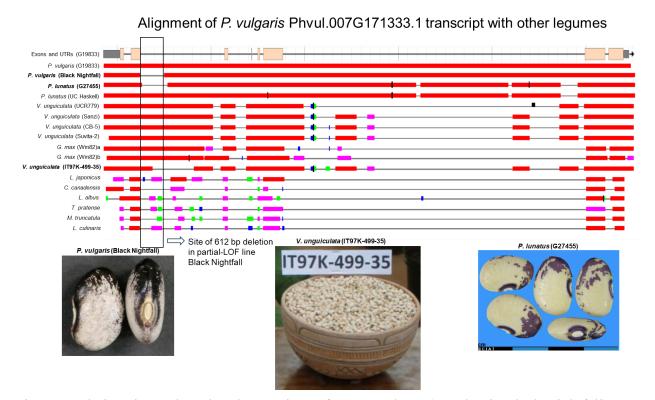


Fig. 10. Deletions in partly-colored accessions of common bean (P. vulgaris Black Nightfall), lima bean (P. lunatus G27455), and cowpea (V. unguiculata IT97K-499-35) occur in the same region of the equivalent gene, whereas fully-colored types of all known legumes in this subfamily have no deletion and high similarity in the area. The top row in the figure shows the gene structure of the common bean 'P' (*Pigment*) gene, with exons (expressed as a protein) in tan and introns (regions removed before making a protein product) as a black line. Lower rows align a variety of other legumes, with areas that are very similar shown in red, less similarity shown in cooler colors, and no similarity shown as a black line. These results show that there is high similarity across legumes in a part of the second intron which is deleted in Black Nightfall (in a vertical black box). This area has been conserved across millions of years of legume evolution, showing that mutations likely have a strong deleterious effect in wild legumes and are removed from the population through natural selection. This region is deleted in three legumes with partly-colored seeds. Understanding of the genetic basis of this parallel mutation across drought-tolerant legumes will help breeders to select for increased seed diversity suited to arid organic conditions.

6. Conclusions and Discussion

Our results have shown and provided precise quantitative data regarding the superior adaptation of tepary beans relative to common beans under arid organic conditions. The one lima bean trialed also was far more adapted to these arid organic conditions than common bean. Cowpea pod set was very heavy before squirrel herbivory immediately prior to harvest. These results helped in selecting accessions with improved yield in these scenarios, and ensured that selection decisions were made under organic conditions, thus selecting types which meet the needs of organic growers, rather than the high-input conditions of conventional agriculture.

Our seed color results led to the development of rapid and inexpensive tests to evaluate seed color genetics, allowing breeders to know which progeny have inherited the high-value seed patterns before even planting seeds. These genetic screening tests also allow breeding programs to know which progeny have inherited these recessive mutations even when their color effects are masked by the original wild-type gene copy. They also inform the process of moving greater environmental stability into these materials by using alternative mutations.

See also previous section for more detailed information.

7. Outreach

We have shared knowledge and resources associated with this project with growers, seed producers, scientists, and the general public. For example, we made and distributed approximately 1000 seed packets of high-yielding tepary and common beans at the California Organic Seed Summit and UC Davis's Picnic Day. We also gave a presentation on the project at the Organic Seed Summit, allowing us to show our results and distribute the relevant seed to organic seed growers, who are pivotal in making varieties available to organic growers and the agricultural community. Our Picnic Day booth

(<u>https://picnicday.ucdavis.edu/events/seed-packet-and-sweet-potato-giveaway</u>) further allowed us to engage directly with hundreds to thousands of consumers on how selection of crop biodiversity in organic conditions can maximize productivity and resource use efficiency in these conditions.

We also discussed our projects and preliminary results at two field days, one being the UC Davis dry bean field day and the other hosted by the Student Organic Plant-breeding and Education (SCOPE) project at the UC Davis organic student farm. These attract growers, farm advisors, and students. Project technician Troy Williams also gave a presentation to a scientific and breeding audience at the UC Davis Plant Sciences Symposium.

PI Travis Parker gave a presentation and interactive discussion with the UCANR Master Gardeners of San Diego County on these organic breeding projects on November 3, 2022.

The project was also publicized online, for example through the UC Davis Department of Plant Sciences website:

https://www.plantsciences.ucdavis.edu/news/parkers-proposal-more-beans-less-water

Results have also been written up to disseminate to legume breeders in the 2024 Bean Improvement Cooperative's annual report.



Fig. 11. Seed packets and promotional materials distributed at the California Organic Seed Summit. A presentation on our field trial and results was also given to attendees.

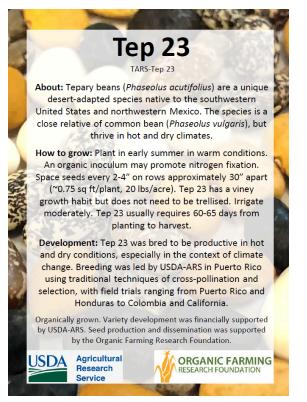


Fig. 12. An example label of the printed seed packets, giving information on the highly drought-tolerant variety, and acknowledging OFRF's support.



Fig. 13. The line leading up to the organic bean seed and sweetpotato giveaway booth at the UC Davis Picnic Day. The event was a great opportunity to engage with and educate the general public about our organic trials and breeding. At least 1,000 people visited the booth.



Fig. 13. Engaging with the public at UC Davis's Picnic Day.

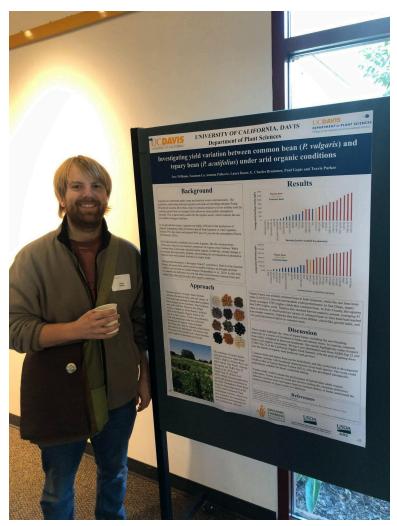


Fig. 14. Technician Troy Williams developed a poster presentation on the project's result for the 2023 UC Davis Plant Sciences Symposium.



Fig. 15. Engaging with growers. Farmer Cooper Brossy (at right) of Ernie's Organics in Shoshone, ID grows several UC Davis varieties commercially. The field shown is of UC Southwest Red, which has a unique seed pattern based on two genetic variants identified in the genetics section of this project. The variety was developed in collaborative trials with organic growers, as done in this OFRF project, and the goal of the breeding materials studied in this project is to release them publicly to organic growers looking for high-value, organic-adapted materials.



Fig. 16. An example of two organically-grown heirloom-type varieties developed by UC Davis. The varieties have unique seed patterns and are sold commercially for a high product value.

8. Financial accounting

Item	Breakdown	Item total	Section subtotal
Employee wages and salary			\$4,693.44
Irrigation supplies (drip tape, connectors)		\$842.85	
Lab consumables	\$76.45 + \$626.22 + \$10 + \$312.12	\$1,024.79	

Seed packet printing and other promotional materials	\$168.46 + \$35.56 + \$253.92 + \$58.95	\$516.89	
Mike Reeske farmer stipend for 1/4 acre trial: Planting, management, harvest, cleaning, weighing yield		\$4,000.00	
DNA sequencing costs	\$2600 + \$3515	\$6,115.00	
Hourly field labor (weeding)		\$2,399.00	
Miscellaneous field supplies (stakes, labels, paper bags)		\$91.07	
Supplies and expenses subtotal			\$14,989.60
Employee Benefits			\$286.96
Total			\$19,970.00

9. Leveraged resources

This project has greatly helped us work on applied breeding projects for farmers. On our more fundamental research side, we have several grants looking at genetics of traits of interest:

USDA-NIFA (AFRI): Genetic control of pod traits distinguishing snap beans from dry beans, and pre-breeding to enhance their genetic stability. \$649,480.

Kirkhouse Trust SCIO, 2022: "Application of cutting-edge genomic technologies to characterize major agronomic traits in common bean and tepary bean, and educate African teams on their use." \$470,701.

Center for People, Food, and the Environment, 2023: Gardener management and microevolution in a Phaseolus vulgaris population in California. \$7,644.

We also received a \$1000 donation from a bean industry representative to support our breeding program during the project period.

10. References: Provide a list of references you used to help develop your project and/or that you referred to in the body of your report.

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11. Photos and other addenda

See previous.