Farmer-based evolutionary participatory plant breeding for organic quinoa, buckwheat and spelt

Photo: Farmer cooperator Zach Wailand threshing quinoa with WSU graduate students Hannah Walters and Adam Peterson

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1. Project Summary

The purpose of this project was to identify varieties of quinoa, buckwheat and spelt optimally adapted to organic farming systems in Washington State. Data from these initial variety trials was used to initiate, or in the case of spelt, to complement, organic breeding trials for each crop. Each of these crops was chosen based on farmer interest combined with a lack of general knowledge of, or research focused on, these alternative/specialty crops in organic systems. From an initial pool of 44 accessions to a current program with over 800 cultivars and advanced breeding lines, quinoa varieties have been identified that perform well in both Eastern and Western Washington. Traits of interest for quinoa include downy mildew resistance, day-length sensitivity and a shorter time to maturity, seed yield and protein content, nitrogen use efficiency and response to irrigation and intercropping. The multi-location quinoa variety trials have led to the establishment of a robust organic quinoa breeding and agronomy program, with multiple students incorporating genetic, agroecological and social aspects into their research. Additionally, stemming from the initial buckwheat trials including 32 varieties of buckwheat from around the world, evolutionary breeding populations were established from intercrossing among all varieties, and established on two organic farms in Washington State. These populations have responded to natural selection pressures in each environment, with marked changes in genetic composition, and are currently growing and continuing to adapt. They will provide an excellent source for buckwheat varieties in the future. Furthermore, nutritional and end-use quality parameters of 10 buckwheat cultivars were identified. The spelt project continued with two additional years of variety trials, including European cultivars and advanced breeding lines from our organic spelt crosses from hybridizations from 2004 and 2005. Elite spelt breeding lines have been identified and are currently in the final stages of selection prior to consideration for variety release.

2. Introduction to Topic

Quinoa, buckwheat, and spelt are ancient grains that have been grown for thousands of years in their native regions. They have served well as highly nutritious staple food sources but have been largely neglected by research universities in the U.S., which typically favor the major cereal and legume crops. Each of these alternative grains is in high demand from consumers and would fill a niche of underrepresented grains in our farming and marketing systems. However, very little is known about locally adapted varieties for buckwheat, quinoa, and spelt. Farmers have requested that research be conducted with these grains in organic systems and to our knowledge, limited research, if any, has been done. From its onset, this project was initiated by organic farmers and relies heavily on farmer participatory research for its success.

Buckwheat, quinoa, and spelt have the potential to diversify cropping systems with underrepresented grain crops destined for local markets; function as valuable high-biomass cover crops; provide locally grown options for nutritious food for humans, particularly those with gluten intolerance or wheat allergies; and serve as a high protein, nutritious animal feed. Intensive variety trials in these crops are being conducted with growers at locations across Washington State and multiple agronomic, nutritional and quality traits are being be evaluated for each crop. These ongoing trials will help identify the varieties with the traits deemed most necessary by farmers at each diverse location.

This project combines research with extension and education to address and improve innovation and diversification of farming systems to increase their resiliency and sustainability. Additionally, this project focuses on breeding within the larger context of organic, sustainable farming systems that increase local and diversified access to food and nutrition.

3. Objectives Statement

The general goal of this project was to evaluate different varieties of three unique and important crops, namely quinoa, buckwheat and spelt, for traits of interest under organic conditions. Scale-sensitive breeding programs were either developed or continued for each crop. Specific objectives are listed below:

1. Create populations in fall/winter 2010-2011 based on current field evaluations by organic farmers and researchers of 40 quinoa varieties, 30 buckwheat varieties, and 30 winter spelt breeding lines on seven to nine organic farms;

2. Plant, grow, and harvest these early-generation, bulk populations on each organic farm utilizing an evolutionary participatory breeding strategy;

3. Develop a coordinated farmer evaluation process for positive and negative field selection of traits of interest in these alternative grain bulk populations;

4. Disseminate information to other interested farmers, grain buyers, students, and consumers.

~~4.~~ Materials and Methods

Quinoa

Preliminary quinoa variety trials, 2010

Quinoa trials were held in 2010 due to the absence of information on quinoa agronomy and variety performance in Washington State, The main goal of these trials was to identify varieties best adapted to Washington State and identify the major challenges in cultivating quinoa in the region.
Forty-four varieties, obtained from the National Plant Germplasm Repository in Ames, Iowa, were selected to be included in the trial. These represented a wide range of varieties from throughout the native range of quinoa in South America, and also included varieties developed in the United States in the 1980s. The trial was planted in Olympia at the Evergreen State College Organic Farm; in Pullman, at the WSU Organic Farm, and in Port Townsend, at the Dryland Farm Research Project. Large environmental differences exist between these locations and are reflective of the major growing environments found across the state. Varieties were replicated twice within each trial. Traits such as early plant vigor, flowering date, flowering uniformity, plant height, lodging, and seed maturity were measured. There were pest pressures from aphids and *Lygus* spp. at all sites, and notes on relative levels of susceptibility to these pests were taken. At the Olympia site, unseasonal rainfall occurred in late summer resulting in a high level of seed sprouting (Figure 1). The opportunity was taken to rate variety differences in their resistance to pre-harvest sprouting.

Initial quinoa nitrogen and larger-scale variety trials, 2011

Given the lack of agronomic data available for quinoa in Washington State, a study was conducted to determine proper fertilization rates for organically grown quinoa in our region. The experiment was conducted on certified organic land at the WSU Organic Farm in Pullman, WA. A split-plot design with

three replicates was used, with variety forming the whole plot and fertilization forming the sub-plot. Sub-plots were 4 ft x 10 ft in size. Sixteen quinoa varieties were grown in the study, comprising 11 varieties grown from the previous year and five commercially available varieties. Using the organic fertilizer Perfect Blend 7-2-2, four fertilization treatments at the rates of 0, 50, 100, and 150 kg N/ha were included. Seed was planted the first week of June. Recorded traits included yield, plant height, aphid susceptibility, leaf greenness, days to first flowering bud, and days to full senescence. Plants were harvested on October 1, 2011 with a 1999 Wintersteiger Nursery Master Elite plot combine.

Quinoa Population Screening, 2012

In 2012, advanced breeding lines from four quinoa populations were planted at the Washington State University certified organic farm located in Pullman, WA. These four breeding populations were contributed by Brigham Young University. In 2012, F2:8 generation of populations 39, 40 and 1 and F2:5 generation of M_3 were planted by hand on May 31 and June 1 in a single replicate with repeating check experimental design. Each population had between 80 - 90 lines. The area planted depended on how much seed was available for each line. Seed availability ranged from 5 to over 20 seeds. There was a set of checks replicated four to five times in each population. Check varieties included Brightest Brilliant Rainbow, Cherry Vanilla, Oro de Valle and Red Head, all sourced from Wild Garden Seed in Philomath, Oregon. Morphological evaluations were recorded throughout the growing season, including plant height (juvenile and mature), aphids (Table 1), downy mildew (Table 1), plant color, leaf shape, developmental stage (Table 2), flowering date (Table 2 & 3), and whether or not the breeding line produced seed or not. The lines which produced seed were harvested by hand and the seed was threshed using a Vogel seed thresher.

Irrigation x Intercropping in Quinoa, 2012

The irrigation x intercropping study in quinoa was conducted in 2012 at the Washington State University certified organic farm located on Tukey Orchard in Pullman, WA. This study consisted of two quinoa varieties and three intercrop treatments that were evaluated under three irrigation regimes in a fully factorial (2×3×3) split block randomized complete block design (RCBD) with four replicates. The two quinoa varieties, Cherry Vanilla and Oro de Valle, along with the intercrops, were planted on 4 foot beds with 12in. space between each bed. Three rows of quinoa were planted in each bed with 1in. row spacing. The intercrop was planted on both sides of each quinoa row for a total of four rows of intercrop per bed. Plot size was 4ft. x 17ft. The quinoa and intercrops were planted on June 22, 2012. The quinoa was planted with a Jang Seeder Model JP-1 one row hand seeder with settings to plant the seed 2in. apart. The intercrop was planted by hand.

Two intercrop treatments and a no-intercrop control were planted between the quinoa rows. The two intercrop treatments were clover mix (clover on Figure 1) and fescue grass clover mix (mix on Figure 1) from Peaceful Valley Farm and Garden Supply. The fescue grass clover mix was Peaceful Valley's Roadway Mix, which included dwarf turf type fescue, tough turf type fescue, New Zealand white clover, strawberry clover, and Kentucky bluegrass. The clover mix was Peaceful Valley's Dry Land Clover Mix, which included crimson clover, rose clover, dalkeith clover, anta sub-clover, Denmark sub-clover, scimitae medic, nitro Persian clover, lightning Persian clover and clare sub-clover. Fescue grass was seeded at a rate of 2lb/1,000sq.ft. and the clover mix at 1lb/1,000sq.ft. per plot. After quinoa harvest the intercrop was left in the field to over winter.

The experiment was watered with overhead sprinklers on June 25, 2012 and drip tape was laid out on July 4. There were three irrigation treatments, including full irrigation, part irrigation and dry land irrigation. Drip tape was used for both the full and part irrigation. Four lines of 15ML high flow with 8" space emitter were laid out per bed and stopped two-thirds of the way down the field. After three plots

(one-third down the field) at the end of the full irrigation, a tape coupler with a valve was placed. This valve was shut off every other week to separate the full and part irrigation regime. The dry land section only received water from the initial overhead watering on June 25, and subsequent rainfall during the season, which consisted of only 1.17in. For the full irrigation regime, quinoa was irrigated once a week for two hours between July 4 and August 29. The part irrigation regime included two hours every other week between July 4 and August 29.

At the start of the season, percent germination per plot was recorded for quinoa and intercrop. Soil samples per plot were collected to get a baseline soil nutrient level. During the season, plant height measurements were taken on July 20, August 16, August 27 and October 8. On October 12, 2012 the quinoa was harvested with a Wintersteiger plot combine. The seed was cleaned and weighed. In the spring, percent survival of the intercrop per plot was recorded (April 30, 2013) and biomass was harvested and weighed (May 2, 2013).

Spelt

Two years of field trials were conducted on certified organic acreage on the Boyd Farm in Pullman, WA. During the 2011 and 2012 seasons, 45 advanced F_7 to F_9 of spelt breeding lines and 5 check varieties were grown in a RCBD with three replicates. The plots were managed using organic methods. Plant height and stripe rust measurements were taken on plants in the field. Harvested grain was analyzed for yield, threshability, thousand kernel weight (TKW), and test weight. Yield was measured before and after dehulling the grain, which allowed the data to be converted to a threshability index (TI). Stripe rust resistance was observed and recorded, using ratings based on a standards booklet. All data was statistically analyzed using SAS ProcGLM to determine the best lines for further evaluation. Following the 2012 season, 22 lines were selected from the initial 45 lines evaluated, and grown for additional testing and development. The current year's evaluation is being conducted at Spillman Agronomy Farm in Pullman. Twenty commercially available varieties are included as checks.

Buckwheat

Buckwheat (*Fagopyrum esculentum*), a highly nutritious pseudocereal rich in bioactive compounds, is principally cultivated in central and eastern European countries. The nutritional composition of buckwheat groats and husks, as well as genetic variation among North American cultivars, has not been extensively studied. In our study, buckwheat whole grains of ten different cultivars, VNS, Gv228, Co903, Gv-Manor, Co901, Gv-Manisoba, Commercial (a commercial buckwheat grain), Co902, Nikko and Ta-1 (*Fagopyrum tataricum*), were provided by McKay Seed Company (Almira, WA). A portion of the whole grains was used for the determination of 1000-kernel weight, and manually dehulled to determine the husk proportion. Husk proportion was expressed as the weight ratio of husk over whole grain. The 1000-kernel weight of cleaned whole buckwheat grains was determined using an electronic seed counter. The remaining buckwheat grains were dehulled using a tangential abrasive dehulling device (TADD) and fractionated into buckwheat groats (grits) and husks by sifting. Groats and husks were ground separately into meals using a cyclone mill (Udy Co., Fort Collins, CO) fitted with 0.5 mm sieve. The digestibility of buckwheat groats was compared with those of whole wheat, brown rice, white rice, pearled barley, and chickpea. Ten buckwheat cultivars were included in the study. Grains were cooked in boiled water with a grain to water weight ratio of 1 to 8. Grains were checked every 5 min by cutting and observing the appearance to determine the cooking time. Completely cooked grain has a translucent endosperm/cotyledon. After cooking, grains were cooled to 23°C and then dried in a convection oven at 60°C for 35 hr. Dried grains were ground into meal using a TADD and passed through a 0.5 mm sieve for determination of digestibility.

Color measurement of buckwheat groats and husks

Buckwheat groats and husks were ground and used for color determination by a CM-2002 spectrophotometer. The color score was recorded using the CIE-Lab standards, where "L" represented lightness, "a" represented redness and greenness, and "b" represented yellowness and blueness.

Determination of ash, protein, total starch, and dietary fiber content of groats and husks
Moisture and ash content of ten buckwheat cultivars were determined according to the AACC International Approved Methods 44-15.02 and 08-01.01 (2010), respectively. Protein content was determined using a nitrogen analyzer (FP-528, Leco Corp. St. Joseph. MI) coupled with a thermo-conductivity detector. A factor of 6.25 was used to calculate the protein content. Total starch content was determined together with resistant starch determination (AACC International Approved Method 32-40). Insoluble (IDF), soluble (SDF) and total dietary fiber (TDF) content determination was performed using a commercially available assay kit (Megazyme International Ireland Ltd, Wicklow, Ireland).

Free, bound, and total phenolics content
Buckwheat groats and husks (0.1 g, db) were extracted with acidified methanol (13 ml for groats and 18ml for husks) (HCl/methanol/water, 1/80/10, v/v) at 23°C for 2 hr with constant shaking. After centrifugation, the supernatant was collected for free phenolics determination. Residue was hydrolyzed with 2N sodium hydroxide for 1 hr under nitrogen gas at 23°C. The solution was then acidified with 2N hydrochloride acid and centrifuged at 2,500 g for 10 min. Supernatant was collected for bound phenolics content determination. Free and bound phenolics content were determined by the Folin-Ciocalteu method. Acidified methanol served as blank and gallic acid was used to create a standard curve. Phenolic content was expressed as milligram gallic acid equivalent per gram dry sample. All analyses were performed in duplicate.

5. Project Results

Quinoa

Preliminary quinoa variety trials, 2010
Of the 44 varieties evaluated, a distinct set of varieties emerged across all sites as the most adapted to Washington conditions. Virtually all of these varieties were of Chilean origin, or were from American varieties developed from Chilean material. Quinoa accessions from Peru, Bolivia, and Ecuador proved to be relatively maladapted. A few distinct problems were observed, namely lack of seed set and continued indeterminate growth.

The Olympia trial revealed a few key challenges that face quinoa cultivation in wetter locations of the maritime Pacific Northwest. Late summer rains posed a significant challenge, causing pre-harvest sprouting in a majority of the quinoa accessions. However, some accessions proved to be quite resistant to pre-harvest sprouting. Among these was accession PI 614880, originally from Chiloe Island, an area in Chile characterized by a similar oceanic climate (~75-83 in. annual precipitation). This accession was also discovered to have high pre-harvest sprouting tolerance by researchers in Argentina (Ceccato et al., 2011). Another challenge was the lack of plant dry down in the field. With the return of autumn rains, plants did not fully dry down and could not be harvested at the Olympia site. Unlike in Olympia, adequate dry down occurred in Pullman and seed was harvested from plants. On the other hand, one challenge discovered in Pullman was the presence of small black honeydew particles produced by aphids. These were found to be similar in size, shape, and weight to quinoa seeds and were rather difficult to separate from the quinoa seed.

Initial quinoa nitrogen and larger-scale variety trials, 2011

Due to high temperatures approaching 35°C during the period of seed filling, yields for all varieties were quite low. This phenomenon has been previously reported in quinoa exposed to high temperatures, and involves reabsorption of seeds (Bonifacio, 1995). No differences appeared for any of the response variables due to fertilization level ($p=0.7796$). Heat was likely a confounding factor, hiding any response due to fertilization. However, differences in yield were found between varieties ($p=0.0002$), which appears to indicate differences in heat tolerance. Many of the varieties that yielded highest originated from northern locations in the Chilean Central Valley, which experiences some of the highest summer temperatures found in central Chile. Seed from the best performing varieties at Boyd Farm were included in regional quinoa variety trials currently underway at locations in Washington, Oregon, Idaho, and Utah. These locations represent an even wider range of environments than those tested initially in 2010. The data gathered since 2010 has greatly informed our choice of location and quinoa variety for these regional trials. These data also form an invaluable knowledge base to which we will continue to add as our work with quinoa continues.

Quinoa Population Screening, 2012

Due to the wide crosses and origin of some of the parents, few of the lines in the populations produced seed. All the lines that set seed were hand harvested. The lines that did set seed and had good agronomic traits were selected and placed in the 2013 multi state variety trails. In 2012, there was an outbreak of downy mildew and notes were taken on disease resistance. It was found that some lines were more resistant than the others. Also, many lines have variable disease reactions to downy mildew. Some lines showed yellowing on the leaves; other lines had purple spotting, while other lines had final leaf loss. There were also a number of insects found on the quinoa, particularly aphids and the lygus bug. The aphids cause a leaf curl, while the lygus bugs feed on developing flower buds and seeds.

Irrigation x Intercropping in Quinoa, 2012

Irrigation had a significant impact on seed yield and plant height. The mean yield (in grams per plot) for the full irrigation and part irrigation plots were 807g/plot and 709g/plot, respectively, which is significantly higher than the dry land mean yield of 511g/plot (Figure 2). Cherry Vanilla (604g/plot) had a higher mean yield than Oro de Valle (511g/plot). The mean yield per plot for each intercrop treatment was 616g/plot for the control plots, 569g/plot for the fescue grass clover mix and 491g/plot for the clover mix. Irrigation also had an effect on plant height (Figure 3). At all four measurement dates the fully irrigated and partly irrigated plots had significantly taller plants than the non-irrigated plots. Over all, there was not a large height difference between the three cover crop treatments. Cherry Vanilla was significantly taller than Oro de Valle when measured on July 20 and August 16. Overall, the fescue grass intercrop had a winter higher survival rate than the clover mix intercrop. Only eight clover mix plots re-grew in the spring and they were all in the non-irrigated section of the field. Twenty-one of the 24 fescue grass clover mix plots survived over the winter and grew in the spring. The three plots that did not overwinter were in the dry land section.

Spelt

Of the 45 breeding lines and five varieties evaluated, several were identified as possessing high yields, high test weights, good threshability index, high kernel weight, and with good stripe rust resistance. These results are shown in Table 4. Many of our organically bred breeding lines performed better than the check cultivars. Of the 45 lines analyzed, 22 have been selected for further evaluation based on several parameters. The primary characteristics determining selection were yield and threshability, but data for multiple traits evaluated from 2012 is in Table 4.

Stripe rust was rated from 0-100% coverage of plants and a scale showing resistance to spreading within the leaf once the plant had been inoculated. The ratings were resistant (r), moderately resistant (mr), moderately susceptible (ms), and susceptible (s). Several stripe rust resistant lines were noted, and have been included in continuing trials. The stripe rust data from 2012 is shown below (Table 4).

Buckwheat

Buckwheat seeds of ten cultivars were fractionated into groats and husks and subjected to nutritional composition analysis and *in vitro* starch digestibility determination. Significant genetic variation was detected in buckwheat groats for 1000-kernel weight (16.5-39.8g), protein content (10.2-17.9%), soluble dietary fiber (SDF) (1.4-3.4%), insoluble dietary fiber (IDF) (2.3-8.6%), total dietary fiber (TDF) (3.6-10.6%), free phenolics content (4.5-17.1mg GA/g) and total phenolics content (6.8-20.7 mg GA/g) (Tables 4-10). Cultivar Co901exhibited the highest total starch content (76.8%), and high resistant starch content (RS) (3.6 g/100g) in cooked groats. Cultivar Ta-1 contained high groat protein, ash, IDF, TDF, and total phenolics content. The buckwheat husks exhibited relatively small differences in ash (2.0-2.5%), IDF (67.4-83.8%), SDF(1.97-3.21%), TDF (69.9-85.7%), and free phenolics content (22.3-34.2 mg GA/g) among cultivars, but large variation in protein content (3.0-6.5%), bound phenolics (6.7-26.1mg GA/g) and total phenolics content (32.4-58.6 mg GA/g). Total phenolics were 1.5-8 times higher in the buckwheat husk than in the groat. Cooked buckwheat groats exhibited lower starch digestibility and greater RS content than raw buckwheat groats. Significant genotypic variation in nutritional composition was detected among buckwheat groats and husks, and cultivars with unique nutritional composition were identified for future breeding.

6. Conclusions and Discussion

Quinoa

Variety trials

From these initial trials, several important discoveries were made that have informed our continued work with the crop. The most valuable discovery was the impact of varietal origin on the adaptation and success of a variety in our location. Chilean and Chilean derived varieties performed best across all three locations and have since formed much of the core of the WSU quinoa breeding program. Quinoa has a high level of genetic diversity, with varieties adapted to a wide range of environmental conditions. Matching the environment of a variety's area of origin with that of the target growing environment is a key step in introducing quinoa varieties with the greatest adaptation.

Some farmers interested in quinoa have planted food quinoa commonly sold in stores. Unfortunately, as quinoa sold in stores is often imported from Bolivia and Peru, the plants typically fail to set seed due to differences in photoperiod requirements. The evaluation and development of new varieties, in particular, those that successfully set seed under Washington growing conditions, will be a crucial step in making quinoa a viable crop for Washington farmers. Pre-harvest sprouting was found to be a major issue for the site in Olympia, indicating that this may be an issue for areas prone to early rains or for drier regions where the seasonal rains trigger pre-harvest sprouting, as quinoa takes a long time to reach maturity. Despite this challenge, there appears to be resistance for this trait. Breeding for earlier maturity will also be necessary to ensure proper plant dry down before harvest.

Although results were not obtained for fertilization response, valuable data was gathered on varietal differences in yield under heat stress. This data will help in future breeding efforts. Previous reports on heat susceptibility in quinoa have focused on the effect of high temperatures on pollen sterility (Johnson

and Croissant, 1990). As high temperatures were avoided during pollination in our experiment, this provides evidence that seed filling is a stage in quinoa development that is critically susceptible to heat.

Seed from the best performing varieties at Boyd Farm were included in regional quinoa variety trials currently underway at locations in Washington, Oregon, Idaho, and Utah. These locations represent an even wider range of environments than those tested initially in 2010. The data provided since 2010 has greatly informed our location and variety choice for these regional trials, and forms an invaluable knowledge base to which we will continue to add as our work with quinoa continues.

Irrigation x Intercropping in Quinoa, 2012

The results of this study showed that irrigation does increase yields of quinoa in Eastern Washington. The fully irrigated and partially irrigated plots did not have significantly different yields so a partial or deficit irrigation regime should be a good option in areas with limited use of water. Also, the success of the overwintered fescue grass showed that an intercrop plant can be planted and utilized as a green manure for the following spring. This is a viable option for farms with short growing seasons, when there is not time for a green manure crop to be planted and established before the end of the growing season. A problem encountered during the project is that in the fall, the clover became a salad bar for the rabbits and mice. At harvest there was hardly any clover left. One other problem that was encountered was poor germination of quinoa in the non-irrigated section of the field. Potential soil surface crusting after the initial overhead irrigation may have caused poor germination.

Quinoa Population Screening, 2012

This project is very useful for starting a quinoa breeding program. The phenotyping of the populations for selected traits, together with existing genotype information, can be used to develop molecular markers for use in the breeding program. Also, evaluating all of the breeding lines allows for selected lines that have the potential to become new quinoa varieties that grow well in the Pacific Northwest. Six lines from the 300+ lines grown in 2012 are currently being grown in our 2013 multi-state organic variety trails. Not only did this project contribute to present and further quinoa breeding efforts, it also presented an excellent opportunity to evaluate quinoa lines for downy mildew resistance, heat tolerance, reaction to insect pressure and many morphological traits. Again, these data will help us choose the lines best adapted to the Pacific Northwest.

Buckwheat

Significant differences in physical properties and nutritional compositions were detected in buckwheat groats and husks among ten selected cultivars. Large genetic variation was observed in TKW, protein content, SDF, IDF, TDF, and free and total phenolics content in buckwheat groats. Two nutritionally unique buckwheat cultivars were detected. Co901 had the highest total starch content as well as high RS content in the cooked groats. Ta-1 exhibited the highest groat protein, ash, IDF, TDF, and total phenolics content, which suggested more desirable nutritional value compared with other cultivars. The genetic background probably accounts for the uniqueness of the two cultivars, Co901 and Ta-1. The high starch content possibly contributed to the higher RS (retrograded starch) of cooked groats in Co901 than others. Ta-1 was a Tataricum origin and exhibited large compositional differences from common buckwheat cultivars.

Large genetic differences were found for protein content, bound phenolics, and total phenolics content of buckwheat husks. Buckwheat husks contained 1.5-8 times higher total phenolics than groats. Free phenolics are the major phenolic compounds both in buckwheat groat and husk, which could be incorporated into food production with improved antioxidant capacity. Cooked buckwheat groats were

digested slower during enzyme incubation compared to raw groats. RS content of cooked buckwheat groats was higher than in raw buckwheat groats.

Spelt

The spelt breeding lines have been selected in organic systems from 2004 to 2012. When compared to the check cultivars 'Schwarzer Winter', 'WA 5768', 'Spelta Hohenheim', '2948' and 'Oe', several of our breeding lines stood out in terms of yield, threshability, thousand kernel weight (TKW), test weight (TW), and stripe rust resistance (Table 4). For example, breeding lines SPYT3 and SPYT8 were found to be very high yielding and with a threshability index at least twice that of the highest yielding check cultivar 'Oe'. Most of the check cultivars were moderately resistant to stripe rust. Breeding lines SPYT10, SPYT12, and SPYT14 proved to be most resistant to current races of stripe rust. Threshability is a potentially valuable trait as it eliminates the dehulling/pearling step, which can remove mineral nutrients from the finished grain.

7. Useful Tools, Information, and Resources for Farmers

Using the most appropriate, regionally adapted quinoa varieties is critically important to the success of organic quinoa production. Sources for quinoa seed are outlined in the webinar Organic Quinoa Production (see Section 8). This seminar also discusses various tools and techniques of use in quinoa production and post-harvest. As quinoa production is relatively new to most of the US, there are many important lessons learned regarding production and post-production methods. These are too extensive to list here and for this reason we prepared a publically available webinar that should address many of the basics of organic quinoa production: http://www.youtube.com/watch?v=V7j_VdwrFSA. An additional source of information is a presentation by John McCamant and Paul New on their extensive experience growing quinoa in Colorado: http://www.youtube.com/watch?v=05hyd2H85BY&list=PLZMuQJAj6rOoftNenC-Iuz3MKsc5H1C2u&index=11. An excellent discussion by plant breeder Frank Morton, which was a component of the 2013 International Quinoa Research Symposium, can be found here: http://www.youtube.com/watch?v=EGXxowjrzhg&list=PLZMuQJAj6rOoftNenC-Iuz3MKsc5H1C2u&index=5.

The development of useful tools and resources for buckwheat and spelt are currently in progress, with updated information on the nutritional value of buckwheat cultivars currently in press and complete spelt variety and breeding line trials expected after the 2014 growing season. Our mineral concentration results for spelt are anticipated to be completed in the spring of 2014.

8. Outreach

Webinar:
In collaboration with eOrganic, Kevin Murphy delivered a webinar in February 2013 on Organic Quinoa Production: http://www.youtube.com/watch?v=V7j_VdwrFSA, and along with Adam Peterson, fielded questions post-webinar. This has proven to be a valuable resource for farmers interested in the basics of quinoa production.

Field days:
July 2010, WSU Organic Farm Field Day, Quinoa Variety Trial
August 2010, Port Townsend, WA Dryland Farming Project, Quinoa Variety Trial
July 2011, WSU Organic Farm Field Day, Quinoa Nitrogen x Variety Trial
July 2012, WSU Organic Farm Field Day, Quinoa Nitrogen x Variety Trial, Quinoa Breeding Line Phenotyping Evaluations, Intercropping x Irrigation Trial

July 2013, Shepherd's Grain Field Day, Clark Farm, Albion, WA, Quinoa Variety Trial
July 2013, WSU Organic Farm Field Day, Quinoa Breeding Line Phenotyping Evaluations, Intercropping x Irrigation Trial
August 2013, International Quinoa Research Symposium Field Days, Pullman, WA and Albion, WA

Presentations:
October 2011, American Society of Agronomy, Nitrogen Use in Quinoa, San Antonio, TX
November 2012, Focus on Farming Conference, Snohomish, WA
August 2013, Fifteen presentations at the International Quinoa Research Symposium, Pullman, WA https://www.etouches.com/ehome/quinoa/. These were recorded by eOrganic and are available beginning September 2013 at the following website: http://www.youtube.com/playlist?list=PLZMuQJAj6rOoftNenC-Iuz3MKsc5H1C2u

9. Leveraged resources

Without support from OFRF, we would not have obtained the additional funding needed to carry on long-term breeding and agronomy research for alternative crops. The additional funding includes a $36,000 grant from the WSU Center for Sustaining Agriculture and Natural Resources and a $1.6M grant from the USDA Organic Research and Extension Initiative.

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Figures

Figure 1. Plant with poor pre-harvesting sprouting tolerance. Olympia, WA (2010).

Figure 2. Yield of quinoa (g/plot) by cover crop, irrigation and variety.

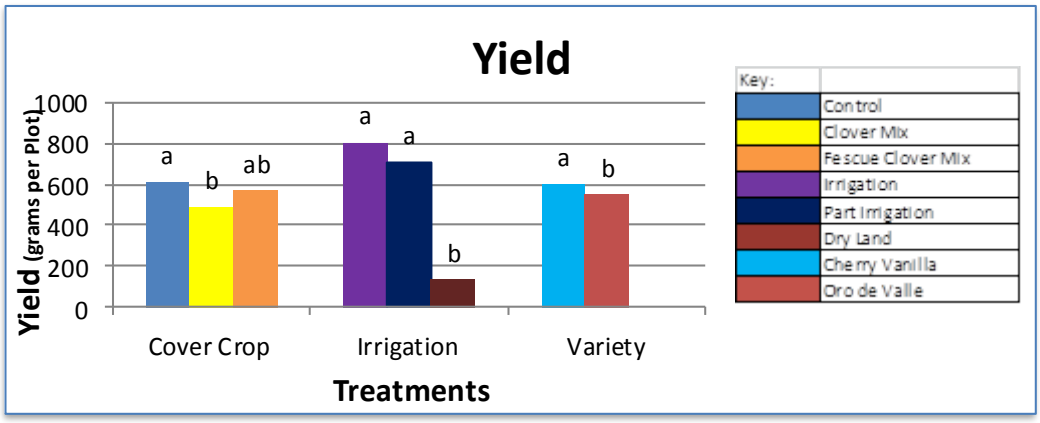
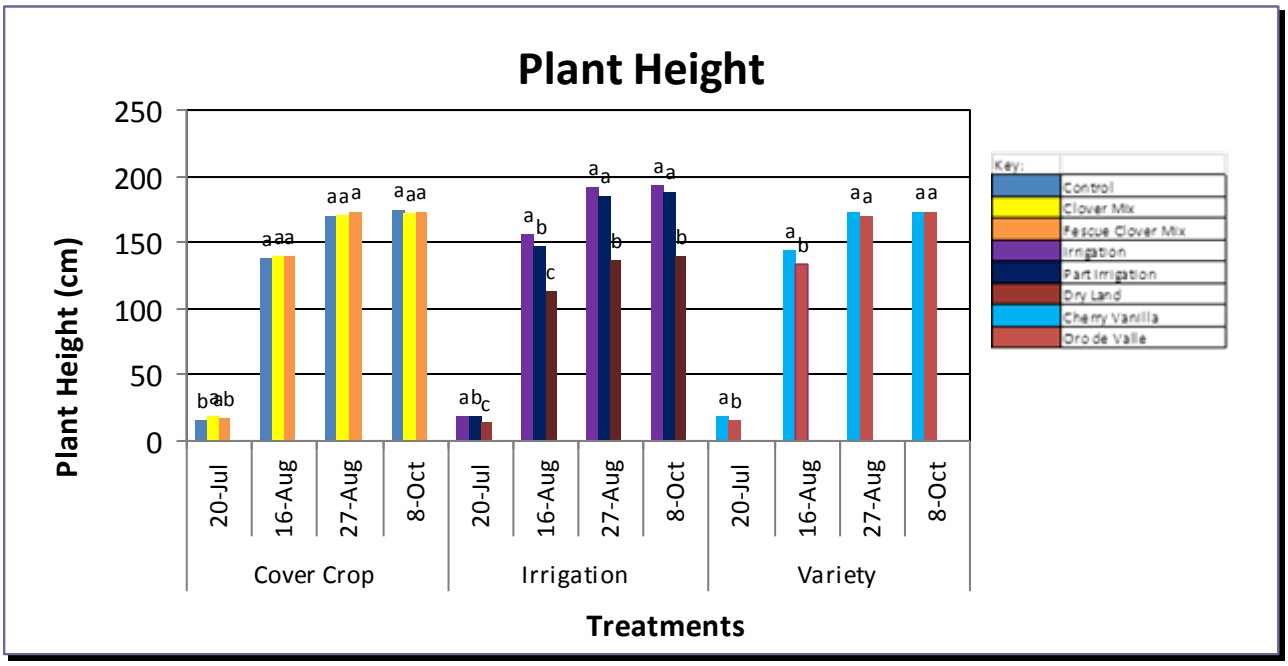


Figure 3. Plant height of quinoa (cm) by cover crop, irrigation and variety across four dates in 2012.



Tables

Table 1. Photos of Common Pests and Diseases of Quinoa.

Aphids on plant Left – Symptoms of Aphids, leaf roll Right – Aphids	
Lygus Bug Usually are located around the flower bud	
Leaf Roller Left- Leaf Roller Right- damage done	
Downy Mildew Left- Downy Mildew on green quinoa Right – Downy Mildew on red/purple quinoa	

Table 2. Quinoa morphological scale, adapted from Jacobsen and Stolen (1993).

Stage	Description	
0	**Vegetative phase**	
1	**Bud formation**	bud covered
2		bud visible
3		bud distinct
4		bud approximately 0.5 cm long
5		bud approximately 1.0 cm long
6		onset of pyramid shape
7		distinct pyramid shape
8	**Anthesis**	onset of flowering
9		50% of flowering
10		100% flowering
11	**Floral dehiscence**	onset
12		majority of flowers dehisced
13		only wilted anthers present
14	**Seed set**	33% seed set
15		50% seed set
16		67% seed set
17		100% seed set
18	**Maturity**	plant color: green > yellow
19		plant color: yellow > green
20		mature
21		wilted

Table 3. Photos of development stages 6, 8, 17, and 20 of quinoa.

On-set of Pyramid **Development stage 6**	
Anthesis **Development stage 8**	
Seed Set **Development stage 17**	
Maturity **Development stage 20**	

Table 4. Spelt breeding lines and variety trial results, Pullman 2012. Weight is in grams/plot; TI = threshability index, TKW = thousand kernel weight, and SR1, 2 and 3 refer to stripe rust evaluations, where s=susceptible, ms=moderately susceptible; mr=moderately resistant; and, 4=resistant.

Name	Weight	T.I.	TKW	TW (Pint)	Plant Ht 1	Plant Ht 2	SR 1	SR 2	SR 3
SPYT1	1377.34	0.62	30.6	395.3	38.7	38.0	90s	60ms	75ms
SPYT2	1445.28	0.72	36.8	404.0	38.3	37.0	75s	30mr	50ms
SPYT3	1912.82	0.65	39.5	405.0	36.7	37.0	50ms	25mr	30mr
SPYT4	1468.24	0.59	32.4	408.3	35.0	36.7	95s	40mr	25mr
SPYT5	1402.68	0.62	32.0	408.5	45.0	43.3	95s	70ms	50mr
SPYT6	1720.74	0.64	37.4	416.2	41.0	36.0	90s	60ms	30mr
SPYT7	1876.37	0.65	42.0	416.7	38.3	41.0	65ms	50mr	25mr
SPYT8	2137.78	0.54	38.2	417.7	38.7	40.7	30mr	15mr	20r
SPYT9	814.94	0.39	30.1	417.7	34.5	34.5	25mr	50mr	25mr
SPYT10	1564.27	0.68	38.9	413.4	35.0	40.3	45mr	10r	0r
SPYT11	1759.93	0.70	37.7	411.0	40.0	39.7	20mr	10r	15mr
SPYT12	1783.72	0.70	38.9	414.8	40.7	40.7	15r	10r	5r
SPYT13	934.24	0.58	35.6	404.2	36.7	38.7	50mr	55ms	20mr
SPYT14	1658.51	0.67	39.9	408.2	42.0	40.0	15mr	5r	5r
SPYT15	1342.75	0.75	40.0	419.7	40.0	38.0	15mr	30mr	20mr
SPYT16	1050.95	0.65	31.2	411.0	37.3	39.3	50mr	70s	50mr
SPYT17	1431.14	0.62	34.9	420.9	42.7	45.3	30mr	70ms	50mr
SPYT18	1263.00	0.50	33.1	414.7	37.7	38.0	50ms	65ms	30mr
SPYT19	821.24	0.57	23.4	407.1	39.0	38.0	30mr	70ms	40mr
SPYT20	1069.64	0.69	24.7	402.9	41.0	41.0	10r	50mr	20mr
SPYT21	1618.15	0.55	37.5	409.0	38.3	36.7	15mr	30mr	30mr
SPYT22	1641.52	0.57	40.2	410.7	36.0	36.0	25mr	50mr	20mr
SPYT23	1624.43	0.66	41.3	415.1	37.7	37.7	25mr	25mr	30mr
SPYT24	1440.50	0.44	37.8	419.2	38.7	39.3	25mr	30mr	20mr
SPYT25	1375.84	0.61	43.9	411.2	34.0	33.3	25mr	30mr	20mr
SPYT26	1435.87	0.48	43.1	410.9	38.3	39.3	20mr	20mr	15r
SPYT27	1366.96	0.69	44.3	423.7	36.3	40.3	30mr	20mr	20mr
SPYT28	1264.28	0.69	41.3	403.4	37.7	40.0	20mr	15mr	30mr
SPYT29	1215.49	0.68	41.8	406.2	35.7	38.3	25mr	10mr	10mr
SPYT30	1432.06	0.60	35.2	.	40.7	42.3	10r	20mr	25mr
SPYT31	1338.72	0.62	37.9	398.5	39.7	40.3	15mr	40mr	20mr
SPYT32	1164.85	0.61	36.7	408.9	37.7	37.0	40mr	25mr	30mr
SPYT33	1864.32	0.61	38.1	408.9	40.3	38.0	40mr	10r	20mr
SPYT34	1170.77	0.60	37.0	411.3	34.0	33.3	35mr	40mr	30mr
SPYT35	1076.38	0.57	31.3	414.5	32.3	31.7	70ms	85s	20mr
SPYT36	1339.72	0.49	43.2	.	37.0	38.0	35mr	15r	20mr
SPYT37	1775.65	0.51	43.2	402.1	40.0	39.7	50mr	40mr	15r
SPYT38	1561.37	0.49	42.8	409.4	39.7	39.3	50mr	30mr	15mr
SPYT39	1221.89	0.57	35.0	413.8	34.7	36.0	70ms	35mr	40mr
SPYT40	1686.50	0.68	41.7	417.3	39.7	42.0	40ms	20mr	30mr
SPYT41	1354.07	0.61	34.8	407.0	36.3	37.0	70ms	20mr	50ms
SPYT42	1265.45	0.66	37.1	404.1	35.7	37.3	70ms	50mr	25mr
SPYT43	1351.29	0.61	35.2	429.9	41.7	39.7	30mr	60ms	15mr
SPYT44	1274.79	0.70	33.7	419.5	39.0	39.0	70ms	50ms	30mr
SPYT45	1702.03	0.79	36.1	427.5	36.7	37.0	50mr	15r	20mr
Schwarzer Winter	1524.76	0.32	39.2	.	44.7	44.7	15r	5r	30mr
WA 5768	1470.72	0.47	34.2	.	43.3	43.3	20mr	25mr	20mr
Spelta Hohenheim	1678.09	0.39	38.3	.	47.0	46.0	15mr	20mr	10r
2948	1686.67	0.32	37.8	.	44.3	44.7	10mr	15mr	20r
Oe	1877.64	0.27	38.4	.	42.7	45.3	35mr	10r	15mr

Table 4. Husk proportion and 1000-kernel weight of ten buckwheat cultivars.

Cultivar	Husk Proportion (%)	1000-Kernel Weight (g)
VNS	17.1f[a]	39.8a
Gv228	23.6ab	33.5bcd
Co903	23.2abc	33.5bc
G-Manor	23.8ab	34.6bc
Co901	22.7bcd	31.4de
Gv-Manisoba	24.3a	33.1bcd
Commercial	21.5de	35.2b
Co902	21.3e	32.3cd
Nikko	22.2cde	29.9e
Ta-1	22.1cde	16.5f

[a]Each value is expressed as mean (n = 2). Mean values with different letters within a column are significantly different ($p < 0.05$).

Table 5. Color of buckwheat groats of ten cultivars**.**

Cultivar	**Groat**		
	L	**a**	**b**
VNS	84.9g[a]	1.0a	7.7c
Gv228	86.0e	0.4f	6.7g
Co903	86.5b	0.4e	7.0f
Gv-Manor	86.2d	0.4d	7.0f
Co901	87.0a	0.3g	7.1f
Gv-Manisoba	86.6b	0.5d	7.3e
Commercial	85.6f	0.6b	7.0f
Co902	86.3c	0.5c	7.6d
Nikko	85.9e	0.2h	7.9b
Ta-1	76.9h	-1.3i	21.3a

[a]Each value is expressed as mean (n = 3). Mean values with different letters within a column are significantly different ($p < 0.05$).

Table 6. Chemical composition of buckwheat groats of ten cultivars.

Cultivar	Ash (%)	Protein (%)	Total Starch (%)	IDF (%)	SDF (%)	Total DF (%)
VNS	1.8de[a]	10.4h	75.9b	4.1b	3.4a	7.6b
Gv228	1.7e	11.1f	76.0b	3.9b	2.8b	6.7bc
Co903	2.1b	12.1d	72.8f	3.9b	2.9ab	6.8bc
Gv-Manor	1.9cd	10.8g	75.5bc	3.9b	2.5bc	6.3bcd
Co901	1.7e	10.2i	76.8a	2.3c	1.4e	3.6e
Gv-Manisoba	2.0b	12.3c	74.5e	3.6b	1.7de	5.3d
Commercial	1.9cd	11.7e	75.0d	4.7b	1.4de	6.1cd
Co902	2.0bc	11.6e	75.1cd	4.1b	1.5de	5.6cd
Nikko	2.0b	13.4b	72.8f	4.8b	1.7de	6.4bcd
Ta-1	2.7a	17.9a	61.2g	8.6a	2.0cd	10.6a

[a]Each value is expressed as mean (n = 2), except for protein (n = 3). Mean values with different letters within a column are significantly different ($p < 0.05$). All values are expressed based on % of dry sample weight.

Table 7. Chemical composition of buckwheat husks of ten cultivars.

Cultivar	Ash (%)	Protein (%)	IDF (%)	SDF (%)	TDF (%)
VNS	2.5bc[a]	3.0g	83.8a	2.0d	85.7a
Gv228	2.0d	6.5a	67.4g	2.5c	69.9h
Co903	2.1cd	5.6b	78.7d	2.6bc	81.3d
Gv-Manor	2.1cd	4.6d	76.7e	2.1d	78.8f
Co901	2.4a	5.2c	76.4e	3.2a	79.6e
Gv-Manisoba	2.0d	4.2e	82.2b	2.2d	84.4b
Commercial	2.1cd	4.5d	81.0c	2.2d	83.2c
Co902	2.3ab	5.0c	79.4d	2.6bc	82.0d
Nikko	2.1cd	5.0c	81.7bc	2.5c	84.2b
Ta-1	2.1cd	3.4f	74.0f	2.9b	76.9g

[a]Each value is expressed as mean (n = 2), except for protein (n = 3). Mean values with different letters within a column are significantly different ($p < 0.05$). All values are expressed based on % of dry sample weight.

Table 8. Free, bound and total phenolics content in buckwheat groats and husks.

Groats Cultivar	Groats			Husks		
	Free Phenolics[b]	Bound Phenolics	Total Phenolics	Free Phenolics	Bound Phenolics	Total Phenolics
VNS	4.9e[a]	2.4cd	7.3e	23.8f	9.6g	33.4h
Gv228	4.9 e	2.5bc	7.4e	22.3g	15.0d	37.3g
Co903	5.3ed	2.6b	7.8d	31.0c	23.9b	54.9b
Gv-Manor	5.6cbd	2.4cd	8.0d	24.3ef	13.5e	37.9g
Co901	4.5f	2.3d	6.8f	32.8b	17.2c	50.0c
Gv-Manisoba	5.5cd	2.5bc	8.0d	24.6e	15.1d	39.7f
Commercial	5.7bc	2.5b	8.3c	25.4d	17.0c	42.4e
Co902	5.4cd	2.4bc	7.9d	32.6b	26.1a	58.6a
Nikko	6.0b	2.4bc	8.4b	34.2a	12.5f	46.7d
Ta-1	17.1a	3.6a	20.7a	25.7d	6.7h	32.4i

[a]Each value is expressed as mean (n = 2). Mean values with different letters within a column are significantly different ($p < 0.05$).

[b]Expressed as mg gallic acid equivalent per g of dry sample (mg GA/g).

Table 9. Digestibility of starch in raw buckwheat groats during incubation with pancreatic α-amylase and amyloglucosidase.

Cultivar	20min[a]	120min[a]	16 hr[a]	RS[a]	Total Starch[a]	RS/Total Starch (%)
VNS	15.0b[b]	48.7c	75.6b	0.3a	75.9b	0.4a
Gv228	13.6de	51.4a	75.7b	0.3a	76.0b	0.4ab
Co903	14.4c	51.5a	72.6e	0.2e	72.8f	0.3de
Gv-Manor	13.1ef	50.6b	75.3b	0.2de	75.5bc	0.3de
Co901	10.8g	49.9b	76.6a	0.2de	76.8a	0.3e
Gv-Manisoba	13.6d	50.3b	74.2d	0.3b	74.5e	0.4b
Commercial	13.6de	50.2b	74.7c	0.3cd	75.0d	0.3cd
Co902	13.0f	49.0c	74.9c	0.3c	75.1cd	0.3c
Nikko	15.6a	49.1c	72.6e	0.2cde	72.8f	0.3cde
Ta-1	6.4h	37.8d	61.0f	0.2f	61.2g	0.3cde

[a]Expressed as digested starch content (g) per 100 g dry raw buckwheat groats within corresponding incubation time.

[b]Each value is expressed as mean (n = 2). Mean values with different letters within a column are significantly different ($p < 0.05$).

Table 10. Digestibility of starch in cooked buckwheat groats during incubation with pancreatic α-amylase and amyloglucosidase.

Cultivar	20min[a]	120min[a]	16 hr[a]	RS[a]	Total Starch[a]	RS/Total Starch (%)
VNS	14.0a[b]	29.8a	71.3a	3.8a	75.0a	5.0a
Gv228	13.4bcd	29.2a	68.9f	3.1f	72.0d	4.3f
Co903	13.2d	29.2a	69.1ef	3.2e	72.3cd	4.41e
Gv-Manor	13.5b	29.0a	70.4cb	3.5c	73.9b	4.7c
Co901	13.5b	29.0a	70.5cb	3.6b	74.1b	4.9b
Gv-Manisoba	13.5bc	29.1a	69.9cd	3.1f	73.0c	4.2g
Commercial	13.3cd	29.1a	69.0ef	3.4d	72.4cd	4.6cd
Co902	13.2d	29.3a	70.7ab	3.4d	74.1b	4.5d
Nikko	13.0e	28.8ab	69.6de	2.9g	72.5cd	4.0h
Ta-1	11.4f	25.8b	56.6g	1.6h	58.3e	2.8i

[a]Expressed as digested starch content (g) per 100 g dry cooked buckwheat groats within indicated incubation time.

[b]Each value is expressed as mean (n = 2). Mean values with different letters within a column are significantly different ($p < 0.05$).