

EVALUATING CHARACTERISTICS OF PROSO MILLET CULTIVARS AND POTENTIAL  
FOR ADOPTION IN THE INLAND PACIFIC NORTHWEST

By  
TAYLER REINMAN

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of TAYLER REINMAN find it satisfactory and recommend that it be accepted.

Kevin Murphy, Ph.D., Chair

Jessica Goldberger, Ph.D.

Stephen Bramwell, Ph.D.

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Abstract

by Tayler Reinman, M.S.  
Washington State University  
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Chair: Kevin Murphy

Increasing cropping system diversity is one way to improve resiliency of our food system in the wake of global population increase and climate change. Proso millet is a small-seeded grain regarded as climate-resilient due to its water efficiency, performance in low fertility soils, and desirable nutritional profile. Proso millet is grown in the Great Plains but underutilized for human consumption in the US. However, it shows potential for adoption in the inland Pacific Northwest (PNW) due to its short growing season, compatibility with regional equipment, and environmental requirements. To better understand this potential, seven commercially available varieties were planted in a researcher-run trial in Pullman, WA and in a series of producer-run trials across the region in 2022. In chapter two, samples were analyzed for agronomic phenotypes, mineral concentrations, and seed morphology phenotypes. Varieties from the researcher-run trial showed significant differences for all traits excluding percent emergence and Ca concentration. Grain yield was correlated with plant height, seed area, and thousand seed weight and was negatively correlated with Zn. Most minerals were positively correlated with one another. Samples from producer-run trials showed differences by location for all seed

morphology phenotypes and for concentration of Zn, Fe, Cu, and Mn. In chapter 3, interviews with producer participants were analyzed to better understand benefits and challenges of working with the crop in the inland PNW. Key benefits shared by participating producers include the resilience of the crop, capacity for rotational weed control, and increased opportunity for on-farm diversity. Key challenges include timing and logistics of harvest, the lack of a reliable market, insufficient infrastructure for storage, and inconsistent stand. Results from these studies can support future proso millet breeding efforts, inform variety selection for stakeholders across the food system, and support producers who are interested in increasing diversity in their cropping systems.

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## **Dedication**

Eternal love and gratitude to P.S. and S. R.

## CHAPTER ONE: GLOBAL INTRODUCTION

As climate change progresses and the global population continues to grow, strategies will need to be implemented to increase global agricultural production and while improving resiliency of agricultural systems. One way to improve resiliency is to increase diversity in rotational cropping systems. However, novel crop adoption is a complex process that hinges on environmental conditions of a given region, existing cropping systems, market demand, producer awareness and interest, and varietal selection or breeding. One crop that has been identified as promising for the inland Pacific Northwest (PNW), a region dominated by dryland wheat production, is proso millet (Habiyaemye, Matanguihan, *et al.*, 2017). Despite desirable agronomic and nutritional characteristics of proso millet, within the US, little research has been conducted to assess its potential outside of the Central Great Plains region of Colorado, Nebraska, and South Dakota. The following thesis presents research conducted through a Western Sustainable Agricultural Research and Education funded project called New Grains Northwest (SW21-926), in which the potential for adoption of proso millet into the PNW food system was assessed. New Grains Northwest research activities considered production, processing, marketing, and consumption, specifically within the context of regional food system development. Additionally, participatory research methods were employed to garner a reciprocal exchange of information between researchers and regional producers and ground research in real-world producer experiences. This global introduction provides an overview of relevant literature supporting the regional, researcher- and producer-run proso millet field trial activities that were conducted in 2022 under this project.

## 1. INCREASING AGRICULTURAL RESILIENCY IN THE FACE OF CLIMATE CHANGE

Global agricultural production must meet the caloric demand of the world's population, and historically, as the population has continued to grow, agricultural technology has been developed to increase production capacity and meet this demand. However, many modern agricultural practices used to increase yields contribute to excess atmospheric greenhouse gases that propel climate change (J. Wang *et al.*, 2018). Climate change, in turn, negatively impacts the productivity and resiliency of the current global agricultural system through biotic and abiotic stressors such as changes in annual rainfall, average temperatures, extreme weather events, and weed, pest, or microbe pressure (Raza *et al.*, 2019).

One strategy to increase resiliency in the face of these stressors is to increase diversity in industrial cropping systems. Agricultural intensification has led to a loss in biodiversity both within production systems (reduced genetic diversity of crop species) and as a result of these production systems (loss of biota on agricultural land due to fertilizers, herbicide, pesticides, and land clearing) (Schmitz *et al.*, 2023). Genetic diversity within agricultural production decreased significantly during the Green Revolution of the 1960s, with a surge of technological advancements that catered to greater uniformity of crops over larger areas, greatly increasing yields but also increasing vulnerability to major threats associated with climate change (Massawe, Mayes and Cheng, 2016). For example, just three major cereal crops, wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and rice (*Oryza sativa* L.), are estimated to sustain 50% of the caloric demand of the world population, and their productivity is projected to be affected by climate change (Bekkering and Tian, 2019; Neupane *et al.*, 2022).

Alternative crops with more climate resilient traits are being explored as an opportunity to replace or substitute major cereals and improve global food security (Kumar and Bhalothia, 2020). Examples of these underutilized crops, sometimes referred to as “orphan crops,” include millets, quinoa, pseudocereals such as buckwheat and amaranth, and a number of legumes (Kumar and Bhalothia, 2020). While adoption of underutilized crops is a promising solution for increasing resiliency of the food system in the wake of climate change, limitations exist surrounding the yield potential, trait improvement, knowledge diffusion, and market buy-in for them, making them potentially riskier and more resource intensive to adopt (Bekkering and Tian, 2019). Additionally, development of these crops must be conducted based on the specific region of adoption in order to optimize production for regional cropping systems and market potential for regional food systems.

## **2. INLAND PACIFIC NORTHWEST CROPPING SYSTEMS**

The inland PNW is a semiarid region including central Washington, northeast Oregon, and northern Idaho that contains geographic features including the Columbia Basin, Columbia Plateau, and the Palouse and Nez Perce Prairies, and is dominated by dryland cereal-based cropping systems (Yorgey and Kruger, 2017; Roesch-Mcnally, 2018). Overall, it tends to have cold wet winters and hot dry summers, but annual precipitation (ranging from 180 mm to 1130 mm) and average temperature vary depending on elevation and local topography (Karimi *et al.*, 2018). Typically, the western-most part of the region, in the lee of the Cascade Mountains, is driest, with annual precipitation increasing toward the east (Kaur *et al.*, 2017). The inland PNW is an important agricultural region, producing around 17% of the nation’s wheat and, based on an estimate from 2015, generating an annual \$1.3 billion (Yorgey and Kruger, 2017; Karimi *et al.*, 2018). Wheat is the dominant crop produced in the region, commonly in a wheat-fallow rotation,

but small quantities of other small grains, legumes, and canola are also incorporated into cropping rotations (Roesch-Mcnally, 2018).

Wheat production, however, will be challenged by predicted climactic changes in the region, which include warmer summer temperatures, more frost-free days, wetter winters, and longer periods of drought in the summer and fall (Roesch-Mcnally, 2018). While some predictions show that increased levels of CO<sub>2</sub> could increase wheat yields in the inland PNW in the short term, expected climactic changes and associated biotic and abiotic pressures are likely to lead to a leveling off or decline in yields by 2100 (Yorgey *et al.*, 2017).

### **3. PROSO MILLET**

Based on the characteristics of the region, existing cropping systems, and anticipated challenges, one crop with potential to increase the diversity and resiliency of inland PNW cropping systems is proso millet. Millets are small-seeded cereal crops that have been cultivated as staple crops in semi-arid environments of Asia, Africa, and Europe since prehistoric times in Asia, Africa, and Europe (Spengler, 1975). Today, they are still an important source of energy and protein for millions of people, particularly in underdeveloped countries and marginal agricultural zones with hot, dry, environments (Amadou, Gounga and Le, 2013). In 2021, 56% of worldwide millet production took place in Asia (led by India and China), followed by 40% in Africa (led by Niger and Nigeria), and 20% in Europe (led by Russia and Ukraine) while only 1% was produced in North America (Amadou, Gounga and Le, 2013; FAOSTAT, 2021). Millets are recognized as promising climate resilient crops as they are highly water efficient, can grow on shallow, low fertile soils with a high range of salinity and acidity, and are C4 cereals meaning that they take more carbon dioxide from the atmosphere than wheat and rice (Kumar *et al.*, 2018). Additionally, they have a comparable nutritional profile to other major cereal grains



(Kumar *et al.*, 2018). Millets show such promise for improving global food security in the wake of climate change that in order to raise awareness and stimulate research and development of the grains, the United Nations declared 2023 “International Year of the Millets” (*International Year of Millets 2023*, 2023).

There are many different species of millets that vary in plant and seed morphology. The most commonly cultivated species for human consumption in the US is proso millet (Myers, 2018). US producers planted an estimated 670,000 acres in 2022, but even as the leading US millet for human consumption, most proso millet grown domestically is funneled into birdseed markets (Das *et al.*, 2019; Flanary and Keane, 2020). Primary production of proso millet has historically taken place in the Central Great Plains, specifically concentrated in Nebraska, Colorado, and South Dakota, however, its agronomic qualities also show compatibility with growing conditions in the inland PNW (Habiyaremye, Matanguihan, *et al.*, 2017; Das *et al.*, 2019). Proso millet is well-adapted to the rainfed, dryland cropping systems characteristic of the region, as well as its well-drained loamy soils (Habiyaremye, Matanguihan, *et al.*, 2017). Additionally, it has a short growing season of approximately 60-100 days and is typically planted in late May or early June, making it viable for insertion into a winter wheat rotation, either as a replacement for summer fallow or as a “catch crop” if a winter or early spring crop were to fail (Lyon *et al.*, 2014; Ventura *et al.*, 2020). When added into a winter wheat rotation, proso millet has been shown to increase yields by controlling winter annual grassy weeds, reducing insect and disease pressures, and preserving moisture in the deep soil (Santra, 2013). Last, as there are no other warm season grasses commonly cultivated in the area, proso millet presents a niche opportunity for producers looking to diversify their rotations.

Desirable agronomics, however, are not enough to justify regional adoption of an underutilized crop. A reliable market must also be developed in order to make adoption financially viable for producers. In addition to its rotational benefits, proso millet has marketable characteristics such as a desirable nutritional profile and gluten-free proteins (Habiyaemye, Matanguihan, *et al.*, 2017).

Proso millet is nutritionally comparable to wheat, but with higher fiber content, lower glycemic index, and richer bioactive compounds, making it marketable in the health food sector (Kumar *et al.*, 2018). It has a comparable protein content to wheat with higher concentrations of certain amino acids such as leucine, isoleucine, and thiamine (Kumar *et al.*, 2018). Additionally, proso millet can be marketed alongside quinoa and amaranth as an “ancient grain”, which is a rapidly growing niche in health-food markets (Cheng, 2018; Das *et al.*, 2019).

Another growing niche in health-food markets is gluten-free products (Das *et al.*, 2019). Rise in the popularity of gluten-free products began with increased diagnosis of celiac disease, a genetic disease that prevents digestion of gluten proteins in grains such as wheat, barley, and rye, but has expanded into a larger market of non-celiac, gluten-intolerant individuals, and those who choose to avoid gluten because of perceived health benefits (Woomer and Adedeji, 2021). An estimated 1% of the US population is diagnosed with celiac disease, while about 6% are reported with non-celiac gluten sensitivity, and a rising number opt for gluten-free products for personal reasons (Das *et al.*, 2019). Proso millet contains gluten-free proteins, making it desirable for incorporation into gluten-free products such as breads, pastas, breakfast cereals, puffed snacks, and beverages (Habiyaemye, Matanguihan, *et al.*, 2017; Woomer and Adedeji, 2021). Due to high amylase activity, proso millet can also be malted as a substitution for barley in gluten free beverages such as beer (Das *et al.*, 2019).

Regardless of marketable characteristics, proso millet cannot be feasibly adopted into a food system without availability of required post-harvest infrastructure, such as seed cleaning and dehulling equipment, that bridges the supply chain between producers and processors. Some large processors are vertically integrated with equipment such as seed cleaners and dehullers specific to proso millet, however, small- to mid-scale processors who do not typically work with the crop may have trouble accessing this vital equipment, hindering them from adoption. However, coordination within regional food systems can create opportunity for shared investment in necessary equipment, expanding the capacity of the regional foodshed.

#### **4. REGIONAL FOOD SYSTEMS**

When considering regional adoption of a crop like proso millet from a production standpoint, it is also important to consider the regional food system as a whole. While local markets can often support unique and novel crops, the scale of grain production and farms in the inland PNW make them better suited to regional food system adoption. Local and regional food systems are often conflated, but when these systems expand to span multiple counties or multiple states, they enter the purview of “regional food systems” which operate on a more comprehensive scale with larger markets, food needs, and volume, and increased variety, land use, and policy impacts (Clancy and Ruhf, 2010). According to Clancy and Ruhf (2010), “An ideal regional food system describes a system in which as much food as possible to meet the population’s food needs is produced, processed, distributed, and purchased at multiple levels and scales within the region, resulting in maximum resilience, minimum importation, and significant economic and social return to all stakeholders in the region.”

Regional food systems have some unique benefits compared with their smaller-scale counterparts, beyond just their ability to meet a higher market demand. Regional food systems

can accommodate large or mid-level farms, often catering to institutional customers such as restaurants, health care facilities, and universities rather than just direct-to-consumer markets (Vogt and Kaiser, 2008). Regional-scale systems are often more efficient in their production, transportation, and marketing, leading to economic benefits for the farmer and environmental benefits for the supply chain (Cumming, Kelmenson and Norwood, 2019). Additionally, regional systems that operate between urban and rural areas have the potential to bring money into those less prosperous regions and increase access to fresher, higher quality food (Vogt and Kaiser, 2008; Cumming, Kelmenson and Norwood, 2019).

Regional food systems also have some unique challenges compared with local food systems. First, more complex physical infrastructure, such as warehouses, machinery, and technology, as well as human resources must be in place for processing and central distribution of regional-scale products (Vogt and Kaiser, 2008; Hermiatin *et al.*, 2022). Regional products can also be harder to market, as the typical “know-your-farmer” narrative that supports direct-to-consumer markets is harder to communicate on a larger scale, with more invisible actors such as truck drivers and food safety managers. (Cumming, Kelmenson and Norwood, 2019). Additionally, regional marketing requires a greater level of education to ensure that consumers understand the economic and environmental benefits of regional food systems, which may contain multiple states and many different communities, than it does for consumers to inherently understand the benefit of “buying local” (Cumming, Kelmenson and Norwood, 2019). However, certain models of regional food systems, such as value chains, use strategies across the supply chain to navigate these marketing and infrastructure-based challenges.

“Value-added” can be used to describe two different phenomenon regarding food products: first, it can mean that a raw product was processed in a way that increased its market

value, or it can mean that the product was differentiated through desirable production practices, such as environmental stewardship (e.g., organically grown, salmon-safe), social justice (e.g., fair-trade), or food safety or functionality (e.g., antibiotic-free), also increasing the market value of the final product (Stevenson and Pirog, 2013). When all actors across a supply chain (including input suppliers, producers, processors, distributors, wholesalers, retailers, and consumers) unite around adding value through both processing and differentiated practices, a “value-based supply chain”, or value chain, is formed (Stevenson and Pirog, 2013). In the case of proso millet, values such as climate resilience and diversity have the potential to unite actors across a value-based supply chain. Producers play a key role in the development of value chains and one way to involve them in this process is through participatory research activities.

## **5. PARTICIPATORY RESEARCH**

Participatory agricultural research approaches “engage people in a community in some or all aspects of the research process – determining research questions, developing technical solutions and approaches to obtain information, and deciding what the research means and how it should be used to benefit the community” (Lilja and Bellon, 2008). The concept of participatory research became popular in the 1980’s and 1990’s in opposition to the more traditional academic agricultural research pipeline, in which knowledge flowed unilaterally from university to extension specialist to producer with no mechanism for feedback on research methods and objectives from non-academic stakeholders (Van De Fliert and Braun, 2002; Pagliarino *et al.*, 2020). Researchers began to bring attention to the way this traditional model, which is geared toward large-scale, mechanized conventional agriculture systems with limited diversity and a high level of control via chemical inputs, was not meeting the needs of more diversified, highly variable systems, such as subsistence agricultural systems in developing countries and

agroecological systems in the United States and Europe (Cuéllar-Padilla and Calle-Collado, 2011; Pagliarino *et al.*, 2020). Critics argue that when traditional research questions are generated by academics and experiments are conducted in highly controlled experiments, the resulting solutions and technology are oversimplified and unable to account for the extreme variation in conditions from farm-to-farm, field-to-field, and season-to-season (Cuéllar-Padilla and Calle-Collado, 2011).

Even within large-scale, mechanized, monocultural systems that traditional agricultural research was developed for, uptake of agricultural technology from formal research can be stymied by producers' *inability* to adopt (due to factors such as high cost of technology, lack of access to information, high labor requirements, and limited support) or *unwillingness* to adopt (due to factors such as incompatibility of technology to a given production system or high risk of negative outcomes for producers) (Lilja and Bellon, 2008). However, when producers are incorporated in the research process, they can help anticipate and problem-solve these barriers to adoption and can disseminate information within their communities, through trusted relationships with their peers, more effectively than researchers (Hoffmann, Probst and Christinck, 2007). In general, participatory research can foster a more holistic exchange of information than traditional research, incorporating experts with scientific, cultural, local, and indigenous perspectives (Cuéllar-Padilla and Calle-Collado, 2011).

In spite of its shortcomings, traditional research does bring its own strengths. The narrow focus of academic research is integral to the goal of sustained, rational, objective inquiry, and to the function of the scientific method. Further, traditional research that supports industrial agriculture has led to increased availability and quality of food around the world (Dlott, Altieri and Masumoto, 1994; Pagliarino *et al.*, 2020). In order to incorporate the benefits of both

traditional and participatory approaches, research activities under New Grains Northwest use both, as depicted in the following chapters.

## **6. INTRODUCTION TO THESIS CHAPTERS**

Chapter Two discusses a 2022 field trial experiment designed to test agronomic, nutritional, and seed morphology phenotypes of seven proso millet varieties. A researcher-led trial was conducted in Pullman, WA in which four replicates of seven varieties (Dawn, Earlybird, Horizon, Huntsman, Plateau, Sunrise, and Sunup) were planted in a replicated complete block design. Agronomic data collected in the field included days to emergence, plant height, days to heading, and days to maturity. Seed was collected from each plot and samples were weighed to record yield. Additionally, five producer-led, unreplicated strip trials containing a subset of these seven varieties were planted using full-scale equipment on producer participants' fields in Edwall, WA, Mansfield, WA, and Genesee, ID. Seed samples from both the researcher-led and producer-led trials were analyzed for mineral content including zinc, iron, copper, manganese, magnesium, calcium, phosphorus, and potassium and seed morphology traits including thousand seed weight, seed area, seed eccentricity, and color.

Chapter Three summarizes key takeaways from interviews with producers who participated in on-farm, producer-run variety trials. All five producer participants completed pre- and post-season interviews about their experience with the crop. Themes regarding the benefits (resilience of the crop, rotation weed control, and increased opportunity for on-farm diversity) and challenges (timing and logistics of harvest, the lack of a reliable market, and insufficient infrastructure) are discussed. Additionally, details regarding the planting and management strategies of each grower are included.

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## CHAPTER TWO: AGRONOMIC, MINERAL, AND SEED MORPHOLOGY PHENOTYPES OF PROSO MILLET GROWN IN THE INLAND PACIFIC NORTHWEST

### **ABSTRACT**

Increasing cropping system diversity can create a more resilient food system. One crop that could add diversity to cropping systems in the inland Pacific Northwest is proso millet, a climate-resilient, small-seeded cereal crop that is highly water efficient, able to grow in low fertility soils, and has a desirable nutritional profile. Proso millet shows potential for adoption in this region due to its short growing season, compatibility with regional equipment, and environmental requirements, however US cultivars have been developed for the Great Plains and little research has been conducted outside of this region. To better understand the potential for adoption in the inland PNW, seven commercially available varieties were planted in a researcher-run trial in Pullman, WA and in a series of producer-run trials across the region in 2022. Samples were analyzed for agronomic phenotypes (grain yield, plant height, days to heading, days to maturity, and percent emergence), mineral concentrations (Zn, Fe, Cu, Mn, Mg, Ca, P, and K), and seed morphology phenotypes (seed area, seed eccentricity, thousand seed weight, and seed color). Varieties from the researcher-run trial showed significant differences for all traits excluding percent emergence and Ca concentration. Samples from producer-run trials were not analyzed for agronomic phenotypes but showed differences by location for all seed morphology phenotypes and for concentration of Zn, Fe, Cu, and Mn. Samples from producer-run trials showed no difference by variety for mineral concentration but showed varietal differences for all seed morphology phenotypes. Grain yield was correlated with plant height, seed area, and thousand seed weight and was negatively correlated with Zn. Most minerals were positively correlated with one another. Results from this study can help support future proso

millet breeding efforts, particularly in this region, and inform variety selection for stakeholders interested in adopting proso millet in the inland PNW.

## 1. INTRODUCTION

Climate change increases abiotic and biotic stressors that will challenge the resiliency of agricultural production systems around the world (Raza *et al.*, 2019). At the same time that these stressors are becoming more severe, our global population continues to rise, creating a mounting global demand for nutrient-dense calories (Noya *et al.*, 2018; Prosekov and Ivanova, 2018; Neupane *et al.*, 2022). In order to meet this demand in the wake of climate change, greater resiliency must be cultivated in industrial agricultural systems. One promising strategy to increase resiliency is by increasing crop diversity within these systems. Just three major cereal crops, wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and rice (*Oryza sativa* L.), are estimated to sustain 50% of the caloric demand of the world population (Neupane *et al.*, 2022). However, there are a number of alternative crops including millets, quinoa, buckwheat, and amaranth with promising nutritional profiles and climate resilient traits that have not been as thoroughly developed for agricultural intensification and are currently being underutilized in certain parts of the world (Kumar and Bhalothia, 2020). Limitations exist in these crops regarding yield potential, trait improvement, knowledge diffusion, and market buy-in, stymieing their potential for adoption (Bekkering and Tian, 2019). Furthermore, solutions to these limitations, including management strategies, breeding for crop improvement, and market development, are often dependent on the specific region where adoption is being considered.

As researchers endeavor to improve agronomic and end-use qualities of underresearched crops, they may find a range of motivations for varietal selection and breeding goals from different actors across the food system. Analysis of seed morphology and mineral

characterization, when considered in concert with agronomic phenotypes, can help inform variety selection for the whole food system, including processors and consumers, rather than narrowly focusing in on increased yields in the field.

The inland Pacific Northwest (PNW) is a semiarid region including Central Washington, Northeast Oregon, and Northern Idaho that is dominated by dryland cereal production (Yorgey and Kruger, 2017; Roesch-Mcnally, 2018). More specifically, rain-fed wheat-fallow cropping systems are pervasive across the landscape, with lesser quantities of other small grains, legumes, and canola incorporated into rotations (Roesch-Mcnally, 2018). The region is characterized by cold, wet winters and hot, dry summers, though average temperature and average precipitation (between 180 mm to 1130 mm) depend on elevation and local topography (Karimi *et al.*, 2018).

Considering the climactic characteristics of the region and existing cropping systems, one crop with potential to increase the diversity and resiliency of the inland PNW is proso millet (*Panicum miliacium* L.) (Habiyaemye, Matanguihan, *et al.*, 2017). Millets are small-seeded cereal crops that grow in semi-arid environments and have gained interest as climate resilient grains as they are highly water efficient, can grow on shallow, low fertile soils with a high range of salinity and acidity, and are C4 crops meaning that they take up more carbon dioxide from the environment than wheat and rice (Kumar and Bhalothia, 2020). Additionally, millets have a comparable nutritional profile to other major cereal grains, making them a promising crop for helping to improve food security in the wake of climate change (Kumar *et al.*, 2018). Millets are currently a staple food source for millions of people in arid and semiarid regions of India, Africa, and China, but in an effort to raise awareness and stimulate research and development of these grains in other parts of the world, the United Nations declared 2023 “International Year of the Millets” (Amadou, Gouna and Le, 2013; *International Year of Millets 2023*, 2023).



There are approximately 20 different species of millets grown around the world for food, feed, forage, and fuel, that vary greatly in plant and seed morphology (Das *et al.*, 2019). However, proso millet is the species of greatest interest for human consumption in the US (Myers, 2018). Production and development of proso millet varieties have been historically concentrated in the Central Great Plains of Nebraska, Colorado, and South Dakota, and despite desirable nutritional characteristics, have been largely siloed into the birdseed market (Das *et al.*, 2019). Fourteen cultivars of proso millet have been developed in the US since the 1960s, and the six most commonly cultivated varieties were developed at the University of Nebraska-Lincoln, which houses the only proso millet breeding program in the country (Rajput and Santra, 2016). North American cultivars have a narrow genetic base because of a limited number of parents in breeding (Rajput and Santra, 2016).

Agronomic qualities and environmental requirements of these Midwest varieties show potential compatibility with production systems in the inland PNW (Habiyaemye, Barth, *et al.*, 2017; Habiyaemye, Matanguihan, *et al.*, 2017; Das *et al.*, 2019). Proso millet is compatible with the winter wheat rotations characteristic of the region, as it is typically planted in late May or early June, and with a short growing season of 60-100 days can be used either as a replacement for summer fallow or as an emergency crop if an earlier seeded crop were to fail (Lyon *et al.*, 2014; Ventura *et al.*, 2020). Proso millet has been shown to benefit these rotations and increase winter wheat yields by controlling winter annual grassy weeds, reducing insect and disease pressures, and preserving soil moisture (Santra, 2013). It also fills a unique niche for producers in the region looking for ways to diversify their rotations, as there are not currently any other warm season grasses commonly cultivated in the area. Finally, proso millet is well-adapted to the

rained, dryland cropping systems characteristic of the region, as well as its well-drained loamy soils (Habiyaemye, Matanguihan, *et al.*, 2017).

In this study we evaluated agronomic, nutritional, and seed morphology phenotypes of seven proso millet varieties grown in the inland Pacific Northwest. Varieties included ‘Dawn’, ‘Earlybird’, ‘Horizon’, ‘Huntsman’, ‘Plateau’, ‘Sunrise’, and ‘Sunup’. The overall goal of the study was to assess agronomic, nutritional, and seed morphology traits of commercially available proso millet varieties grown in the inland Pacific Northwest to better understand their potential for adoption into the regional food system. Specific objectives were to: 1) compare yield, plant height, days to heading, days to maturity, and percent emergence of each variety in this environment; 2) evaluate differences in zinc, iron, copper, manganese, magnesium, calcium, and phosphorus concentration in each variety; and 3) compare the area, eccentricity, color, and thousand seed weight of seed from each variety.

## **2. MATERIALS AND METHODS**

### **2.1 Location**

#### *2.1.1 Researcher-led Trial*

Samples for all research activities were collected from a single-year, researcher-run trial conducted in 2022 at Spillman Agronomy Farm in Pullman, WA (46.69743 °N Lat., -117.14720 °W Long.). Meteorological data were obtained from Pullman meteorological station located at 46.7 N Lat., -117.15 W Long, and elevation 760 m. Pullman received a total of 522 mm of total precipitation in 2022 and the average temperature was 8.3 °C (*WSU AgWeatherNet*, 2023) (Table 1.1). The growing season was preceded by an uncharacteristically cold and wet spring, recorded at Washington state’s third coldest June on record and above average precipitation in April, May,

and June 2022 (NCEI, 2022). In contrast, Washington also experienced its hottest average temperature for the month of August in the same growing season of 2022 (NCEI, 2022).

### *2.1.2 Producer-led Trial*

Samples for seed morphology and mineral characteristics were collected from both the researcher-led trial in Pullman, WA, and a series of on-farm, producer-led trials across the region (Table 2.3). One site was located in Edwall, WA (47.44474, -117.887), one in Mansfield, WA (47.91997, -119.795), and three in Genessee, ID (46.62175, -116.895; 46.56439, -116.831; 46.50229, -116.811). Elevation ranged from 713 m to 866 m and annual precipitation ranged from 6 to 22 in.

## **2.2 Plant materials**

The 2022 trial at Spillman farm in Pullman, WA included seven test varieties, all of which were commercially available (Table 2.2). Dawn (Nelson, 1976), Earlybird (Baltensperger, Nelson and Frickel, 1995), Horizon (Baltensperger *et al.*, 2004), Huntsman (Baltensperger *et al.*, 1995), Plateau (Santra *et al.*, 2015), and Sunrise (Baltensperger *et al.*, 1997) were sourced from Kriesel Seen Inc. in Gurley, Nebraska, and Sunup (Nelson, 1990) was sourced from Perry Brothers Seed Inc. in Otis, Colorado. Dawn, Earlybird, Huntsman, Sunup, and Plateau were all developed by the Nebraska Agricultural Experiment Station (Lyon *et al.*, 2014). Horizon was developed by the Nebraska Agricultural Experiment Station in cooperation with the University of Wyoming, South Dakota State University, and the USDA-ARS Central Great Plains Research Station (Baltensperger *et al.*, 2004). Sunrise was released jointly by the Institute of Agriculture and Natural Resources, University of Nebraska, and USDA-ARS (Baltensperger *et al.*, 1997). Dawn is the earliest developed variety, released in 1975 and originally introduced as an experimental line from the Soviet Union, and is one of the parents of most varieties in the study

(Nelson, 1976; Lyon *et al.*, 2014). Sunup was released in 1989 and the leading variety at the time. Earlybird an early-maturing, short-stature variety, and Huntsman, a high-yielding late-maturing, tall variety, were both released in 1994, followed shortly thereafter by Sunrise, a large-seeded, high-yield variety released in 1995. Horizon was developed as an earlier-maturing, short stature variety in 2003. Most recently, Plateau, a cross between Huntsman and a Chinese waxy accession, was developed in 2014 for applications in food and industrial use (Lyon *et al.*, 2014; Santra *et al.*, 2015). Commercially available varieties were selected as they are accessible to growers who may be interested in adoption, but their performance has not been thoroughly assessed in the inland PNW environment.

## **2.3 Experimental Design and Data Collection**

### *2.3.1 Experimental Design: Researcher-led trial*

Varieties were planted in a randomized complete-block design with four replicated and blocks arranged in a grid format. Each plot was 7.4 m<sup>2</sup> (80 ft<sup>2</sup>) split into 4 rows with 25.4 cm (10 in) between rows and one 76.2 cm (30 in) alley. Each row contained 2.4 g of seed to approximately represent a seeding rate of 19.1 kg/ha (17 lb/acre).

Percent emergence was estimated visually for each plot based on expected density of plants in each row. Heading was quantified by the number of days from planting to 50% heading. Plant length of five randomly selected individuals was measured from the base of the stem to the end of the panicle and mean length was recorded (102 days after planting in 2022). Maturity was determined when approximately 75% of plants had dry panicles and “ripe” seed (grain hard, difficult to divide with thumbnail) (Ventura *et al.*, 2020).

Plots were harvested by hand, cut with sickles at the base of the stem, bundled, and placed in a greenhouse for between three days and two weeks to facilitate drying of material.

Plots were harvested over the course of twelve days based on maturity. Dried bundles from each plot were threshed using a Vogel thresher (Bill's Welding, Pullman, WA, USA). Each sample of threshed seed was run through a 2021 Wintersteiger Classic Plus Plot Combine and tabletop sifter (Clipper Separation Technologies, Office Tester Seed Cleaner) to remove excess plant matter and other debris before yield weight was recorded.

### *2.3.2 Experimental Design: Producer-led trials*

On-farm, producer-led trials were planted in unreplicated, side-by-side strips using full-scale planting equipment belonging to the producer. Producers were given the opportunity to plant between three to seven varieties depending on their capacity and were provided with 50 pounds of seed per variety. Varieties were randomly assigned to each producer participant, except Huntsman, which was included at each site as a control (Table 2.4). The order in which varieties were planted was also randomly assigned by the research team. While researchers provided instruction on trial layout, all other planting and management decisions throughout the season were made by producer participants.

When each trial reached maturity, researchers conducted a site visit and collected subsamples from each variety strip. Strips were walked from one end to the other and a 1 m<sup>2</sup> quadrat was used to hand-harvest five subsamples distributed evenly throughout the strip. Subsamples were cut with sickles at the base of the stem, bundled, and placed in a greenhouse for between three days and two weeks to facilitate drying of material. Dried bundles from each plot were threshed using a Vogel thresher (Bill's Welding, Pullman, WA, USA). Each sample of threshed seed was run through a 2021 Wintersteiger Classic Plus Plot Combine and tabletop sifter (Clipper Separation Technologies, Office Tester Seed Cleaner) to remove excess plant

matter and other debris. Further processing was conducted on subsamples for seed scanning and MP analysis.

### *2.3.3 Mineral Phenotypes*

Subsamples of seed from each plot were collected and then further processed for nutritional analysis. The hull was removed from whole seed using a household rice polishing machine (Takumuajiami White MB-RC52, Michiba Kitchen, Yamamoto Electric, Fukushima, Japan) which separates seed from hull through the abrasion of a spinning, stainless-steel mesh basket. Hulls and broken seeds were then separated from samples using a series of stainless-steel sieves, and seeds that were not successfully dehulled through polishing were manually removed from each sample. Dehulled samples were then milled down into flour using a IKA A 10 Basic Mill (IKA Works Inc., Wilmington, NC, USA).

Analysis of flour from each sample was then conducted using an Agilent MP-AES 4200 (Agilent Technologies, Santa Clara, CA, USA) equipped with a double pass glass cyclonic spray chamber, OneNeb V2 Nebulizer, and an SPS-3 autosampler (Agilent Technologies, Santa Clara, CA, USA) (Braden, personal comm.). For each sample, 250 gm ( $\pm$  5 mg) of flour was added to a 75mL PTFE digestion vessel containing 2mL DIW, and an additional 10 mL of DIW and 2 mL of HNO<sub>3</sub> were added. Vessels were then capped and vortexed for one minute in order to mix flour and acid, before an additional 2 mL of H<sub>2</sub>O<sub>2</sub> was added. Caps were removed, and samples were pre-digested for 15 minutes. The Mars6 Xpress Microwave System (CEM Corporation, Matthews, NC, USA) with 40 PTFE vessel holders was used to digest each sample.

### *2.3.4 Seed Morphology Phenotypes*

Subsamples for seed scanning were dehulled using a Tangential Abrasive Dehulling Device (Saskatoon, Sask., Canada).

Seed morphology data was generated with a system of flatbed scanners, using methods developed for comparable morphology analysis of quinoa (Craine *et al.*, 2023). Two 1-2 g subsamples of clean seed were collected from each plot of the trial and distributed across the glass surface of a scanner and covered with a black background. Scanners then captured an 8-bit red, green, and blue (RGB) image at a resolution of 1,200 dots per inch (dpi) for each sample. These images were analyzed using the All Grains tool from the phytoMorph Image Phenomics Toolkit. This tool, developed by (Moore *et al.*, 2013), generated average seed area, major axis (length), minor axis (width), and eccentricity (length:width ratio). The tool also generated a count of individual seeds in each image, using an approach originally developed for the analysis of maize kernels (Miller *et al.*, 2017). The tool produced average values for the intensity of red, green, and blue (i.e. RGB) of each pixel within each seed (Craine *et al.*, 2023). RGB decimal codes were generated by multiplying intensity averages by 255, creating a quantitative value corresponding to a specific color within the RGB color model. Principal Component Analysis was performed on RGB color space to reduce the three values to two latent factors. Thousand seed weight (TSW) was calculated with the weight of each sample divided by the algorithmically-counted seed number, multiplied by 1,000.

## **2.4 Statistical Analysis**

Statistical analyses were conducted using the R statistical software (R Core Team, 2023).

Levene's test was conducted separately for researcher-run trial data and producer-run trial data for all outcomes. Analysis of variance (ANOVA) was conducted with function 'aov' to determine if any of the given outcomes (agronomic, mineral, and morphological phenotypes) differed by variety for both trial groups.

Based on results from Levene's test and ANOVA's, further analysis was only conducted for researcher-run trial data. Effect size was calculated with function 'etaSquared' from the 'lsr' package. The package 'LSD.test' from R package 'agricolae' was used to produce means, coefficient of variation (C.V.), and least significant difference (LSD) values for each outcome. Finally, Pearson correlation analysis was performed with functions 'cor' and 'cor.test' to assess the relationship between all agronomic, mineral, and morphological traits for researcher-run trial samples. Statistical significant level was set at  $\alpha = 0.05$ .

It should be noted that there may be discrepancies in significance indicated by groupings and LSD values. LSD calculations require an even dataset and groupings do not. Therefore, varieties with missing data points (uneven data sets) were eliminated for LSD calculations but were included for grouping calculations. Both are provided for reference.

### **3. RESULTS AND DISCUSSION**

#### **3.1 Agronomic Phenotypes**

##### *3.1.1 Grain Yield*

There was a significant difference in grain yield by variety (Table 2.5). Sunup yielded more than Dawn and Plateau (Table 2.6). Plateau also yielded less than Huntsman, Sunrise, and Earlybird. Mean grain yield was 653 g/m<sup>2</sup> with a least significant difference of 110. However, since LSD requires a balanced data set for calculation, and one of four samples of Dawn was missing, LSD was calculated with all Dawn samples excluded.

Sunup was high yielding in our study, which was unexpected as Huntsman, Earlybird, Horizon, and Sunrise were all bred as high-yielding replacements for this older variety (Lyon *et al.*, 2014). In a meta-analysis of dryland proso millet variety trials from Sidney, NB, Akron, CO, and Lingle, WY between 2002 and 2013, Sunup yielded more than Dawn on average, which is



consistent with our results (Santra *et al.*, 2015). However, this same analysis showed Plateau yielding more than Sunup, contrary to our results. A 2017 proso millet trial in Musanze, Rwanda showed Sunup as lower yielding than Huntsman but higher yielding than Earlybird (Habiyaremye *et al.*, 2022). Habiyaremye *et al.* (2017), conducted an irrigated proso millet trial in Pullman, WA. Results cannot be directly compared as this study did not include Plateau or Dawn, but it showed Sunup as yielding more than Huntsman and Sunrise in 2012, more than Sunrise in 2013, and less than Huntsman and Sunrise in 2014 (Habiyaremye, Barth, *et al.*, 2017).

### 3.1.2 Plant Height

There was a significant difference in plant height by variety (Table 2.5). Sunup was taller than Dawn and Plateau (Table 2.6). Huntsman and Sunrise were also taller than Plateau.

Sunup and Huntsman were released after Dawn with one marketable phenotype being greater height that increases potential for direct harvest using a combine equipped with a stripper-header (Lyon *et al.*, 2014). However, too much height, greater than 150 cm according to Zhang *et al.* (2019), can increase susceptibility to lodging. Mean plant height in the 2022 Pullman, WA trial was 123 cm and maximum plant height across varieties was 140 cm (Sunup), which did not exceed this upper limit (Zhang *et al.*, 2019). Consistent with the findings in our study, Santra *et al.* (2015) found Sunup and Huntsman to be taller than Plateau in dryland trials in NB, CO, and WY.

A 2007 study of the world's core collection of proso millet showed a range in mean plant height from 33-92 cm (Reddy, Upadhyaya and Gowda, 2007), which is lower than the range of means in our study (112-132 cm), however a later core collection study found mean plant height ranging from 64-175 cm which includes the range of our results (Zhang *et al.*, 2019).

### *3.1.3 Days to Heading*

There was a significant difference in days to heading by variety (Table 2.5). Plateau headed earlier than all other varieties (Table 2.6). Dawn headed earlier than Huntsman.

Plateau did not form heads in significantly less day than other varieties in Midwest trials (Santra *et al.*, 2015). If proso millet were being grown as a forage crop, it would need to be harvested soon after heading to optimize forage quality (Lyon *et al.*, 2014). Analysis of the global proso millet germplasm collection found a high Shannon-Weaver diversity index (H') value for days to 50% flowering, indicating opportunity for breeding for fewer days to heading (Vetriventhan *et al.*, 2019).

### *3.1.4 Days to Maturity*

There was a significant difference in days to maturity by variety (Table 2.5). Dawn matured more quickly than Sunup, Huntsman, Sunrise, and Earlybird (Table 2.6). Plateau and Horizon also matured more quickly than Sunup.

Early maturity is a desirable trait for producers in the inland PNW who need to harvest their crop before the rainy fall season begins. The 2022 researcher-run trial was harvested on a plot-by-plot basis as they reached maturity, however all remaining plots had to be harvested on day 113 after planting, regardless of if full maturity had been reached. These samples (n=5) were logged as DM=114, which could have slightly altered means by variety for DM. However, proso millet is frequently swathed or chemically desiccated before full maturity is reached in order to expedite harvest, which was simulated in early harvest of these five samples (Lyon *et al.*, 2014).

### *3.1.5 Percent Emergence*

There was no significant difference in percent emergence by variety (Table 2.5). Effect size calculation suggests that 34% of percent emergence can be explained by variety, which is

lower than effect size for other agronomic outcomes in the study, but still very large (Cohen's  $f = 0.71$ ) (Kotrlík, Williams and Jabor, 2011) (Table 2.6). Sample size may have been too small to produce significant results at this effect size. Further research with a larger sample size could be conducted to clarify results.

## **3.2 Mineral Concentration**

### *3.2.1 Researcher-run trial*

Significant differences were found for all minerals by variety, excluding calcium (Table 2.7). Plateau had a high concentration of every element, and was higher than all other varieties for Zn, Mn, and K (Table 2.8). It was higher than all but one other variety for Cu and P. Plateau is the only test variety that was developed for waxy starch end-use quality, bred using a waxy Chinese accession as a parent, potentially factoring into its standout characteristics (Lyon *et al.*, 2014; Santra *et al.*, 2015).

Macronutrients, such as K, Ca, P, and Mg, and micronutrients such as Zn, Cu, Fe, and Mn all serve important roles in human nutrition. While deficiency of macronutrients can result in hunger, wasting, and stunted growth, micronutrient deficiencies have less detectable physical manifestations, and are therefore easier to overlook (Kumar *et al.*, 2022). Approximately three billion people worldwide suffer from micronutrient deficiencies (Chasapis *et al.*, 2020). Studies have been more frequently conducted on the macronutrients and micronutrients of pearl millet than proso millet, but some studies compare proso millet mineral concentration with other millets and other grains (Demirbas, 2005; Anuradha *et al.*, 2018; Vali Pasha *et al.*, 2018; Kumar *et al.*, 2022; PA *et al.*, 2023). In a comparison of foxtail millet, little millet, barnyard millet, kodo millet, finger millet, and sorghum, with three Indian cultivars of proso millet, foxtail and barnyard millet had less Fe than proso millet while little millet and barnyard millet had higher Zn

(Vali Pasha *et al.*, 2018). Finger millet and kodo had higher Mn than proso millet and all millets excluding kodo had more P than proso millet (Vali Pasha *et al.*, 2018). All small millets, including proso millet, had higher Zn, Fe, K, Mn, Mg and Cu than sorghum (Vali Pasha *et al.*, 2018). Mean mineral concentration for proso millet in this study was higher for all tested elements (Ca, P, K, Mg, Fe, Cu, Zn, Mn) than varieties in our study.

Proso millet has been shown to have higher concentrations of Mg, Fe, Mn, and Zn than rice, comparable levels of P, Mg, Fe, and Zn to maize, and lower levels of Ca, P, Fe, Mn, and Zn than wheat (Devi *et al.*, 2014; Kumar *et al.*, 2018). However, a study of Turkish cereal grains shows proso millet as also having a higher concentration of Ca and P than spring and winter wheat (Demirbas, 2005).

While different cultivars of a crop can vary in their macronutrient and micronutrient profiles, mineral concentration in crops has been shown to be linked to soil organic matter and management practices (Kwiatkowski *et al.*, 2015; Moharana, Sharma and Biswas, 2017) A Polish study comparing mineral concentration of proso millet in conventional and organic systems showed higher concentration of Cu, Mn, Fe, and Zn in proso millet produced organically, suggesting that production practices may influence mineral content for these elements regardless of variety (Kwiatkowski *et al.*, 2015). Mean results from conventional samples from this study, which used a proso millet variety ‘Jagna’, fell within variety averages from our study for Mg, Cu, and Zn while mean results were higher than variety averages from our study for Ca, Mn, and Fe (Kwiatkowski *et al.*, 2015).

### 3.2.2 Producer-run trials

Levene’s test was conducted on producer-run trial data for each mineral. Results suggested equal variance between groups, however one-way ANOVA with variety as the

independent variable did not produce significant results for any elements within this data set. Two-way ANOVA was also conducted, with variety and location as independent variables. Location had a significant effect on Zn ( $p < 0.01$ ), Fe ( $p < 0.001$ ), Cu ( $p < 0.05$ ), and Mn ( $p < 0.01$ ) (Table 2.9) (Table 2.10). However, as each trial look place at a different location, contained a different subset of varieties, and received different treatments (such as fertilizer application), we are unable to isolate what variable within ‘location’ led to significant differences in these elements. ANOVA was also conducted for the interaction of trial and variety but did not produce significant results for any elements.

### **3.3 Seed Morphology Phenotypes**

#### *3.3.1 Researcher-run trial*

There was a significant difference in seed area by variety (Table 2.11). Dawn was larger than all varieties except for Sunrise and Plateau was the smallest variety (Table 2.12). There was a significant difference in seed eccentricity by variety (Table 2.11). The length:width ratio of Plateau was furthest from 1, indicating that it was the least round (Table 2.12). There was a significant difference in thousand seed weight by variety (Table 2.11). Plateau weighed less than all other varieties, coinciding with its smaller area (Table 2.12). Varietal means for TSW ranged from 4.17- 5.15 g.

An analysis of the proso millet world core collection found a range in TSW from 3.9-6.6 g (Vetriventhan *et al.*, 2019). Compared to other grains planted in the region, proso millet is more similar in weight to canola, which typically ranges from 2 to 6 mg seed<sup>-1</sup>, what wheat seed planted in the inland Pacific Northwest, which typically ranges from 31 to 38 mg seed<sup>-1</sup> (Pan *et al.*, 2016).

There was a significant difference in seed color by variety (Table 2.11) (Figure 2.1). Principle component analysis was performed to compare red, green, and blue values with a single value. PCA was performed with both researcher-led and producer-led sample values. Plateau has a higher PC value, indicating a lighter color. Sunup had the lowest PC value, indicating darker color than the other varieties.

All varieties in our study were “white” proso millet cultivars, ranging in shades of straw or light brown, however world core collections are also made up of accessions with light red, dark olive green, dark red, olive green, dark brown, dark green, brown, and black (Reddy, Upadhyaya and Gowda, 2007; Vetriventhan *et al.*, 2019). One study showed about 80% of accessions to be light brown, straw, or white (Reddy, Upadhyaya and Gowda, 2007) while a later study showed about 50% of *miliaceum* accessions to be in this group (Vetriventhan *et al.*, 2019).

### 3.3.5 Producer-run trials

Differences were found for seed area, seed eccentricity, thousand seed weight, and seed color by location and by variety in the producer-run trials when two-way anova was run on producer-run trial data (Table 2.13) (Table 2.14) (Figure 2.3). A one-way anova did not show differences in these traits for the producer-run trial. Sample size was low, as each location planted a single replicate of a subset of varieties (Table 2.4). Figures 2.2 and 2.4 show grouping by location and seed size. Each of these locations received different treatments (environmental conditions, planting and harvest date, fertilizer, chemical desiccation etc.), so differences cannot be attributed to any specific treatment, but do suggest that traits can be affected by treatment.

### 3.4 Correlation

#### 3.4.1 Agronomic phenotypes

Grain yield and plant height were highly correlated ( $r = 0.62$ ,  $p < 0.001$ ) (Table 2.15). Days to heading and days to maturity were also highly correlated ( $r = 0.55$ ,  $p < 0.01$ ). Percent emergence and days to maturity were moderately negatively correlated ( $r = -0.40$ ,  $p < 0.05$ ), suggesting that the best emergers were also quickest to mature, which is desirable as producers are looking for early-maturing varieties and good emergence.

Other studies of proso millet similarly found a positive correlation between grain yield and plant height, suggesting that plant height can be used for simple selection (Salini *et al.*, 2010; Calamai *et al.*, 2020; Boukail *et al.*, 2021). Plant height has also been associated with rate of maturity, but we did not see this in our study (Boukail *et al.*, 2021). A study of pearl millet also found positive correlation for grain yield with plant height and thousand seed weight, which we saw in our study (Anuradha *et al.*, 2018). Risk of lodging should be taken into consideration when selecting for plants with greater height.

#### 3.4.2 Mineral concentration

Many minerals show high correlations. Zn is most highly correlated with Cu ( $r = 0.80$ ,  $p < 0.001$ ) and K ( $r = 0.82$ ,  $p < 0.001$ ). Fe is most highly correlated with Cu ( $r = 0.75$ ,  $p < 0.001$ ), Mg ( $r = 0.67$ ,  $p < 0.001$ ), and P ( $r = 0.74$ ,  $p < 0.001$ ). In addition to Fe, Cu is most highly correlated with Mg ( $r = 0.72$ ,  $p < 0.001$ ), P ( $r = 0.77$ ,  $p < 0.001$ ), and K ( $r = 0.74$ ,  $p < 0.001$ ). Mn is most highly correlated with Mg ( $r = 0.74$ ,  $p < 0.001$ ), P ( $r = 0.77$ ,  $p < 0.001$ ), and K ( $r = 0.73$ ,  $p < 0.001$ ). In addition to Fe and Mn, Mg is highly correlated with K ( $r = 0.78$ ,  $p < 0.001$ ) and extremely highly correlated with P ( $r = 0.91$ ,  $p < 0.001$ ). In addition to Fe, Cu, Mn, and Mg, P is very highly correlated with K ( $r = 0.8$ ,  $p < 0.001$ ). We didn't see negative correlation of any minerals and all

correlations were greater than  $r = 0.38$ , suggesting that breeders can seek to increase mineral concentration of select minerals without hampering concentration of others.

Zn and Fe were shown to be strongly positively correlated in studies of hard winter wheat, and were correlated in our study ( $r = 0.52$ ,  $p < 0.01$ ) (Morgounov *et al.*, 2007; Guttieri *et al.*, 2015). Zn and Fe were also highly correlated in two studies of pearl millet, (Anuradha *et al.*, 2018; Kumar *et al.*, 2020). One of these studies also showed strong correlation with Zn and Cu in pearl millet, which we also observed in our study (Kumar *et al.*, 2020). Our study is consistent with the later of these two pearl millet studies, which showed a significant positive correlation between Fe and Cu, while the earlier study did not (Anuradha *et al.*, 2018; Kumar *et al.*, 2020). In one study of hard winter wheat, correlation of phosphorus was  $>0.05$  with Mg, K, Fe, and Zn (Guttieri *et al.*, 2015). This is consistent with our results. Strong positive correlations of Zn, Fe, and Cu, observed in our study and in the studies of other grains, reflect the underlying physiology that links the accumulation of Zn, Fe, and Cu in grain (Guttieri *et al.*, 2015).

#### *3.4.3 Seed morphology*

Seed color is highly correlated with seed eccentricity ( $r = 0.67$ ,  $p < 0.001$ ). Seed eccentricity is negatively correlated with seed area ( $r = -0.53$ ,  $p < 0.001$ ) and thousand seed weight ( $r = -0.48$ ,  $p < 0.001$ ), indicating that larger seeds are more round. As expected, seed area is extremely highly correlated with thousand seed weight ( $r = 0.98$ ,  $p < 0.001$ ), indicating that larger seeds weigh more.

#### *3.4.4 Correlations across agronomic phenotypes, mineral concentration, and seed morphology*

No agronomic and seed morphology traits were correlated at a rate higher than  $r = 0.57$ , however plant height showed correlation with seed area ( $r = 0.57$ ,  $p < 0.01$ ), thousand seed weight



( $r = 0.51$ ,  $p < 0.01$ ), and negative correlation with seed color ( $r = -0.51$ ,  $p < 0.01$ ). Grain yield was negatively correlated with seed eccentricity ( $r = -0.54$ ,  $p < 0.01$ ). Grain yield was moderately positively correlated with TSW ( $r = 0.45$ ,  $p < 0.05$ ) and seed area ( $r = 0.45$ ,  $p < 0.05$ )

In many crops, associations have been shown with traits important in emergence (such as seed germination and seedling vigor) and size, density, or weight of seeds (Lawan *et al.*, 1985), however, we did not observe association between these traits. A 2014 study showed wheat with large seed size associated with more promising agronomic performance than wheat with small seed size, which is consistent with our results (Shahwani *et al.*, 2014). This same study showed wheat emerged from larger seeds also resulted in taller plants, which is also consistent with our results, and that wheat sown from larger seed resulted in higher yield (Shahwani *et al.*, 2014). This final association could be assumed from the results of our study, although sown seeds were not measured or included in our correlation.

Several agronomic traits were correlated with mineral content. Zinc was negatively correlated with plant height ( $r = -0.67$ ,  $p < 0.01$ ), days to heading ( $r = -0.57$ ,  $p < 0.01$ ), and grain yield ( $r = -0.55$ ,  $p < 0.01$ ). Days to heading was also negatively correlated with potassium ( $r = -0.64$ ,  $p < 0.01$ ), copper ( $r = -0.53$ ,  $p < 0.01$ ), and manganese ( $r = -0.52$ ,  $p < 0.01$ ).

A study of pearl millet found positive correlation of grain yield with Cu and Mn, and found genotypic, but not phenotypic, correlation with Fe (Anuradha *et al.*, 2018). Grain yield was not correlated with Cu and Mn in our study.

Studies on mineral concentration of wheat have shown negative correlation between grain yield and Zn, which we observed in this proso millet study (Garvin, Welch and Finley, 2006; Fan *et al.*, 2008; Guttieri *et al.*, 2015; S. Wang *et al.*, 2018). However, decreasing trends in concentration of Cu, Fe, and Mg have also been observed in high yielding varieties, which we

did not observe in our study (Fan *et al.*, 2008; Guttieri *et al.*, 2015). Lower mineral varieties in wheat also correspond to release date, dropping off significantly in the late 1960s when semi-dwarf, high yielding varieties were introduced, which has been attributed to breeders targeting grain yield without accounting for mineral content (Garvin, Welch and Finley, 2006; Fan *et al.*, 2008). Proso millet breeding in the US has been limited compared to wheat, so there is an opportunity to consider mineral concentration in the development of new varieties.

Days to heading was negatively correlated with all minerals besides Fe and Ca, indicating that quicker-developing varieties had higher mineral concentration. However, 2018 study of pearl millet found contradictory results, where Zn, P, Cu, and Mn were positively associated with days to 50% flowering (Anuradha *et al.*, 2018). Another study of proso millet found no association between days to 50% flowering and Zn, Cu, or Mn (Kumar *et al.*, 2022). A study of common wheat also found no association between Zn and days to heading (Morgounov *et al.*, 2007).

Seed morphology traits showed both negative and positive correlations with mineral content. Zinc and potassium showed the strongest correlations across seed traits. All minerals besides Ca and Fe showed some degree of negative correlation with seed area and thousand seed weight, indicating that larger seed samples have lower mineral concentration. Strongest negative correlations with seed area include P ( $r = -0.84$ ,  $p < 0.001$ ) and Zn ( $r = -0.7$ ,  $p < 0.001$ ). Thousand seed weight, which coincides with seed area, showed strong correlation with P ( $r = -0.82$ ,  $p < 0.001$ ), Zn ( $r = -0.64$ ,  $p < 0.001$ ), and Mn ( $r = -0.53$ ,  $p < 0.001$ ). This negative correlation could be attributed to the ‘dilution effect’, where mineral concentration decreases with grain size due to an increase only in the endosperm of the grain and not the bran or germ which contains most minerals (Murphy *et al.*, 2009). A study of mineral concentration in perennial and annual wheat

cultivars showed a negative association with TSW and Ca, Cu, and Zn when both perennial and annual cultivars were analyzed, but found no correlation when just perennial lines were analyzed, suggesting that dilution effect may not apply across grains (Murphy *et al.*, 2009). However, our study provides evidence supporting dilution effect in proso millet.

Several minerals were positively correlated to some degree with seed color, suggesting that higher mineral concentrations were associated with lighter seed color. Seed color was most strongly correlated with zinc ( $r = 0.64$ ,  $p < 0.001$ ) and potassium ( $r = 0.59$ ,  $p < 0.01$ ), and calcium ( $r = 0.51$ ,  $p < 0.01$ ). Seed eccentricity was most highly correlated with zinc ( $r = 0.59$ ,  $p < 0.01$ ) and potassium ( $r = 0.51$ ,  $p < 0.01$ ).

#### **4. CONCLUSION**

From this study, commercially available proso millet varieties grown in the inland PNW appear distinct in their agronomic phenotypes, mineral concentrations, and seed morphology phenotypes. Varieties from the researcher-run trial showed significant differences for all traits excluding percent emergence and Ca concentration. However, no specific variety excelled across the board. Unfortunately, one of the varieties with highest mineral concentration, Plateau, was also one of the lowest yielding. Mineral concentration has been historically overlooked by plant breeders, and since there has been limited breeding for proso millet there is an opportunity to incorporate this into breeding goals, however breeders will have to work against dilution effect when looking toward high-yielding, high-mineral cultivars. As most minerals were positively correlated with one another, working toward increasing specific minerals in breeding may help to improve overall mineral richness of a cultivar.

Seed morphology has been shown to be associated with germination physiology, nutrient quality, and yield, and can be easily targeted by breeders as they are less impacted by the

environment (Boukail *et al.*, 2021). We saw this association in our study, in a positive correlation of grain yield with seed area and thousand seed weight. However, environment and different treatments did seem to effect seed morphology phenotypes, as we saw varietal differences in seed area, seed eccentricity, thousand seed weight, and seed color by location. Further investigation is required to explain these results, as producer-run trials had a small sample size and high variability in treatments.

Proso millet core collections have high phenotypic and molecular diversity, which means there is a lot of potential for crop improvement in future breeding programs (Zhang *et al.*, 2019; Boukail *et al.*, 2021). Breeding efforts in the US have been limited and those that have occurred have taken place exclusively in Central Great Plains region of the US. While commercially available varieties were successfully grown out in this region in the 2022 season, further research is required to home in on breeding goals specific to cropping systems in the inland PNW, as well as end-use qualities desired by processors and consumers in the regional food system.

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**Table 2.1** Total precipitation (TP) and average maximum day temperature (MT) recorded during the growing season (May to September 2022) in Pullman, WA.

| <b>Year</b> | <b>Month</b> | <b>TP [mm]</b> | <b>AT [°C]</b> |
|-------------|--------------|----------------|----------------|
| 2022        | June         | 93.98          | 20.5           |
|             | July         | 10.92          | 28.4           |
|             | August       | 0.51           | 31.2           |
|             | September    | 36.32          | 24.8           |

Meteorological data were collected from Pullman, WA meteorological station situated at 46.7°N Lat., -117.15°W Long., and elevation 759.86m. Source: WSU AgWeatherNet, 2023.

**Table 2.2** Proso millet variety used for variety trials to evaluate agronomic, mineral, and seed morphology phenotypes when grown in the inland Pacific Northwest in 2022.

| <b>Entry Name</b> | <b>Seed Source</b> | <b>Release Date</b> | <b>Developer</b>                       | <b>Pedigree</b>  | <b>Marketed Traits</b>   |
|-------------------|--------------------|---------------------|--|--|--|
| 'Dawn'            | KS                 | 1976                | NAES                                   | Initially introduced as PI 260053 from the USSR  | Early maturing<br>Short<br>Moderate yield<br>Large seed size<br>Compact panicle type |
| 'Earlybird'       | KS                 | 1993                | NAES                                   | Selected from the cross 'Minco'/NE76010//RiseV NE 79017; NE76010 was a selection from 'Dawn'/Panhandle' and NE79017 was a selection from Dawn/NE76010  | Early maturing<br>Short<br>Good yield<br>Large seed size                             |
| 'Horizon'         | KS                 | 2003                | NAES<br>UoW<br>SDSU<br>CSU<br>USDA-ARS | Single-plant F4 selection from bulk population including 'Sunup', 'Rise', 'Dawn', 'Cope', and three lines later released as 'Earlybird', 'Sunrise', and 'Huntsman'   | Early maturing<br>Short  |
| 'Huntsman'        | KS                 | 1994                | NAES<br>USDA-ARS                       | Selected from the cross NE79012/NE79017/3/'Cope'// 'Dawn'/'Common'; NE79012 is a selection from a Dawn/NE76004 cross and NE79017 is a selection from the cross Dawn/NE76010. NE76004 is a selection from a Dawn/'Min 402' cross and NE76010 is a selection from Dawn/'Panhandle' | Late maturing<br>Tall<br>Excellent yield<br>Large seed size                          |

| <b>Entry Name</b> | <b>Seed Source</b> | <b>Release Date</b> | <b>Developer</b>      | <b>Pedigree</b>  | <b>Marketed Traits</b>  |
|-------------------|--------------------|---------------------|-----------------------|--|---|
| 'Plateau'         | KS                 | 2014                | NAES                  | Cross 'Huntsman'/PI 578074// PI 436626 (catalogued as 'Lung Shu #18' in Germplasm Research Institute of China)                           | Waxy starch<br>Medium height<br>Moderate yield<br>Good yield<br>Small seed size |
| 'Sunrise'         | KS                 | 1995                | IANR, UNARD, USDA-ARS | Selected from the cross NE83014/NE83007, and has the expanded pedigree 'Minn402V2*'Dawn'// 'Panhandle72*'Dawn/3/ 'Minco'//Dawn/Panhandle | Mid-season maturing<br>Good yield<br>Large seed size<br>Lodging tolerance       |
| 'Sunup'           | PB                 | 1989                | NAES                  | Increase of an F4 derived proso line from the cross 'Rise' X 'Dawn'  | Good yield (at time of release)<br>Small seed size                              |

KS: Kriesel Seed Inc. (Gurley, NB); PB: Perry Brothers Seed Inc. (Otis, CO). IANR: Institute of Agriculture and Natural Resources, NAES: Nebraska Agricultural Experiment Station; UNARD: University of Nebraska Agricultural Research Division, USDA-ARS: U.S. Department of Agriculture, Agricultural Research Service; UoW: University of Wyoming; SDSU: South Dakota State University; CSU: Colorado State University

**Table 2.3** Producer-run trial data including location (LOC), latitude (LAT), longitude (LONG), elevation (ELV in m), annual precipitation (AP) (in), planting date (PD), and harvest date (HD).

| <b>Trial</b> | <b>LOC</b>    | <b>LAT</b> | <b>LONG</b> | <b>ELV</b> | <b>AP</b> | <b>PL</b> | <b>HD</b>  |
|--------------|---------------|------------|-------------|------------|-----------|-----------|------------|
| 1            | Edwall, WA    | 47.44474   | -117.887    | 713        | 11        | 6/12/2022 | 10/4/2022  |
| 2            | Mansfield, WA | 47.91997   | -119.795    | 866        | 6-9       | 6/20/2022 | 9/27/2022  |
| 3            | Genesee, ID   | 46.62175   | -116.895    | 850        | 22        | 6/1/2022  | 10/15/2022 |
| 4            | Genesee, ID   | 46.56439   | -116.831    | 853        | 18-22     | 5/26/2022 | 9/28/2022  |
| 5            | Genesee, ID   | 46.50229   | -116.811    | 745        | 22        | 5/24/2022 | 9/13/2022  |

Elevation data source: USGS National Map Viewer, 2023

Annual precipitation data source: Self-reported from producer participants

**Table 2.4** Varieties included in 2022 producer-run trials.

| <b>Variety</b> | <b>Location 1</b> | <b>Location 2</b> | <b>Location 3</b> | <b>Location 4</b> | <b>Location 5</b> |
|----------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| Dawn           | x                 |                   | x                 |                   | x                 |
| Earlybird      | x                 |                   |                   | x                 |                   |
| Horizon        | x                 | x                 | x                 |                   |                   |
| Huntsman       | x                 | x                 |                   | x                 |                   |
| Plateau        | x                 |                   | x                 |                   | x                 |
| Sunrise        |                   | x                 |                   | x                 |                   |
| Sunup          |                   |                   | x                 |                   |                   |

**Table 2.5** Analysis of variance for grain yield (GY), plant height (PH), days to heading (DH), days to maturity (DM), and percent emergence (PE) for proso millet varieties grown in Pullman, WA in 2022.

|           | <b>Df</b>       | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F-Value</b> | <b>Pr(&gt;F)</b> | <b><math>\eta^2</math></b> | <b>Significance</b> |
|-----------|-----------------|---------------|----------------|----------------|------------------|----------------------------|---------------------|
| <b>GY</b> |                 |               |                |                |                  |                            |                     |
| Entries   | 6               | 111922        | 18654          | 3.198          | 0.0229           | 0.49                       | *                   |
| Residuals | 20 <sup>1</sup> | 116675        | 5834           |                |                  |                            |                     |
| <b>PH</b> |                 |               |                |                |                  |                            |                     |
| Entries   | 6               | 992.8         | 165.47         | 2.9            | 0.0321           | 0.45                       | *                   |
| Residuals | 21              | 1198.3        | 57.06          |                |                  |                            |                     |
| <b>DH</b> |                 |               |                |                |                  |                            |                     |
| Entries   | 6               | 240.4         | 40.07          | 4.573          | 0.00406          | 0.57                       | **                  |
| Residuals | 21              | 21            | 184.0          | 8.76           |                  |                            |                     |
| <b>DM</b> |                 |               |                |                |                  |                            |                     |
| Entries   | 6               | 207.4         | 34.56          | 3.845          | 0.0096           | 0.52                       | **                  |
| Residuals | 21              | 188.8         | 8.99           |                |                  |                            |                     |
| <b>PE</b> |                 |               |                |                |                  |                            |                     |
| Entries   | 6               | 2016          | 336.0          | 1.783          | 0.151            | 0.37                       |                     |
| Residuals | 21              | 3957          | 188.4          |                |                  |                            |                     |

Significant level at ( $p < 0.05$ ) while \* $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ .

<sup>1</sup> One observation deleted due to missingness (missing data for one observation of “Dawn”).



**Table 2.6** Mean data of proso millet varieties for grain yield (GY), plant height (PH), days to heading (DH), plant height (PH), and percent emergence (PE) from the 2022 researcher-run trial.

| <b>Variety</b> | <b>GY (g/m<sup>2</sup>)</b> | <b>PH (cm)</b> | <b>DH (days)</b> | <b>DM (days)</b> | <b>PE (%)</b> |
|----------------|-----------------------------|----------------|------------------|------------------|---------------|
| Sunup          | 749 (a)                     | 132 (a)        | 67 (ab)          | 114 (a)          | 70 (ab)       |
| Huntsman       | 685 (ab)                    | 127 (ab)       | 69 (a)           | 112 (ab)         | 65 (b)        |
| Sunrise        | 683 (ab)                    | 127 (ab)       | 67 (ab)          | 112 (ab)         | 86 (a)        |
| Earlybird      | 679 (ab)                    | 123 (abc)      | 67 (ab)          | 111 (abc)        | 90 (a)        |
| Horizon        | 638 (abc)                   | 121 (abc)      | 67 (ab)          | 108 (bcd)        | 85 (ab)       |
| Dawn           | 599 (bc)                    | 119 (bc)       | 64 (b)           | 106 (d)          | 82 (ab)       |
| Plateau        | 535 (c)                     | 112 (c)        | 59 (c)           | 107 (cd)         | 75 (ab)       |
| C.V. %         | 11                          | 6              | 5                | 3                | 17            |
| LSD (0.05)     | 110*                        | 11             | 4                | 4                | 21            |
| Mean           | 653                         | 123            | 66               | 110              | 79            |

LSD: Least Significant Difference; LSD comparisons are significant at the  $p < 0.05$  level.

\* LSD for GY excludes entries of ‘Dawn’ (n=3) due to one missing entry. All other varieties contain four entries (n=4) and LSD calculation requires an even dataset.

**Table 2.7** Analysis of variance for mineral concentration (mg/kg) for proso millet varieties grown in researcher-run trial in Pullman, WA in 2022.

|           | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F-Value</b> | <b>Pr(&gt;F)</b> | <b><math>\eta^2</math></b> | <b>Significance</b> |
|-----------|-----------|---------------|----------------|----------------|------------------|----------------------------|---------------------|
| <b>Zn</b> |           |               |                |                |                  |                            |                     |
| Entries   | 6         | 86.85         | 14.475         | 10.82          | 2.14e-05         | 0.76                       | ***                 |
| Residuals | 20        | 26.75         | 1.337          |                |                  |                            |                     |
| <b>Fe</b> |           |               |                |                |                  |                            |                     |
| Entries   | 6         | 171.62        | 98.27          | 5.821          | 0.00121          | 0.64                       | **                  |
| Residuals | 20        | 98.27         | 4.914          |                |                  |                            |                     |
| <b>Cu</b> |           |               |                |                |                  |                            |                     |
| Entries   | 6         | 4.704         | 0.7840         | 6.464          | 0.000656         | 0.66                       | ***                 |
| Residuals | 20        | 2.426         | 0.1213         |                |                  |                            |                     |
| <b>Mn</b> |           |               |                |                |                  |                            |                     |
| Entries   | 6         | 9.706         | 1.6177         | 2.882          | 0.0344           | 0.46                       | *                   |
| Residuals | 20        | 11.226        | 0.5613         |                |                  |                            |                     |
| <b>Mg</b> |           |               |                |                |                  |                            |                     |
| Entries   | 6         | 66007         | 11001          | 2.684          | 0.0446           | 0.45                       | *                   |
| Residuals | 20        | 81984         | 4099           |                |                  |                            |                     |
| <b>Ca</b> |           |               |                |                |                  |                            |                     |
| Entries   | 6         | 10839         | 1806.6         | 2.227          | 0.0829           | 0.40                       |                     |
| Residuals | 20        | 16226         | 811.3          |                |                  |                            |                     |
| <b>P</b>  |           |               |                |                |                  |                            |                     |
| Entries   | 6         | 316733        | 52789          | 3.267          | 0.021            | 0.49                       | *                   |
| Residuals | 20        | 323156        | 16158          |                |                  |                            |                     |
| <b>K</b>  |           |               |                |                |                  |                            |                     |
| Entries   | 6         | 2395507       | 399251         | 43053          | 1.92e-10         | 0.93                       | ***                 |
| Residuals | 20        | 183453        | 9173           |                |                  |                            |                     |

Significant level at ( $p < 0.05$ ) while \* $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ .

**Table 2.8** Mean data of proso millet varieties for zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), magnesium (Mg), calcium (Ca), phosphorus (P), and potassium (K) from the 2022 researcher-run trial.

| Variety        | Zn<br>(mg/kg) | Fe<br>(mg/kg) | Cu<br>(mg/kg) | Mn<br>(mg/kg) | Mg<br>(mg/kg) | Ca<br>(mg/kg) | P<br>(mg/kg) | K<br>(mg/kg) |
|----------------|---------------|---------------|---------------|---------------|---------------|---------------|--------------|--------------|
| Dawn           | 19.4<br>(bc)  | 30.3 (a)      | 5.76 (b)      | 9.11 (b)      | 931 (b)       | 129 (b)       | 2185<br>(b)  | 1873<br>(bc) |
| Earlybird      | 19.4<br>(bc)  | 27.2<br>(bc)  | 5.37<br>(bc)  | 8.74 (b)      | 971<br>(ab)   | 126 (b)       | 2126<br>(b)  | 1853<br>(bc) |
| Horizon        | 19.3<br>(bc)  | 23.3 (d)      | 5.21 (c)      | 8.60 (b)      | 900 (b)       | 128 (b)       | 2014<br>(b)  | 1817<br>(c)  |
| Huntsman       | 20.1 (b)      | 30.2<br>(ab)  | 5.77 (b)      | 8.73 (b)      | 968<br>(ab)   | 149<br>(ab)   | 2196<br>(ab) | 1993<br>(b)  |
| Plateau        | 24.2 (a)      | 30.6 (a)      | 6.33 (a)      | 10.35<br>(a)  | 1043<br>(a)   | 170 (a)       | 2372<br>(a)  | 2715<br>(a)  |
| Sunrise        | 20.3 (b)      | 27.4<br>(abc) | 5.78<br>(ab)  | 8.36 (b)      | 900 (b)       | 168 (b)       | 2092<br>(b)  | 1896<br>(bc) |
| Sunup          | 18.2 (c)      | 26.1<br>(cd)  | 5.00 (c)      | 8.99 (b)      | 901 (b)       | 114 (b)       | 2079<br>(b)  | 1903<br>(bc) |
| C.V. %         | 5.8           | 8.0           | 6.22          | 8.32          | 7             | 20            | 6            | 5            |
| LSD<br>(0.05)* | 1.6           | 3.4           | 0.53          | 1.13          | 100           | 40            | 197          | 149          |
| Mean           | 20.1          | 27.9          | 5.60          | 9.01          | 947           | 139           | 2154         | 2011         |

LSD: Least Significant Difference; LSD comparisons are significant at the  $p < 0.05$  level.

\* LSD excludes entries of ‘Sunrise’ (n=3) due to one missing entry. All other varieties contain four entries (n=4) and LSD calculation requires an even dataset. Groupings were calculated with all data points, including ‘Sunrise’ entries.

**Table 2.9** Analysis of variance for mineral concentration (mg/kg) for proso millet varieties grown in producerr-run trial in Pullman, WA in 2022.

|           | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F-Value</b> | <b>Pr(&gt;F)</b> | <b>Significance</b> |
|-----------|-----------|---------------|----------------|----------------|------------------|---------------------|
| <b>Zn</b> |           |               |                |                |                  |                     |
| Location  | 4         | 249.95        | 62.49          | 8.044          | 0.00216          | **                  |
| Variety   | 6         | 77.30         | 12.88          | 1.658          | 0.21451          |                     |
| Residuals | 12        | 93.22         | 7.77           |                |                  |                     |
| <b>Fe</b> |           |               |                |                |                  |                     |
| Location  | 4         | 6941          | 1735.3         | 17.200         | 6.56e-05         | ***                 |
| Variety   | 6         | 519           | 86.5           | 0.857          | 0.552            |                     |
| Residuals | 12        | 1211          | 100.9          |                |                  |                     |
| <b>Cu</b> |           |               |                |                |                  |                     |
| Location  | 4         | 8.005         | 2.0012         | 4.469          | 0.0193           | *                   |
| Variety   | 6         | 1.425         | 0.2375         | 0.530          | 0.7754           |                     |
| Residuals | 12        | 5.373         | 0.4478         |                |                  |                     |
| <b>Mn</b> |           |               |                |                |                  |                     |
| Location  | 4         | 282.79        | 70.70          | 6.678          | 0.00456          | **                  |
| Variety   | 6         | 62.75         | 10.46          | 0.988          | 0.47488          |                     |
| Residuals | 12        | 127.03        | 10.59          |                |                  |                     |
| <b>Mg</b> |           |               |                |                |                  |                     |
| Location  | 4         | 204471        | 51118          | 2.543          | 0.0943           |                     |
| Variety   | 6         | 37731         | 6289           | 0.313          | 0.9181           |                     |
| Residuals | 12        | 241212        | 20101          |                |                  |                     |
| <b>Ca</b> |           |               |                |                |                  |                     |
| Location  | 4         | 2491          | 622.6          | 0.670          | 0.625            |                     |
| Variety   | 6         | 6007          | 1001.1         | 1.078          | 0.427            |                     |
| Residuals | 12        | 11148         | 929.0          |                |                  |                     |
| <b>P</b>  |           |               |                |                |                  |                     |
| Location  | 4         | 1102856       | 275714         | 2.833          | 0.0725           |                     |
| Variety   | 6         | 220744        | 36791          | 0.378          | 0.8793           |                     |
| Residuals | 12        | 1167938       | 97328          |                |                  |                     |
| <b>K</b>  |           |               |                |                |                  |                     |
| Location  | 4         | 1092706       | 273177         | 2.400          | 0.108            |                     |
| Variety   | 6         | 843764        | 140627         | 1.236          | 0.354            |                     |
| Residuals | 12        | 1365749       | 113812         |                |                  |                     |

Significant level at ( $p < 0.05$ ) while \* $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ .

**Table 2.10** Mean data of proso millet by location for zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), magnesium (Mg), calcium (Ca), phosphorus (P), and potassium (K) from the 2022 producer-run trials.

| <b>Location</b> | <b>Zn<br/>(mg/kg)</b> | <b>Fe<br/>(mg/kg)</b> | <b>Cu<br/>(mg/kg)</b> | <b>Mn<br/>(mg/kg)</b> | <b>Mg<br/>(mg/kg)</b> | <b>Ca<br/>(mg/kg)</b> | <b>P<br/>(mg/kg)</b> | <b>K<br/>(mg/kg)</b> |
|-----------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|----------------------|----------------------|
| 1               | 23.3                  | 27.2                  | 4.51                  | 7.97                  | 1080                  | 159                   | 2471                 | 2581                 |
| 2               | 29.7                  | 81.4                  | 5.27                  | 18.39                 | 1272                  | 144                   | 3089                 | 2733                 |
| 3               | 19.6                  | 33.1                  | 3.91                  | 7.95                  | 1070                  | 137                   | 2594                 | 2591                 |
| 4               | 24.6                  | 31.8                  | 5.10                  | 8.59                  | 1054                  | 138                   | 2590                 | 2218                 |
| 5               | 27.6                  | 30.5                  | 5.58                  | 7.58                  | 928                   | 160                   | 2335                 | 2126                 |
| Mean            | 25.0                  | 40.8                  | 4.87                  | 10.10                 | 1081                  | 148                   | 2616                 | 2450                 |

**Table 2.11** Analysis of variance for seed morphology traits seed area (SA), seed eccentricity (SE), thousand seed weight (TSW), and seed color (SC) for proso millet varieties grown in researcher-run trial in Pullman, WA in 2022.

|            | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F-Value</b> | <b>Pr(&gt;F)</b> | <b><math>\eta^2</math></b> | <b>Significance</b> |
|------------|-----------|---------------|----------------|----------------|------------------|----------------------------|---------------------|
| <b>SA</b>  |           |               |                |                |                  |                            |                     |
| Entries    | 6         | 4835319       | 805886         | 46.19          | <2e-16           | 0.85                       | ***                 |
| Residuals  | 49        | 854977        | 17449          |                |                  |                            |                     |
| <b>SE</b>  |           |               |                |                |                  |                            |                     |
| Entries    | 6         | 0.001667      | 2.779e-04      | 5.559          | 0.000186         | 0.41                       | ***                 |
| Residuals  | 49        | 0.002450      | 4.999e-05      |                |                  |                            |                     |
| <b>TSW</b> |           |               |                |                |                  |                            |                     |
| Entries    | 6         | 5.590         | 0.9317         | 47.9           | <2e-16           | 0.86                       | ***                 |
| Residuals  | 47        | 0.914         | 0.0194         |                |                  |                            |                     |
| <b>SC</b>  |           |               |                |                |                  |                            |                     |
| Entries    | 6         | 1974.0        | 329.0          | 49.37          | <2e-16           | 0.86                       | ***                 |
| Residuals  | 49        | 326.5         | 6.7            |                |                  |                            |                     |

Significant level at ( $p < 0.05$ ) while \* $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ .

**Table 2.12** Mean data of seed morphology traits seed area (SA), seed eccentricity (SE), thousand seed weight (TSW), and seed color (SC) for proso millet varieties grown in researcher-run trial in Pullman, WA in 2022.

| Variety    | SA (mm <sup>2</sup> ) | SE          | TSW (g)   | SC         |
|------------|-----------------------|-------------|-----------|------------|
| Dawn       | 7729 (a)              | 1.079 (d)   | 5.11 (a)  | -21.70 (d) |
| Earlybird  | 7531 (cd)             | 1.082 (bcd) | 5.03 (ab) | -18.90 (c) |
| Horizon    | 7562 (bc)             | 1.087 (b)   | 5.10 (a)  | -13.60 (b) |
| Huntsman   | 7427 (de)             | 1.080 (cd)  | 4.91 (b)  | -19.06 (c) |
| Plateau    | 6777 (f)              | 1.095 (a)   | 4.17 (d)  | -9.15 (a)  |
| Sunrise    | 7670 (ab)             | 1.087 (bc)  | 5.15 (a)  | -15.92 (b) |
| Sunup      | 7365 (e)              | 1.080 (d)   | 4.75 (c)  | -29.38 (e) |
| C.V. %     | 1.78                  | 3.75        | 2.85      | -14.15     |
| LSD (0.05) | 132.73                | 0.013       | 0.14*     | 2.59       |
| Mean       | 7436.88               | 0.362       | 4.89      | -18.24     |

LSD: Least Significant Difference; LSD comparisons are significant at the  $p < 0.05$  level.

\* TSW LSD excludes ‘Sunrise’ (n=3) and ‘Sunup’ (n=3) due to two missing data entries. All other varieties contain four entries (n=4) and LSD calculation requires an even dataset.

Groupings were calculated with all data points, including ‘Sunrise’ and ‘Sunup’ entries.

**Table 2.13** Analysis of variance for seed morphology traits seed area (SA), seed eccentricity (SE), thousand seed weight (TSW), and seed color (SC) for proso millet varieties grown in producer-run trials in 2022

|            | <b>Df</b> | <b>Sum Sq</b> | <b>Mean Sq</b> | <b>F-Value</b> | <b>Pr(&gt;F)</b> | <b>Significance</b> |
|------------|-----------|---------------|----------------|----------------|------------------|---------------------|
| <b>SA</b>  |           |               |                |                |                  |                     |
| Location   | 4         | 8242708       | 2060677        | 48.16          | 7.16e-13         | ***                 |
| Variety    | 6         | 4714380       | 785730         | 18.36          | 5.67e-09         | ***                 |
| Residuals  | 31        | 1326389       | 42787          |                |                  |                     |
| <b>SE</b>  |           |               |                |                |                  |                     |
| Location   | 4         | 0.008092      | 0.0020230      | 21.787         | 1.22e-08         | ***                 |
| Variety    | 6         | 0.001473      | 0.0002455      | 2.644          | 0.0345           | *                   |
| Residuals  | 31        | 0.002879      | 0.0000929      |                |                  |                     |
| <b>TSW</b> |           |               |                |                |                  |                     |
| Location   | 4         | 7.220         | 1.8049         | 43.33          | 2.86e-12         | ***                 |
| Variety    | 6         | 4.418         | 0.7363         | 17.68          | 8.84e-09         | ***                 |
| Residuals  | 31        | 1.291         | 0.0417         |                |                  |                     |
| <b>SC</b>  |           |               |                |                |                  |                     |
| Location   | 4         | 9271          | 2317.8         | 55.615         | 1.05e-13         | ***                 |
| Variety    | 6         | 1111          | 185.1          | 4.442          | 0.00236          | **                  |
| Residuals  | 31        | 1292          | 41.7           |                |                  |                     |

Significant level at ( $p < 0.05$ ) while \* $p < 0.05$ , \*\*  $p < 0.01$ , and \*\*\*  $p < 0.001$ .



**Table 2.14** Mean data of seed morphology traits seed area (SA), seed eccentricity (SE), thousand seed weight (TSW), and seed color (SC) for proso millet varieties grown in producer-run trials.

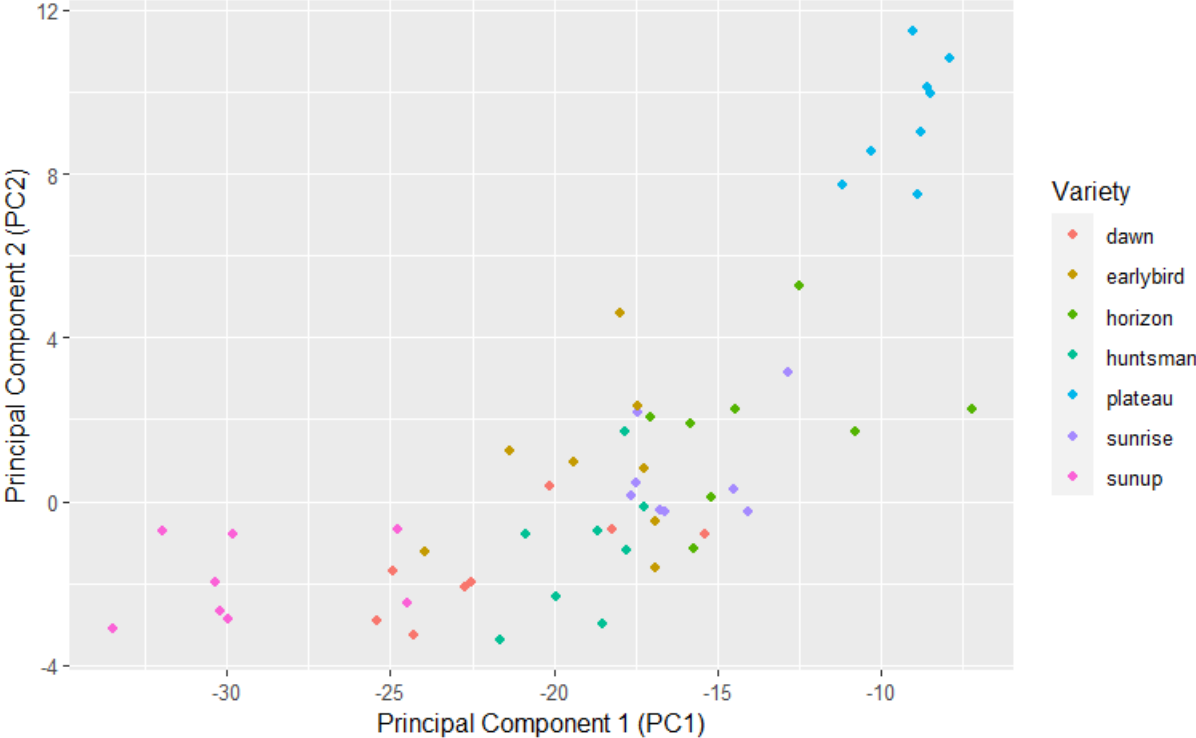
| <b>Variety</b> | <b>SA (mm<sup>2</sup>)</b> | <b>SE</b>    | <b>TSW (g)</b> | <b>SC</b>    |
|----------------|----------------------------|--------------|----------------|--------------|
| Dawn           | 7959                       | 1.067        | 5.13           | 9.96         |
| Earlybird      | 7407                       | 1.089        | 4.76           | 25.25        |
| Horizon        | 7404                       | 1.091        | 4.87           | 32.85        |
| Huntsman       | 7334                       | 1.084        | 4.69           | 33.19        |
| Plateau        | 6985                       | 1.083        | 4.29           | 23.30        |
| Sunrise        | 7603                       | 1.083        | 4.97           | 23.35        |
| Sunup          | 7221                       | 1.082        | 4.52           | 17.67        |
| <b>Mean</b>    | <b>7416</b>                | <b>1.082</b> | <b>28.26</b>   | <b>22.94</b> |

**Table 2.15** Pearson correlation for all phenotypes: grain yield (GY), plant height (PH), days to heading (DH), plant height (PH), percent emergence (PE), seed area (SA), seed eccentricity (SE), seed color (SC), thousand seed weight (TSW), zinc (Zn), iron (Fe), copper (Cu), manganese (Mn), magnesium (Mg), Calcium (C), phosphorus (P), and potassium (K) from the 2022 researcher-run trial.

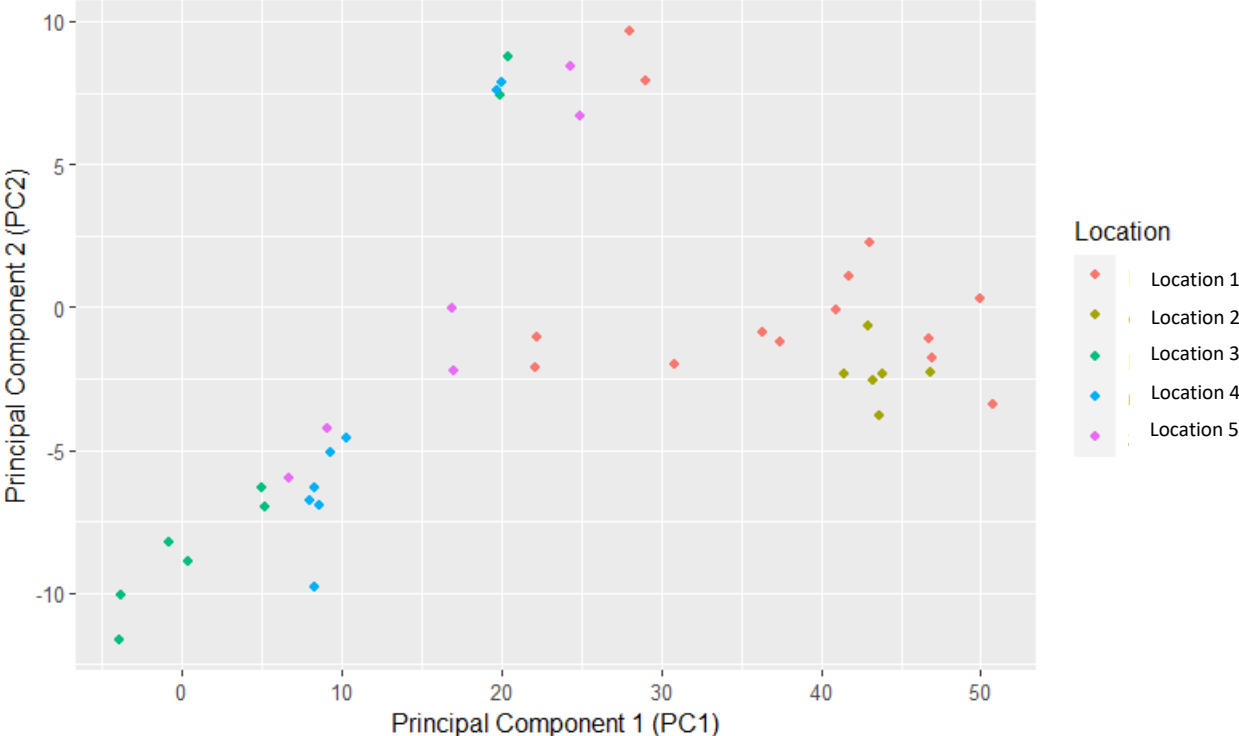
|            | GY          | PH           | DH           | DM      | PE    | SA           | SE           | SC          | TSW          | Zn          | Fe          | Cu          | Mn          | Mg          | Ca     | P      |
|------------|-------------|--------------|--------------|---------|-------|--------------|--------------|-------------|--------------|-------------|-------------|-------------|-------------|-------------|--------|--------|
| <b>PH</b>  | 0.62<br>*** |              |              |         |       |              |              |             |              |             |             |             |             |             |        |        |
| <b>DH</b>  | 0.24        | 0.21         |              |         |       |              |              |             |              |             |             |             |             |             |        |        |
| <b>DM</b>  | 0.3         | 0.35         | 0.55 **      |         |       |              |              |             |              |             |             |             |             |             |        |        |
| <b>PE</b>  | 0.11        | 0            | -0.21        | -0.4 *  |       |              |              |             |              |             |             |             |             |             |        |        |
| <b>SA</b>  | 0.45 *      | 0.57 **      | 0.43 *       | 0.12    | 0.27  |              |              |             |              |             |             |             |             |             |        |        |
| <b>SE</b>  | -0.54<br>** | -0.34        | -0.36        | -0.16   | 0.25  | -0.53<br>*** |              |             |              |             |             |             |             |             |        |        |
| <b>SC</b>  | -0.48 *     | -0.51<br>**  | -0.42 *      | -0.43 * | 0.28  | -0.4 **      | 0.67<br>***  |             |              |             |             |             |             |             |        |        |
| <b>TSW</b> | 0.45 *      | 0.51 **      | 0.45 *       | 0.12    | 0.29  | 0.98<br>***  | -0.48<br>*** | -0.33 *     |              |             |             |             |             |             |        |        |
| <b>Zn</b>  | -0.55<br>** | -0.67<br>*** | -0.57<br>**  | -0.4 *  | -0.06 | -0.7<br>***  | 0.59 **      | 0.64<br>*** | -0.64<br>*** |             |             |             |             |             |        |        |
| <b>Fe</b>  | -0.13       | -0.11        | -0.36        | -0.21   | -0.29 | -0.27        | -0.05        | 0.15        | -0.3         | 0.52 **     |             |             |             |             |        |        |
| <b>Cu</b>  | -0.36       | -0.41 *      | -0.53<br>**  | -0.37   | -0.18 | -0.42 *      | 0.32         | 0.51 **     | -0.41 *      | 0.8 ***     | 0.75<br>*** |             |             |             |        |        |
| <b>Mn</b>  | -0.18       | 0.06         | -0.52<br>**  | -0.25   | -0.2  | -0.47 *      | 0.33         | 0.3         | -0.53<br>**  | 0.39 *      | 0.36        | 0.53 **     |             |             |        |        |
| <b>Mg</b>  | 0           | -0.09        | -0.45 *      | -0.24   | -0.21 | -0.45 *      | 0.2          | 0.42 *      | -0.41 *      | 0.54 **     | 0.67<br>*** | 0.72<br>*** | 0.74<br>*** |             |        |        |
| <b>Ca</b>  | 0.03        | -0.02        | -0.3         | 0.07    | -0.27 | -0.2         | 0.22         | 0.41 *      | -0.17        | 0.38        | 0.4 *       | 0.59 **     | 0.34        | 0.52 **     |        |        |
| <b>P</b>   | -0.2        | -0.1         | -0.43 *      | -0.22   | -0.37 | -0.48 *      | 0.22         | 0.33        | -0.49<br>**  | 0.59 **     | 0.74<br>*** | 0.77<br>*** | 0.77<br>*** | 0.91<br>*** | 0.47*  |        |
| <b>K</b>   | -0.36       | -0.4 *       | -0.64<br>*** | -0.25   | -0.21 | -0.84<br>*** | 0.51 **      | 0.59 **     | -0.82<br>*** | 0.82<br>*** | 0.54 **     | 0.74<br>*** | 0.73<br>*** | 0.78<br>*** | 0.49** | 0.8*** |

\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001

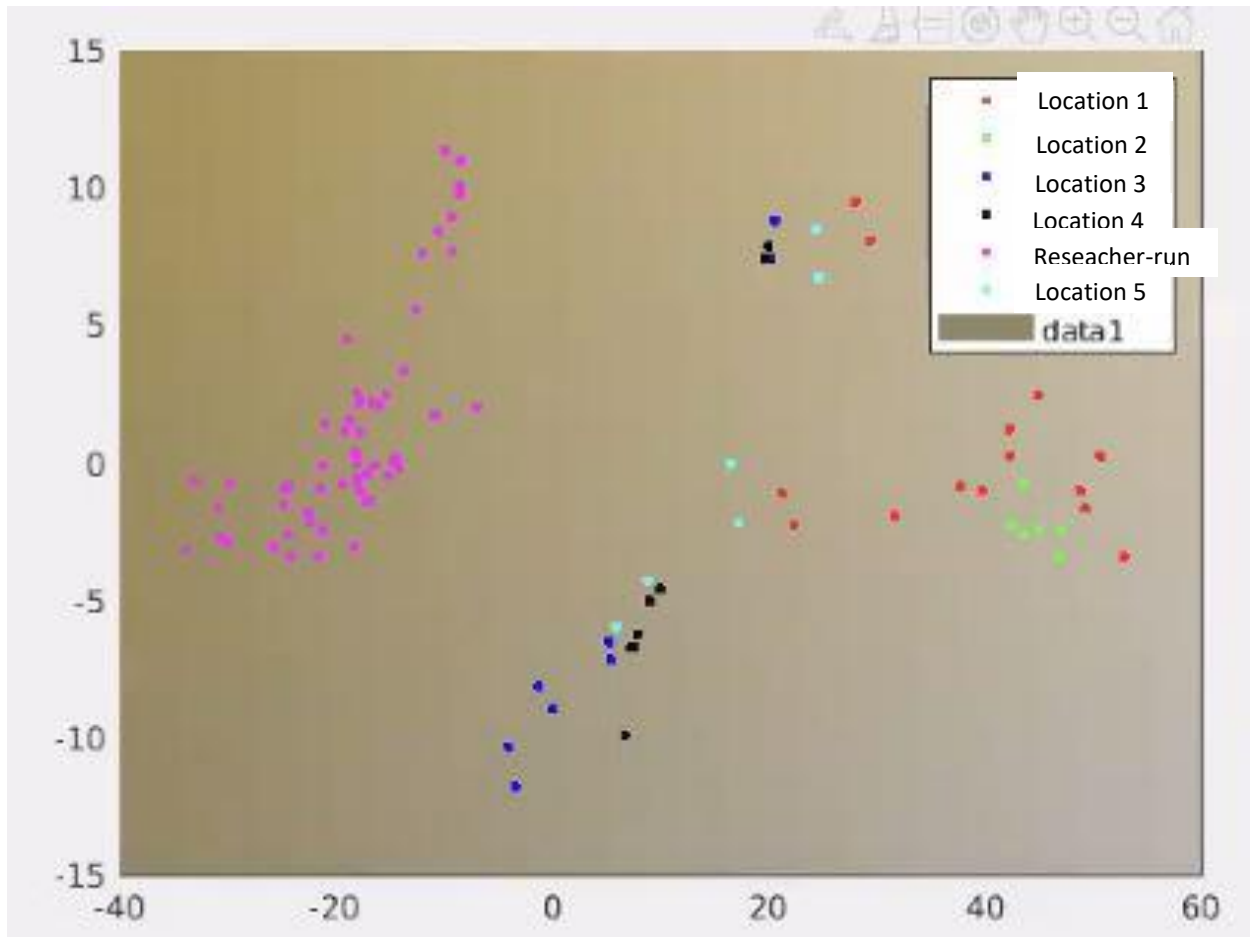
**Figure 2.1** Principal component analysis of red, green, and blue values for researcher-run proso millet trial samples.



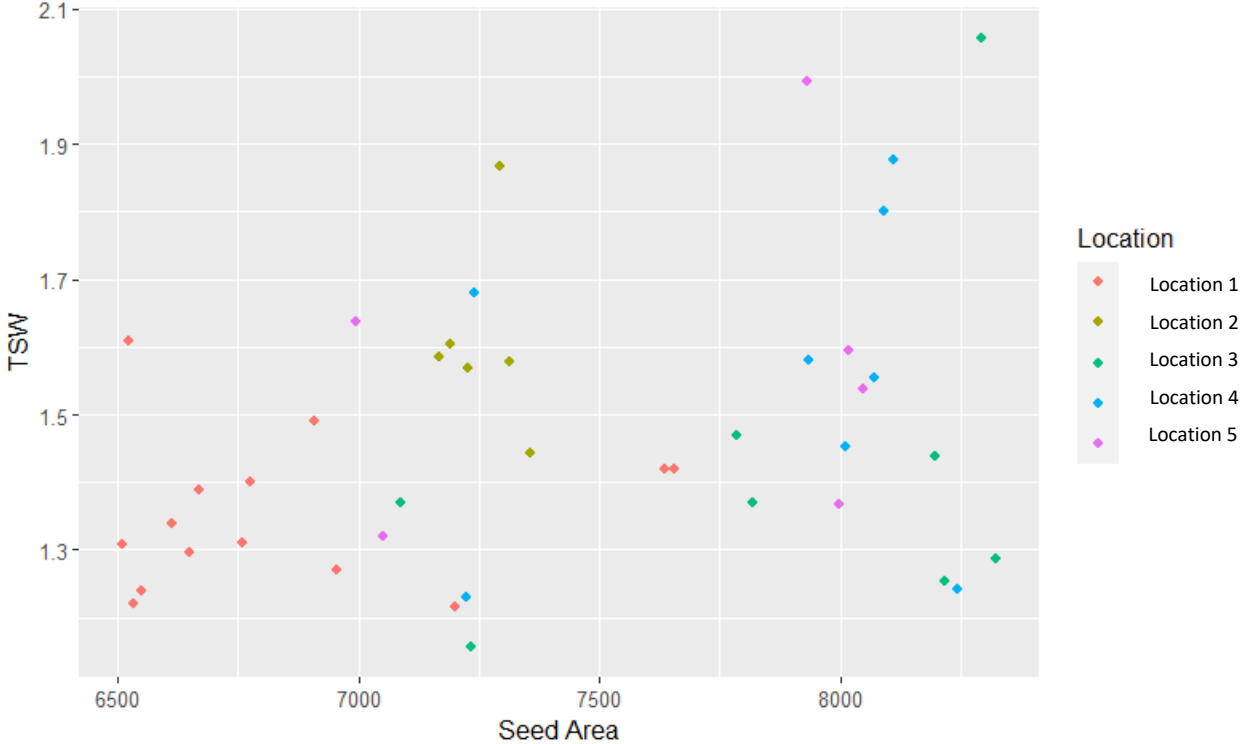
**Figure 2.2** Principal component analysis of red, green, and blue values for producer-run proso millet trial samples.



**Figure 2.3** Principal component analysis of red, green, and blue values for researcher-run and producer-run proso millet trial samples by location.



**Figure 2.4** Seed area by thousand seed weight for producer-run proso millet trial samples.



## CHAPTER THREE: PRODUCER EXPERIENCES WITH PROSO MILLET IN THE INLAND PACIFIC NORTHWEST

### 1. INTRODUCTION

Since Fall 2021, producers, processors, and researchers from Washington State University have been working together to understand the benefits and challenges of integrating proso millet (*Panicum miliaceum* L.) into the regional food system of the Pacific Northwest (PNW), a region where limited research and production of this crop has occurred. This chapter draws on interviews with five producers who participated in on-farm trials to highlight key takeaways regarding production and marketing of proso millet in this region. The objective of the chapter is to share relevant experiences with future PNW producers who may be interested in working with the crop. Furthermore, experiences of these producers may be relevant to potential adopters in other regions, or to other regionally underutilized crops.

This chapter contains an overview of proso millet, background on the project under which this research took place, a description of relevant methods, a summary of participant characteristics, and a breakdown of key benefits and challenges of working with proso millet collected from producer participants. Key perceived benefits shared by participating producers include the resilience of the crop, capacity for rotational weed control, and increased opportunity for on-farm diversity. Key perceived challenges shared by participants include timing and logistics of harvest, the lack of a reliable market, insufficient infrastructure for storage, and inconsistent stand. Last, for those interested in specific information regarding planting and management of on-farm trials, summary tables of farm characteristics, field history, and trial management are included.

## 1.1 What is Proso Millet?

Millets are small-seeded cereal crops that have been cultivated and used for food, feed, and forage for thousands of years, particularly in the semi-arid tropics of India, China, and Africa (Cheng, 2018). Even today, millets are a staple crop for communities in these regions, ranking sixth among the world's most important cereals and sustaining about one-third of the world's population (Habiyaremye, Matanguihan, *et al.*, 2017; Boukail *et al.*, 2021). Millets are recognized for their drought tolerance, low input requirement, and ability to grow in marginal agricultural zones, in addition to their rich nutrient composition (J. Wang *et al.*, 2018). These grains show such promise for providing food security in the wake of climate change that the United Nations declared 2023 “International Year of the Millets” in order to raise awareness and direct policy attention to research, market development, and production of millets globally (*International Year of Millets 2023*, 2023).

There are many different species of millets that vary widely in plant shape, seed color, and seed size. Some commonly cultivated species include pearl millet, finger millet, kodo millet, foxtail millet, little millet, and barnyard millet, but the most commonly cultivated species for human consumption in the United States (US) is proso millet (Myers, 2018; Das *et al.*, 2019). Even so, proso millet is underutilized in the US and is primarily channeled into the birdseed market, despite desirable agronomic and nutritional characteristics (Das *et al.*, 2019).

Proso millet is a warm-season grass with a short growing season of 60-100 days (Habiyaremye, Matanguihan, *et al.*, 2017). It is typically planted in the late spring or early summer, making it compatible in rotation with winter annual crops (such as winter wheat), as a replacement for summer fallow or a “catch crop” if an earlier crop were to fail (Lyon *et al.*, 2014;



Ventura et al., 2020). It has a shallow root system and as a C4 species is water efficient and well-adapted to dryland cropping systems (Lyon et al., 2014; Nielsen and Vigil, 2017).

Proso millet's capacity to grow with limited water, on marginal soil, and with minimal agronomic inputs, in addition to its short growing season, make it a valuable prospect for producers in the inland PNW (Santra, 2013). This region is characterized by a semi-arid environment with hot, dry summers that are predicted to be exacerbated by climate change (Pan et al., 2016). The inland PNW is dominated by dryland wheat production, and when added to a rotation, proso millet has been shown to increase wheat yields by controlling winter annual grassy weeds, reducing insect and disease pressures, and preserving moisture deep in the soil (Santra, 2013; Lyon et al., 2014). Proso millet is compatible with the well-drained loamy soils characteristic of the region, and there aren't currently any other warm season grasses commonly planted in this region, making it a niche opportunity for producers looking to diversify their rotations (Habiyaremye, Matanguihan, *et al.*, 2017).

Historically, North American proso millet has been grown and researched in the Central Great Plains region of Colorado, Nebraska, and South Dakota, which are also dominated by dryland wheat production, but little research has been conducted to understand the performance of varieties developed for the Great Plains for use in the inland PNW (Habiyaremye, Matanguihan, *et al.*, 2017).

## **1.2 Project Background: New Grains Northwest**

The 2022 on-farm, producer-run variety trials were conducted under a Western Sustainable Agriculture Research and Education (SARE) funded project called New Grains Northwest (SW21-926). New Grains Northwest is an interdisciplinary project that aims to increase diversity of the Pacific Northwest food system by exploring the opportunity to integrate

underutilized crops into regional cropping systems and food products. Proso millet was selected as one of the crops of interest based on its compatibility with regional cropping systems, desirable nutritional profile, and varied food applications. This work builds on previous research at WSU that tested diverse proso millet accessions (originating from Bulgaria, Czechoslovakia, Morocco, the former Soviet Union, Turkey, and the United States) for suitability to be grown in irrigated and dryland conditions in the Palouse (Habiyaemye, Barth, *et al.*, 2017).

In order to identify best-suited varieties for the region, researchers used commercially available proso millet varieties to conduct a small-scale replicated plot variety trial and facilitated a series of large-scale, on-farm, producer-led variety trials. In addition to allowing participants to observe agronomic performance of proso millet varieties, these trials provided an opportunity to interview participants about the benefits and challenges of adopting proso millet in the inland PNW.

## **2. METHODS**

Participant recruitment for the proso millet variety trials began at the end of 2021. The WSU research team conducted outreach through social media, organizational listservs, meeting announcements, and personal networks to recruit grain producers with access to full-scale equipment in the inland PNW. Producers were offered a \$500 stipend for the growing season, a 50-pound bag of seed for each test variety (between three and seven, depending on the producer's preference), and a packet of management recommendations based on publicly available extension publications from other regions (McDonald, Hofsteen and Downey, 2003; Lyon et al., 2014). While general guidance was provided, each participant was tasked with managing the trial as they saw fit, making individual decisions around planting, fertilizing, weed

management, and harvest based on management style, field history, and personal preference. See appendix for details regarding farm characteristics, field history, and trial management for each trial. Specific instructions were provided regarding trial layout, with varieties planted in long, unreplicated, parallel strips (one to two drill passes) using full-scale planting equipment.

By February 2022, a group of nine producers expressed interest in participating in the on-farm trials. A research assistant from the crop science team conducted hour-long, pre-season interviews via zoom or phone with each participant to gather information regarding farm characteristics, producer knowledge of the crop, and expectations for the project. Interviews were recorded and transcribed for later reference. Additionally, pre-planting questionnaires regarding field history (e.g., previous crops, recent chemical application, and soil characteristics) and intended trial management (e.g., fertilizer application, seeding depth and spacing) were distributed and collected from each producer.

Seed was distributed to participants in May 2022, at which time researchers conducted an initial visit to trial sites. Seed for six of seven proso millet varieties (Dawn, Earlybird, Horizon, Plateau, and Sunrise) was purchased from Kriesel Certified Seed in Gurley, NE and the seventh variety (Sunup) was purchased from Perry Brothers Seed Inc. in Otis, CO. Despite their earlier interest and intention to participate, several producers dropped out due to undesirable weather, equipment malfunctions, and limited capacity, leaving a total of five growers who ultimately planted on-farm trials. Researchers conducted a final site visit at each farm in late September, shortly before trials were harvested, and hand-collected five quadrat samples and phenotypic data from each variety. After samples were collected, producer participants were free to harvest and use or sell seed as they wished.

In January of 2023, the same research assistant conducted individual post-season interviews with each producer participant via Zoom or phone call regarding in-season management decisions, crop performance, and trial experience. Additionally, a group Zoom call was conducted with all five participants in which producers were able to share their trial experiences and discuss management decisions and overall takeaways with one another. Individual interviews and the group call were both recorded and transcribed.

Transcriptions from pre-season interviews, post-season interviews, and the group grower call were reviewed and relevant quotes were selected and organized by recurring themes. These themes broke down into benefits of working with proso millet (including resilience, weed control, and diversity) and the challenges of working with proso millet (market, harvest, storage, and inconsistent stand). Summaries of each theme and relevant quotes can be found in Producer Experiences and Perspectives, below.

### **3. PRODUCER CHARACTERISTICS**

Of the five producers who ultimately planted on-farm trials, three had never worked with proso millet before, but all five had experience with similar small-seeded and/or spring-planted crops. Producers managed between 1,500 and 16,000 acres. Three participants were located in Genesee, ID (average rainfall: 22 in/year), one in Edwall, WA (average rainfall: 11 in/year), and one in Chelan, WA (average rainfall: 7.4 in/year). The nature of this project attracted producers who were exploring a range of practices to increase sustainability of their farms. All five participants practiced some degree of no-till production and were working to implement diverse cropping rotations, three used integrated livestock, and three use cover crops. Three growers

were Food Alliance certified, three were Farmed Smart certified, and three were privately certified by a regeneratively grown wheat company in the region called Shephard's Grain.

#### **4. PRODUCER EXPERIENCES AND PERSPECTIVES**

Throughout the course of the 2022 trials, producers learned a great deal about the benefits and challenges of working with proso millet and honed their management strategies. Key points from one-on-one interviews with each of the producers, in addition to a group call with all producers, are summarized below.

##### **4.1 Benefits**

Overall, participants were pleasantly surprised with the performance of proso millet in their fields. Four of the participants planned to plant a second trial in the 2023 growing season. The most prominent perceived benefits that surfaced during post-season interviews were the resilience of the crop, rotation weed control, and increased opportunity for on-farm diversity.

##### *4.1.1 Resilience*

Four participants commented on the resilience that proso millet showed in undesirable growing conditions. Two of those participants expected a complete crop failure because of poor weather at planting but were surprised to see a productive crop later in the season. In 2022, the PNW had an uncharacteristically cold and wet spring. Washington State experienced its third coldest June on record and April, May, and June brought above average precipitation across the PNW (*NOAA National Centers for Environmental Information, Monthly National Climate Report, 2022*). However, later in the season, Washington, Oregon, and Idaho all experienced their hottest August temperatures on record (*NOAA National Centers for Environmental Information, Monthly National Climate Report, 2022*). These climactic anomalies not only put

stress on crops in the field, but also threw off typical planting and harvesting schedules and put additional stress on producers. Given the conditions, participants were generally pleased with the performance of the crop and took note of its capacity to withstand high temperatures.

#### Participant Quotes: Resilience

“When we got our heat wave this year the millet looked like it was happy has a clam out there. The crop really took off in August and September. It looked like a nice crop, not like one of my weird experiments.”

– *Producer 1*

“Seeded it and then got 1-1.5 inches of rain on June 4 in 45 minutes. I wrote the crop off. It was hard as a rock. Probably didn’t even go back up there until the 1st of July and here I had a stand of everything, a beautiful stand ... I was amazed that a seed that small had that much vigor.”

– *Producer 2*

“Once the heat hit, I loved driving around seeing something green. It just took off.”

– *Producer 2*

“For seeding it on June 22 – very, very late -- it did alright. I would not want to do that normally or as a general practice but it showed that you can seed it extremely late and still get a crop.”

– *Producer 3*

“As little attention as I gave it, I really mismanaged it and it still lived and did okay. I think it increased my likelihood of continuing to work with this crop.”

– *Producer 5*

#### *4.1.2 Weed Control*

Because proso millet is typically planted in the late spring, participants found that including it in a cropping rotation creates a valuable opportunity to control secondary flushes of weeds that typically emerge once their spring crop has already been planted. Due to herbicide resistance,

many of these weeds are increasingly difficult to control with available chemicals. All five participants used this opportunity to spray a late application of glyphosate (Roundup) before planting, which helped them control problem weeds such as Italian rye grass (*Lolium multiflorum* Lam.) and mayweed chamomile (*Anthemis cotula* L.).

#### Participant Quotes: Weed Control

“I felt like the weed control was much better than I expected it to be. There weren’t that many weeds out there.”

– *Producer 1*

“I was able to hit that one last time and it really made a big difference. I had so much [mayweed chamomile], I was dreading putting something in there and that second Round-up application really cleaned it up.”

– *Producer 1*

“It also addressed weed resistance issues. The number one weed I have problems with is Italian rye grass and because I’m able to seed [proso millet] quite a bit later, I am able to control later season flushes with pre-planting application of Round Up and then just rely on good crop canopy to control it in-season rather than relying on herbicide.”

– *Producer 3*

“It did seem pretty competitive canopy-wise once it got up and got going, even though at the beginning it didn’t look like it was really going to compete much.”

– *Producer 4*

“I didn’t even put a broadleaf herbicide on it. I Round Up-ed it before we seeded it, seeded it, and didn’t touch it the rest of the season. I wanted to see how well it competed against the weeds that we have and I was very impressed. I think it did a good job competing. But I still think it would need an [in-season] herbicide application.”

– *Producer 5*

### 4.1.3 On-Farm Diversity

The producers who volunteered to participate in these trials were interested in adding diversity to their cropping systems. At the conclusion of the trials, an increase in diversity was seen as one of the most notable benefits of working with proso millet. Proso millet is a warm season grass, which fills a rotational niche that is currently vacant in the inland PNW. Proso millet can be integrated into inland PNW cropping systems as a replacement for summer fallow, or as one producer noted, as a catch crop in the case of poor conditions for earlier spring plantings. In addition to crop diversity, two participants noted an increase in insect and wildlife activity that they welcomed on their land.

#### Participant Quotes: On-Farm Diversity

“We are starting to grow a lot of cover crops so I see it as my own market, I’ll just turn right around and it keeps that seed in my microbiome that I am trying to keep as a closed system, so I couldn’t be more thrilled.”

– *Producer 2*

“You can’t quantify that but it just had a buzz, there were insects and grouse... I had never seen anything that big in a crop.”

– *Producer 2*

“You’re getting to have in rotation a warm season grass which we do not have on the Palouse, so having another leg in crop rotation for no-till direct seeding is very important.”

– *Producer 3*

“In 2017 there was a lot of preventative planting around here. People couldn’t get crops in at a normal time. It could be an emergency fit for something like that, if it’s like ‘alright, I’ve got 100 acres and I couldn’t meet planting deadlines for everything else... I could see this as a fit as a rescue.”

– *Producer 4*



“I personally noticed a whole bunch more birds, doves, pheasants - they really liked that, and it’s just something different on the ground so I was pretty excited to just watch it, and I’m excited to see what it looks like in years to come.”

– *Producer 5*

## **4.2 Challenges**

While participants saw notable benefits of working with proso millet, they also experienced some significant challenges that could limit their ability to continue working with the crop. Among these challenges, timing and logistics of harvest, the lack of a reliable market, insufficient infrastructure for storage, and inconsistent stand were most frequently discussed.

### *4.2.1 Harvest Logistics*

Proso millet must be left in the field long enough to fully mature, but it also must be harvested before the inland PNW’s rainy season begins in the fall. This window is different each year depending on the weather and can be difficult to predict. Additionally, proso millet harvest must be managed around other key seasonal activities. Typically for producers in this region, proso millet can be harvested later than other crops and will not interfere with other harvest activities, however, this late harvest can interfere with planting activities for winter crops. In order to navigate this limited harvest window, producers may choose to expedite dry-down of the crop by swathing or chemically desiccating. One participant in the 2022 trial chose to swath while one chose to chemically desiccate with glyphosate. Two growers who direct harvested experienced issues with high moisture in their harvested seed.

### Producer Quotes: Harvest Logistics

“The harvest timing is right when I should be putting in my winter wheat crop. I thought it would be nice to spread out my acres having different times but I forgot that I’m going to have to do seeding at the same time”

– *Producer 1*

“I have swathed before when I thought it was going to be a food grade market. Since then I have used glyphosate and that has worked out pretty well.”

– *Producer 3*

“If we could cut it earlier that would help. The seeding isn’t too bad because that time of year we aren’t really pressed to do something else. We have things we normally do, we grow hay crops, but the harvest was the tough one because we need to be on tractors doing wheat just like everybody else I’m sure.”

– *Producer 4*

“Man if we just had another month in the fall, I think we would be set around here.”

– *Producer 4*

“Comes down to buying two more pieces of equipment, a header for a combine and a swather.”

– *Producer 4*

“I’m not crazy about desiccation, swathing would be an option... We desiccate garbs sometimes, so it’s not the end of the world but I try to eliminate it. I think the less Round Up we can use the better.”

– *Producer 5*

“Some of the concern I have with swathing it is if we get a big windstorm and you have a bunch of millet swathed. Where I farm out on my rim, we’ve raised blue grass and/or hay in the past and had it blow in the canyon and lose all of it. So, desiccation would be probably the most realistic on larger scale, or an earlier maturing variety that will yield.”

– *Producer 5*

#### *4.2.2 Lack of Reliable Market*

A producer’s decision to grow proso millet hinges on the market and whether or not they can sell their crop. Some may be able to justify production based on their own needs for forage or cover crop seed, but most, especially at any significant scale, need a reliable buyer with

adequate prices in the region. Currently, the most prominent market for proso millet in the US is bird seed. Some participants have explored selling to a bird seed company in Spokane, Global Harvest Foods, but for others, this wasn't an attractive enough prospect to warrant working with the crop. One participant is confident in the prospect of selling to a nearby cover crop company, while others didn't find competitive pricing at the cover crop seed companies in their area. Producers briefly explored the prospect of selling to a regional malting facility but, unfortunately, it suddenly shut down. Clearly, producers are willing to get creative in finding outlets for proso millet seed, but adoption will likely remain limited until a more lucrative market emerges in the PNW.

Infrastructure limitations are another notable factor stymying the regional market. Millet has a hull that must be removed for consumption and there is not currently a dehulling facility in the PNW. This makes it more difficult for food processors to adopt regionally grown millet into their products, limiting demand from this sector of regional processors. Additionally, while proso millet could be adopted into the niche gluten-free market, producers have been unable to identify a regional cleaning facility that can clean grain without significant risk of gluten contamination. Without these important pieces of infrastructure, the regional supply chain cannot function continuously from farm to end-use

#### Participant Quotes: Lack of a Reliable Market

“I have no qualms growing it, the problem is selling it. The market. I need it to make financial sense to do it.”

– *Producer 1*

“If its lower [in price] than the current commodities then it will be a really hard sell. Certain people might see the rotational or the soil health benefit that would

go through the extra trouble to grow this, but at a loss, I would have to sell that to my landlord.”

– *Producer 1*

“If its ever going to work [there would have to be market demand for a very large scale], around 400 acres for a grain bin (quick rough math). That would be the size and scale that it would be worth considering buying a swather and a pickup header.”

– *Producer 4*

“The biggest challenge is definitely finding a local market and use for it... if its something we can at least break even on for a rotational-type crop, and/or grazing, I think people will do it. That’s like a lot of this stuff... you can raise all of the [cover crops] you want but if you’re not getting a [return on investment] then it doesn’t make much sense to do it. We kind of have to make money. It is a business unfortunately.”

– *Producer 5*

#### *4.2.3 Storage Infrastructure*

Participants experienced challenges storing proso millet seed after harvest. In February 2023, several months after the 2022 harvest, all five participants reported that their harvested proso millet had been left in covered trucks, and two of those participants reported that seed turning sour because of high moisture content and lack of ventilation. Both of these participants had intended to use their proso millet crop for livestock feed but were ultimately unable to. For those who intended to sell their proso millet seed, they still needed somewhere to keep it covered and at an acceptable moisture level until they found a suitable buyer. Part of this obstacle has to do with scale, as one participant expressed: many producers have their grain hoppers with capacity for either a small-scale crop or a large-scale crop, and nothing in between.

### Participant Quotes: Storage Infrastructure

“On our farm we have either home storage (500-bushel hopper bottom bins that we use for hog food) or a 10,000 bushel grain bin. This crop is currently somewhere in between those. If we went big, we would have to budget using a 10,000 bushel grain bin which is a lot of acres of millet... basically, five acres is the limit unless we can get to 400 [acres].”

– *Producer 4*

“We cut it all, put it in the truck, put it in the shop and it kind of went sour. We raise hogs during the summer but we didn’t feel like we should put it in the pig pen so we just dumped it.”

– *Producer 4*

“My hope [was to feed it to the cows] but its still sitting in my trailer. The tarps rolled, I haven’t rolled the tarp and looked at it. I’m afraid to. I’m sure mines sour and was not dry enough.”

– *Producer 5*

### *4.2.4 Inconsistent Stand*

Most participants noticed an inconsistent stand in their proso millet trials across all varieties. However, participants attributed the inconsistencies to a range of factors, including moisture, soil depth, and equipment. One producer who has grown proso millet many times in the past noted that this year seemed much more inconsistent than usual.

### Participant Quotes: Inconsistent Stand

“My stand was very spotty, some that were up mid chest level and some that were 5-6 inches tall and didn’t even look like they made a head. It didn’t seem to have rhyme or reason, there were spots that it looked like it should have had moisture but it was short and... I don’t know it seemed kind of hit and miss.”

– *Producer 1*

“[I planted into variable soil and] it was more sensitive to soil than probably anything else that I have. I’ve never seen anything follow the contour (for lack of a better term).”

– *Producer 2*

“I think it all came down to moisture, areas where it was thin stand it was just way too wet. The opposite can happen if the whole field is too dry.”

– *Producer 3*

“I need to use different rollers... that may have also contributed to the uneven stand. If I used different rollers, it might be more even.”

– *Producer 3*

“Where I noticed the inconsistent stand heights is 100% related to soil depth. I have been doing mine on the rim where its either rock or good dirt.”

– *Producer 5*

## **5. CONCLUSION**

Working with producer participants through the 2022 on-farm proso millet variety trials created a unique opportunity to understand the benefits and challenges of working with this crop in the inland PNW, a region where limited research has been conducted.

Pre- and post-season interviews indicated that participants saw the resilience of the crop, weed control potential, and the opportunity to increase diversity in their rotations as the most prominent benefits of working with proso millet. These benefits are particularly desirable in the wake of climate change, as resilience will help crops withstand severe weather conditions and increased diversity within cropping rotations can increase food security. Weed control potential is also critical for producers struggling with herbicide resistance.

However, producers also discussed challenges of working with proso millet, including timing and logistics of harvest, the lack of a reliable market, insufficient infrastructure for storage, and inconsistent stand. In particular, absence of market demand stands out as a

prominent issue, as equipment to improve harvest, storage, and management could be justified with proper financial incentive. Market development is a complex objective that will require strategizing and cooperation across producers, researchers, government entities, and processors from private industry. However, at the regional scale, just one substantial buyer could dramatically shift the dynamic of demand and create financial incentive for producers to troubleshoot challenges and integrate proso millet into their rotations. While reliable market and regional management recommendations have yet to be established, the participation of the producers in this study was a valuable first step in understanding the opportunity for adoption of proso millet in the inland PNW. Furthermore, these producer experiences can help us better understand some of the benefits and challenges that producers who are attempting to increase diversity of their farms may experience in other regions and with other crops.

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**Table 3.1** Characteristics of farms from 2022 producer-run trials.

|                         | <b>Producer 1</b>                        | <b>Producer 2</b>                 | <b>Producer 3</b>                                | <b>Producer 4</b>                     | <b>Producer 5</b>  |
|-------------------------|--|-----------------------------------|--|---------------------------------------|--|
| Location                | Edwall, WA                               | Chelan, WA                        | Genesee, ID                                      | Genesee, ID                           | Genesee, ID  |
| Acreage                 | 1,500 acres                              | 16,000 acres                      | 2,200 acres                                      | 2,800 acres                           | 4,900 acres  |
| Certifications          | NA                                       | Farmed Smart                      | Food Alliance,<br>Farmed Smart                   | Food Alliance,<br>Shephard's<br>Grain | Food Alliance,<br>Farmed Smart   |
| Management<br>Style     | No till, some<br>conservation<br>farming | No till, diverse<br>crop rotation | No till, cover<br>crop, diverse<br>crop rotation | No till and<br>minimum till           | No till, cover<br>crop, integrated<br>livestock, some<br>biological inputs |
| Annual<br>Precipitation | 11 in                                    | 6-9 in                            | 22 in  | 18-22 in                              | 22 in  |

Annual precipitation self-reported by producers.

**Table 3.2** Field history of trial sites from of farms from 2022 producer-run trials.

|   | <b>Producer 1</b>  | <b>Producer 2</b>                                       | <b>Producer 3</b>  | <b>Producer 4</b>  | <b>Producer 5</b> |
|---|--|---|--|--|-------------------|
| Plot size                                   | 4.5 acres  | 3.5 acres   | 5 acres  | 2.86 acres   | 8.28 acres        |
| Landscape position                          | SW-NE; ditches, hilly  | Flat /rocky   | Draw   | Slight N slope   | Slope             |
| Previous crop (most recent to least recent) | Spring wheat, spring wheat, winter wheat                                       | Winter wheat, canola, sunflower, spring wheat           | Spring wheat   | Soft white winter wheat (rotation: fall grain, spring grain, legume, fall grain, legume) | Fall wheat        |
| Recent chemical history                     | No in-crop herb in '21 drought year, 24 oz RT3 + molasses                      | Round up 2 weeks ago - 12 oz with reverse osmosis water | 114.3 lb N; 31.9 lb S; 6.18 lb P. 22 oz RoundUp and 1.5 oz sharpen | Last fall - 120 lb N; this spring - 15 lb phosphate, 30 lb sulfur                        | NA                |
| Soil  |  |   |  |  |                   |
| Texture                                     | Silt loam  | Sandy loam  | Silt loam  | Loamy silt, a little clay  | NA                |
| Drainage                                    | Before no-till (15 years ago) lots of pooling, but not any more                | Never rains, slopes south                               | Poorly drained   | Corner wet spot, but otherwise well-drained  | NA                |
| Fertility                                   | Low in calcium and high in manganese/magnesium; usually apply zinc and boron   | No known issues; added biosolids in 2014                | NA   | Low on zinc and magnesium  | NA                |
| Organic Matter                              | 2.7%   | Up to 2%  | 3.50%  | 3% range   | NA                |
| Weeds                                       | Most severe: may weed and cheat grass; Manageable: China lettuce and wild oats | NA  | Italian ryegrass   | Italian rye grass (rotate hay and spot spray for management)                             | NA                |



**Table 3.3** Trial management details from 2022 producer-run trials.

|   | <b>Producer 1</b>                        | <b>Producer 2</b>  | <b>Producer 3</b>                  | <b>Producer 4</b>   | <b>Producer 5</b>                                   |
|---|--|--|------------------------------------|---|---|
| Planting Date                             | 6/12/22                                  | 6/1/2022   | 6/20/2022                          | 5/26/2022   | 5/24/2022   |
| Harvest Date                              | 10/4/22                                  | 10/15/22   | 9/27/2022                          | 9/28/2022   | 9/13/2022   |
| Residue Management                        | No till                                  | No till  | No till                            | Till with fertilizer close to seeding (9 in shank, 3-4 in deep) | No till   |
| Fertilizer                                |  |  |                                    |   |   |
| Product                                   | 2 gal UAN, 2 gal orthophos + molasses    | 100 lb N, 15 lb P, 20 lb S<br>Gypsum, humic, sugar; Mn, Mg, and seed water | 100 lb N, 15 lb P, 20 lb S         | 100 lb N, 10 lb phosphate, zinc                                 | 50 lbs N, 15 lbs S, 20 lbs P                        |
| Placement                                 | Paired row 3", banded in middle 1" below | Ran through drill; came over the top                                       | NA                                 | Through drill kit   | NA  |
| Timing                                    | At planting                              | At planting  |                                    | At planting   | At planting   |
| Preplant Herbicide                        |  |  |                                    |   |   |
| Product                                   | Round up                                 | Round up   | Clarity                            | NA  | NA  |
| Rate                                      | 24 oz; a couple days before planting     | 1 application; month before planting                                       | 4 oz                               |   |   |
| In-season herbicide                       | NA                                       | NA   | NA                                 |   | NA  |
| Product                                   |  |  |                                    | 2,4-D Amine   |   |
| Rate                                      |  |  |                                    | NA  |   |
| Planting Equipment                        | 23 ft Airdrill; Flexicoil hoe drill      | Ag pro 4312-SL drill   |                                    | John Deere 1890- 2012   | 1870 John Deere no-till drill                       |
| Seeding Rate                              | 25 lbs/acre                              | 15 lbs/acre  | 30 lbs/acre                        | 11 lbs/acre   | 35 lbs/acre   |
| Row Spacing                               | 9.5 in paired rows                       | 9 in paired row  | 4.5 in paired rows                 | 7.5 in  | 12 in   |
| Seeding Depth                             | .5-.75 in                                | 1.5 in   | 1 in (varies in rocky ground)      | .75-1.25 in   | .75 in  |
| Harvest Method                            | Swath/pickup                             | Direct   | Chemical dry down, direct          | Direct  | Direct  |
| Harvest Equipment                         | NA                                       | John Deere S780 combine with 45' Mac Don FD240 draper header               | Case 9120 combine with draper head | John Deere S680 combine   | John Deere S680 with direct cut normal wheat header |
| Average yield across varieties (lbs/acre) | NA                                       | 961  | 861                                | 1700  | NA  |

**Figure 3.1** Maturing proso millet panicle.



**Figure 3.2** Mature, on-farm proso millet trial in Edwall, WA.



**Figure 3.3** No-till proso millet planting into wheat stubble in Genesee, ID. Photo by: Participant 5.



**Figure 3.4** Maturing proso millet crop in Edwall, WA. Photo by: Participant 1.



**Figure 3.5** Proso millet harvest in Genesee, ID.



**Figure 3.6** Dehulled proso millet seed.



## APPENDIX



## **Appendix 1: Pivoting from mother-baby trial design**

At the conception of the project, varieties were intended to be assessed using a mother and baby trial design in which a researcher-run “mother” trial, which can be closely monitored and controlled, is strategically linked with on-farm, producer-run “baby” trials, that facilitate observation of varieties under a wide range of farm practices and environmental contexts. Researchers aimed to recruit 10 producer participants to host trials in 2022, but despite sufficient interest from producers in the region, impacted schedules, equipment limitations, and challenging weather conditions at planting meant that only five producers ultimately planted on-farm trials. These trials were unreplicated and contained a subset of total varieties (between 3-7) depending on producer capacity. Considering the low number of replications for each variety across on-farm trials and the high variability between sites, researchers decided to exclude on-farm samples from agronomic data analysis, focusing instead on data from the more standardized, replicated, researcher-run trial. On-farm samples were included, however, in nutritional and morphological analysis, in an effort to increase sample size for these analyses. Furthermore, as agronomic analysis is based on a single site year, they can be considered preliminary results.

**Appendix 2:** Note on seed color

Samples were dehulled using a a Tangential Abrasive Dehulling Device (Saskatoon, Sask., Canada) before seed morphology data was obtained. This process uses abrasion to remove hulls, and subsequently causes some of the outer seed layer to be abraded down. It should be noted that seed color and other seed morphology data could have been affected by the slight abrasion of the seeds, as demonstrated in the photos below.



Sample A, dehulled with Tangential Abrasive Dehulling Device.



Sample A, dehulled with stainless steel rice polisher.



Sample B, dehulled with Tangential Abrasive Dehulling Device.



Sample B, dehulled with stainless steel rice polisher.