

## Article

# Phenotypic Responses of Twenty Diverse Proso Millet (*Panicum miliaceum* L.) Accessions to Irrigation

Cedric Habiyaremye <sup>1</sup>, Victoria Barth <sup>1</sup>, Kelsey Highet <sup>1</sup>, Todd Coffey <sup>2</sup> and Kevin M. Murphy <sup>1,\*</sup>

<sup>1</sup> Department of Crop and Soil Sciences, College of Agricultural, Human, Natural Resource Sciences, Washington State University, P.O. Box 646420, Pullman, WA 99164-6420, USA; cedric.habiyaremye@wsu.edu (C.H.); victoria\_marshall@wsu.edu (V.B.); kelsey.highet@wsu.edu (K.H.)

<sup>2</sup> Department of Mathematics and Statistics, Center for Interdisciplinary Statistical Education and Research, Washington State University, Pullman, WA 99164-3113, USA; todd.coffey@wsu.edu

\* Correspondence: kmurphy2@wsu.edu; Tel.: +509-335-9692; Fax: +509-335-8674

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**Abstract:** To date, little research has been conducted on the phenotypic responses of proso millet to drought and deficit irrigation treatments in the dryland wheat-based cropping systems of the Palouse bioregion of the U.S. The objectives of this study were to evaluate critical agronomic traits of proso millet, including emergence, plant height, days to heading, days to maturity, and grain yield, with and without supplemental irrigation. Twenty diverse proso millet accessions, originating from Bulgaria, Czechoslovakia, Morocco, the former Soviet Union, Turkey, and the United States, were grown in irrigated and non-irrigated treatments under organic conditions in Pullman, WA, from 2012 to 2014. Irrigation was shown to significantly improve emergence and increase plant height at stem extension and to hasten ripening of all the varieties, whereas heading date was not affected by irrigation in two of the three years tested. Irrigation resulted in higher mean seed yield across all varieties, with ‘GR 665’ and ‘Earlybird’ performing best under irrigation. Seed yield was highest in ‘GR 658’ and ‘Minsum’ in the non-irrigated treatment, suggesting the importance of identification and utilization of varieties adapted to low rainfall conditions. The highest yielding varieties in irrigated systems are unlikely to be the highest yielding in dryland systems. Our results suggest that millet has potential as a regionally novel crop for inclusion in traditional dryland cropping rotations in the Palouse ecosystem, thereby contributing to increased cropping system diversity.

**Keywords:** irrigation; proso millet; Pacific Northwest; crop rotation; nutrition and health benefits

## 1. Introduction

Frequent and unpredictable drought conditions, combined with often inadequate access to irrigation, are permanent constraints to the optimization of agricultural production in many regions of the world [1,2]. Development and further refinement of dryland crop production approaches and strategic substitutions of water demanding crops with drought adapted crops can enhance the sustainability of future agricultural production in regions without a reliable supply of water [3,4]. Several species of millets are cultivated primarily in marginal agronomic environments due to their high-water use efficiency and can be grown in arid environments ranging from 200 mm to over 500 mm of average annual rainfall [1]. In addition to drought tolerance, millets can withstand intense heat and are resilient to the extreme climatic and soil conditions prevalent in semi-arid regions [5,6].

Proso millet (*Panicum miliaceum* L.), also known as white millet, red millet, broomcorn millet, common millet, broomtail millet, and hog millet, is a warm-season grass adapted to diverse soil and climatic conditions. The shallow root system of proso millet, usually limited to the upper 90 cm of soil, confers a comparatively high water use efficiency, and millet varieties are capable of producing seeds

from 60 to 110 days after planting [6–8]. Proso millet in the U.S. was grown, primarily as a dryland crop, on 204,366 ha in 2016 [9]. Irrigation is typically applied on approximately 4000 ha and about half of these hectares are located in Nebraska [10]. Close to 100 mm of water is required for the initiation of seed formation in proso millet, which is lower than for wheat (127 mm), sunflower (177 mm), and corn (228 mm) [8]. McDonald et al. [11] found that in Colorado, the total annual crop water requirement of proso millet is approximately 330 mm to 355 mm. Proso millet has been shown to produce grain using as little as 152 mm of total water, which is among the lowest of any major cereal [8].

Proso millet is considered the most suitable rotational crop in the majority of dryland wheat production areas in the semi-arid High Plains region of the U.S. [12]. When planted in a wheat/fallow rotation in the High Plains, proso millet improves the control of volunteer wheat and winter annual grassy weeds, reduces insect and disease pressure, and maintains adequate soil moisture for deeper rooted crops due to its shallow root systems and high water use efficiency [8,13,14].

In this study, we evaluated seed yield and important agronomic characteristics of 20 diverse proso millet accessions under irrigated and non-irrigated conditions in the wheat-based farming Palouse environment of eastern Washington, U.S. The overall goal of this study was to explore the potential for proso millet to be included as a rotational crop in dryland farming systems in the Palouse. Our specific objectives were to: (1) identify proso millet genotypes with enhanced emergence, early maturity, and optimal yield under dryland conditions; (2) evaluate proso millet seed yield in the Palouse environment when soil moisture is not the major limiting factor; and (3) determine whether the highest yielding millet varieties under irrigation were also the highest yielding varieties in dryland systems.

## 2. Materials and Methods

### 2.1. Location

A three-year study (2012–2014) was conducted on a certified organic research farm located at Tukey Orchard in Pullman, Washington (46.7325°N Lat., 117.1717°W Long.). Meteorological data were obtained from Pullman meteorological station situated at 46.7°N Lat., −117.15°W Long, and elevation 759.86 m. Pullman received total annual precipitation of 496 mm in 2012, 349 mm in 2013, and 430 mm 2014 [15]. The majority of this precipitation occurred during the winter and early in the spring. As is typical, summers were mostly dry and hot (Table 1).

**Table 1.** Precipitation and temperature recorded during the growing season (June to September of 2012, 2013, and 2014) in Pullman, WA.

Year	Months	Growing Degree Days at Planting	Total Precipitation [mm]	Average Maximum Day Temperature [°C]
2012	June	748	42.93	19.8
	July		0	27.8
	August		0	29.5
	September		0	24.6
2013	June	862	54.36	21.6
	July		4.83	29.6
	August		6.35	28.9
	September		55.12	22.1
2014	June	881	19.05	21.2
	July		11.18	30.8
	August		9.14	28.8
	September		4.06	24.2

Meteorological data were collected from Pullman, WA meteorological station situated at 46.7°N Lat., −117.15°W Long, and elevation 759.86 m. Source: [15].

## 2.2. Experimental Design and Data Collection

The experimental design was a split-plot randomized complete block design with three replicates. Main plots were irrigated and non-irrigated treatments. Sub-plots were 20 accessions (henceforth to be termed varieties) of proso millet (Table 2) obtained from USDA-ARS, National Resource Program, Iowa State University Regional Plant Introduction Station (Ames, IA, USA). The 20 varieties originated from six different countries including Bulgaria, Czechoslovakia, Morocco, the former Soviet Union, Turkey, and the United States. In years two and three of this experiment, seed was used from the previous year's trial. Care was taken to maintain varietal integrity and seed quality. Seed germination rate was above 98% for all accessions each year in germination tests performed at WSU. Plot size was 0.45 m<sup>2</sup>. Each plot was hand planted using 33 seeds each, with 30 cm spacing between plots. Growing degree days (GDD) at planting is shown in Table 1. GDD was calculated using a base temperature of 10 °C [15] and millet plots were harvested individually upon maturity.

**Table 2.** Twenty accessions of proso millet grown at the organic research farm at Tukey Orchard in Pullman, Washington in 2012, 2013, and 2014.

Item	Accession Number	Accession Name	Year Collected	Origin	Latitude	Longitude
1	PI 171727	Dari	1948	Bolu, Turkey	40.6792°N	31.5583°E
2	PI 346937	Tlicevskoje	1969	Former Soviet Union	N/A	N/A
3	PI 517017	GR 658	1986	Ouarzazate, Morocco	30.9167°N	6.9167°W
4	PI 517018	GR 664	1986	Ouarzazate, Morocco	30.9335°N	6.9370°W
5	PI 517019	GR 665	1986	Ouarzazate, Morocco	30.9335°N	6.9370°W
6	PI 531398	Bolgar 159	1989	Bulgaria	42.7500°N	25.5000°E
7	PI 531410	Kamusinszkoe 67	1989	Former Soviet Union	N/A	N/A
8	PI 531411	Komsomolskoe 996	1989	Former Soviet Union	N/A	N/A
9	PI 531412	Kazanskoe 176	1989	Former Soviet Union	N/A	N/A
10	PI 531429	Tuvinskoe	1989	Former Soviet Union	N/A	N/A
11	PI 531430	Veszelopodoljanskoe 403	1989	Former Soviet Union	N/A	N/A
12	PI 531431	Unikum	1989	Czechoslovakia	50.0833°N	14.4167°E
13	PI 536011	Sunup	1989	Nebraska, United States	41.2324°N	98.4160°W
14	PI 578073	Earlybird	1994	Nebraska, United States	41.2324°N	98.4160°W
15	PI 578074	Huntsman	1994	Nebraska, United States	41.2324°N	98.4160°W
16	PI 583347	Sunrise	1994	Nebraska, United States	41.2324°N	98.4160°W
17	PI 649382	Turghai	1961	North Dakota, United States	47.0000°N	100.0000°W
18	PI 649385	Minsum	1980	Minnesota, United States	46.0000°N	94.0000°W
19	PI 654403	TU-85-074-03	1986	Bitlis, Turkey	38.4000°N	42.1083°E
20	PI 654404	TU-85-087-01	1986	Bitlis, Turkey	38.4000°N	42.1083°E

N/A: Not available (The latitude and longitude information are not available).

Irrigation was applied at a rate of 24 mm/week using 15 mL high flow drip tape with 20 cm emitter spacing (Drip Works, Willit, CA, USA). Initial watering dates varied from year to year, 25 June 2012 (655 GDD), 9 July 2013 (641 GDD), and 9 June 2014 (872 GDD), and irrigation was applied twice weekly until harvest.

Percent emergence was estimated by counting the number of emerged seedlings per plot and dividing by 33. Plant height was measured using three randomly selected subsamples per plot at two growth stages: (1) Feekes 8, stem extension/flag leaf visible (PH1); and (2) Feekes 11.4, ripening (PH2) [16,17]. Plant height measurements at stem extension were taken on the following dates: 20 July 2012 (439 GDD), 30 July 2013 (465 GDD), and 10 August 2014 (347 GDD). Heading was quantified by the number of days from planting until 100% heading emergence. Maturity was measured as number of days from planting until harvest.

Plots were harvested individually at maturity using sickles to cut the stems of the plants. All plants in a plot were then bundled and threshed using a Vogel thresher (Bill's Welding, Pullman, WA, USA). The seeds were then processed with a 3.18 mm (8/64 inch) screen to separate the seed from the larger stem pieces by hands. Seeds were next rubbed to remove the remainder of the seed chaff from the seeds until clean. Seeds were further cleaned using a homemade air-blower to separate the smaller

particles and immature seeds from the mature seeds, then the seed was sieved through a 2.78 mm (7/64 inch) screen (Seedburo, Des Plaines, IL, USA) for final removal of any foreign plant material.

### 2.3. Statistical Analysis

Statistical analysis was performed using the statistical software SAS 9.2 (32) (SAS Institute Inc., Cary, NC, USA). Mixed effects methodology was used to analyze the response data. Continuous responses (plant height, days to heading and maturity, and yield) were either normally distributed or could be made sufficiently normal using a logarithmic transformation and were analyzed using a linear mixed model with PROC MIXED. The binary outcome (plant emergence) was analyzed using a logistic mixed model with PROC GLIMMIX (SAS 9.2). In two cases (plant emergence in 2013 and 2014), no random effects could be estimated and PROC GENMOD—which does not allow random effects—was used to estimate this simpler model. Fixed effects for each model included irrigation, variety, and their interactions. If an interaction and/or main effect were not significant, those terms were removed from the model. A random effect of replicate nested within irrigation status was included in the estimation of each model to account for correlation of measurements within plots. Due to yearly differences in the plants that did not emerge, interaction analyses for variety and year were not estimable and analyses were performed separately for each year. Model assumptions were verified using marginal and conditional studentized residuals from PROC MIXED and studentized residuals from PROC GLIMMIX. A logarithmic transformation was used for yield and plant height to satisfy the homogeneity of variance assumption. Contrasts were calculated to show which of the 20 varieties differed by irrigation status. The statistical significance level was set at  $\alpha = 0.05$ . In addition to raw  $p$ -values, to ensure an overall false positive level of 0.05 for each trait,  $p$ -values were adjusted for multiple comparisons using Hommel's procedure [18,19].

Spearman's rank correlation coefficient ( $R_s$ ) was used to determine the level of rank correlation between yield of all the 20 varieties in irrigated and non-irrigated treatments.  $R_s$  was calculated using the following equation:

$$R_s = 1 - \frac{6 \sum d^2}{n^3 - n}$$

where  $\sum d^2$  is the difference in rank change of each variety and summed for all 20 varieties, and  $n$  is the number of varieties. Statistical significance was assessed at the 5% significance level.

## 3. Results

### 3.1. Yield

In 2012, there was a significant variety  $\times$  irrigation interaction for yield ( $p = 0.0057$ ) (Table 3). When comparing responses of varieties across treatments, the results indicated that most of the varieties were significantly affected by irrigation except for Dari, Tlicevskoje, GR 658, GR 664, Bolgar 159, Tuvinskoe, Turghai, Minsum, TU-85-74-03, and TU-85-087-01 ( $p > 0.05$ ) (Table 4). In addition, the  $p$ -values adjusted for multiple comparisons further showed that GR 665, Komsomolskoe 996, and Earlybird were also not affected by irrigation ( $p > 0.05$ ). In contrast, Kamusinszkoe 67, Kazanskoe 176, Veszelopodoljanskoe 403, Unikum, Sunup, and Sunrise were significantly affected by irrigation ( $p < 0.05$ ). The irrigated treatment and non-irrigated treatment yielded a total average of 55 g/plot and 18 g/plot, respectively. The highest yielding variety within irrigated treatment was Veszelopodoljanskoe 403 (100 g/plot) and Minsum (50 g/plot) was the highest yielding in the non-irrigated treatment. In 2012, grain yield was moderately correlated with PH1 ( $r = 0.53$ ;  $p = 0.0004$ ) and strongly correlated with PH2 ( $r = 0.75$ ;  $p \leq 0.0001$ ); however, no correlation was found between grain yield and either days to heading or days to maturity.

**Table 3.** Analysis of Variance with F value for plant height, days to maturity, days to heading, and yield for proso millet varieties grown with and without irrigation over three crop years.

Years	Effect	DF	Emergence Rate	PH1	PH2	DH	DM	Yield
2012	Irrigation	1			197.77 **			81.89 ***
	Variety	19	N/A	18.06 *	3.34 ***	46.94 ***	12.87 ***	2.51 **
	Irrigation × Variety	19		3.20 ***	1.78 *			2.30 **
2013	Irrigation	1	22.26 ***		2062.74 ***	13.31	101.94 ***	
	Variety	19	248.74 ***	1304.54 ***	8.36 ***	17.59 ***	40.39 ***	3.35 ***
	Irrigation × Variety	19	39.42 **	3.37 ***	4.46 ***	6.05 *** (DF = 11)	3.13 ** (DF = 14))	2.37 **
2014	Irrigation	1	739.35 ***		122.48 ***		13.27 ***	43.03 ***
	Variety	19	91.04 ***	608.05 ***	2.16 *	96.97 ***	41.60 ***	2.99 ***
	Irrigation × Variety	14	76.58 ***				6.35 ***	

DF: Degrees of freedom; PH1: plant height at stem extension; PH2: plant height at ripening. DH: days to heading, DM: days to maturity. Significant level at ( $p < 0.05$ ) while \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ .

**Table 4.** Mean Difference between irrigation and no irrigation for each variety of each trait: plant height, days to maturity, days to heading, and yield.

Plant Name/Year	Emergence	PH1	PH2	DH	DM	Yield
<b>2012</b>						
Dari	NDC		4.12 ***	NIE	NIE	0.63
Tlicevskoje	NDC		4.45 ***	NIE	NIE	1.68
GR 658	NDC		6.20 ***	NIE	NIE	0.71 *
GR 664	NDC		6.00 ***	NIE	NIE	1.60
GR 665	NDC		8.16 ***	NIE	NIE	2.76 **
Bolgar 159	NDC		4.00 ***	NIE	NIE	1.18
Kamusinszkoe 67	NDC		4.85 ***	NIE	NIE	4.00 ***
Komsomolskoe 996	NDC		5.14 ***	NIE	NIE	2.89 **
Kazanskoe 176	NDC		6.32 ***	NIE	NIE	3.82 ***
Tuvinskoe	NDC	22.98 *	2.32 *	NIE	NIE	0.05
Veszelopodoljanskoe 403	NDC		5.18 ***	NIE	NIE	4.11 ***
Unikum	NDC		5.55 ***	NIE	NIE	4.00 ***
Sunup	NDC		3.18 **	NIE	NIE	3.80 ***
Earlybird	NDC		4.12 ***	NIE	NIE	2.83 **
Huntsman	NDC		5.38 ***	NIE	NIE	2.17 *
Sunrise	NDC		3.34 **	NIE	NIE	3.21 **
Turghai	NDC		4.40 ***	NIE	NIE	0.35
Minsum	NDC		5.10 ***	NIE	NIE	0.29
TU-85-074-03	NDC		4.57 ***	NIE	NIE	−0.50
TU-85-087-01	NDC		5.55 ***	NIE	NIE	1.26
<b>2013</b>						
Dari	1.04		14.39 ***	−2.86 **	−2.71 **	
Tlicevskoje	8.73 **		n/a	n/a	−3.25 **	
GR 658	11.22 ***		17.42 ***	3.44 **	n/a	
GR 664	6.41 *		17.43 ***	−0.42	−3.83 ***	
GR 665	1.93		19.35 ***	n/a	−4.75 ***	
Bolgar 159	3.09		10.61 ***	0.19	0.02	
Kamusinszkoe 67	6.87 **		9.73 ***	0.07	−0.21	
Komsomolskoe 996	3.83		16.20 ***	−3.23 **	−4.53 ***	
Kazanskoe 176	1.51		15.17 ***	−0.00	−0.00	
Tuvinskoe	2.52	63.32 ***	17.05 ***	−3.64 ***	−6.61 ***	95.04 ***
Veszelopodoljanskoe 403	1.55		16.04 ***	−3.61 **	−2.65 *	
Unikum	2.86		13.32 ***	−4.21 ***	−4.75 ***	
Sunup	0.07		13.44 ***	n/a	−2.10 *	
Earlybird	0.60		12.60 ***	n/a	−2.10 *	
Huntsman	0.13		n/a	n/a	n/a	
Sunrise	1.14		n/a	n/a	n/a	

Table 4. Cont.

Plant Name/Year	Emergence	PH1	PH2	DH	DM	Yield
Turghai	0.21		16.98 ***	n/a	n/a	
Minsum	3.88*		9.70 ***	n/a	−4.64 ***	
TU-85-074-03	0.07		n/a	−3.49 **	−1.88	
<b>2014</b>						
Dari	18.43 ***			NIE	−0.41	
Tlicevskoje	1720.2 ***			NIE	n/a	
GR 658	32.63 ***			NIE	−0.65	
GR 664	21.14 ***			NIE	−0.00	
GR 665	34.63 ***			NIE	−0.65	
Bolgar 159	1554.7 ***			NIE	n/a	
Kamusinszkoe 67	1516.2 ***			NIE	n/a	
Komsomolskoe 996	27.58 ***			NIE	−6.86 ***	
Kazanskoe 176	33.89 ***			NIE	−7.03 ***	
Tuvinskoe	12.93 ***	2.47 ***	65.49 ***	NIE	0.87 *	3.37 ***
Veszelopodoljanskoe 403	29.20 ***			NIE	−1.31 *	
Unikum	40.80 ***			NIE	−0.46	
Sunup	14.59 ***			NIE	0.44	
Earlybird	14.89 ***			NIE	1.23 *	
Huntsman	8.65 **			NIE	0.00	
Sunrise	16.22 ***			NIE	0.00	
Turghai	1.59			NIE	0.55	
Minsum	1431.1 ***			NIE	n/a	
TU-85-074-03	1521.0 ***			NIE	n/a	
TU-85-087-01	15.86 ***			NIE	0.00	

PH1: plant height at stem extension; PH2: plant height at ripening. DH: days to heading, DM: days to maturity. NDC: No data was recorded (in 2012, emergence data were not recorded); n/a: not available (missing data). Significant level at ( $p < 0.05$ ) while \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$  ( $p$ -values not adjusted). When interaction effect was significant, mean differences are shown for each variety; when irrigation effect was significant but interaction was not, mean differences are the same for each variety; when no interaction or irrigation main effect was significant, NIE (no irrigation effect) was displayed.

In 2013, grain yield was significantly affected by irrigation. When comparing responses of varieties across treatments, all varieties were significantly affected by irrigation ( $p = 0.0002$ ) (Table 3). This is different from 2012, where some varieties did have significantly higher yields in response to irrigation. These results are interesting due to the increased precipitation in 2013 (~121 mm from June to September) compared to 2012 (~43 mm from June to September). Average yields of irrigated and non-irrigated treatments were 207 g/plot and 5 g/plot, respectively. The highest yielding cultivar within the irrigated treatment was Earlybird (365 g/plot) and Unikum (14 g/plot) was the highest yielding in the non-irrigated treatment. Similar to 2012, grain yield in 2013 was strongly correlated ( $p < 0.0001$ ) to both PH1 and PH2 ( $r = 0.81$  and  $r = 0.87$ , respectively). Again, no relationship was found between yield and either days to heading or days to maturity; however, a marginally significant and relatively weak correlation was found between yield and emergence ( $r = 0.28$ ;  $p = 0.09$ ).

In 2014, yield was significantly affected by irrigation; when comparing responses of varieties across treatments, all varieties were significantly affected by irrigation ( $p = 0.0001$ ), similar to the results found in 2013 (Table 3). The irrigated and non-irrigated treatments had mean plot yields of 21 g/plot and 4 g/plot, respectively. The highest yielding variety within the irrigated treatment was GR 665 (112 g/plot), and Turghai and Sunup (12 g/plot each) were the highest yielding varieties in the non-irrigated treatment (Table 5). Pearson correlation results in 2014 were quite different from 2012 and 2013. For example, no relationship was found between grain yield and PH1, or emergence, but correlations were found between grain yield and days to heading ( $r = 0.55$ ;  $p = 0.005$ ), days to maturity ( $r = 0.47$ ;  $p = 0.02$ ), and PH2 ( $r = 0.45$ ,  $p < 0.05$ ).

**Table 5.** Mean data across years 2012, 2013, and 2014 for each trait in irrigated and non-irrigated treatments.

Plant Name/Year	Emergence Rate (%)		PH1 (cm)		PH2 (cm)		DH (Day)		DM (Day)		Yield (g/Plot)	
	Ir.	N-Ir.	Ir.	N-Ir.	Ir.	N-Ir.	Ir.	N-Ir.	Ir.	N-Ir.	Ir.	N-Ir.
<b>2012</b>												
Dari	n/a	n/a	93	68	135	101	47	46	90	88	28	16
Tlicevskoje	n/a	n/a	74	58	129	93	71	75	110	105	71	25
GR 658	n/a	n/a	71	56	145	95	69	73	108	108	54	29
GR 664	n/a	n/a	80	57	153	104	72	69	110	108	69	21
GR 665	n/a	n/a	69	48	149	82	71	73	108	110	95	19
Bolgar 159	n/a	n/a	85	59	127	95	44	45	81	82	68	26
Kamusinszkoe 67	n/a	n/a	86	65	129	89	50	50	80	88	35	2
Komsomolskoe 996	n/a	n/a	90	70	135	93	50	49	81	90	30	6
Kazanskoe 176	n/a	n/a	92	59	124	72	45	44	79	90	40	4
Tuvinskoe	n/a	n/a	92	81	129	110	50	48	82	79	67	38
Veszelopodoljanskoe 403	n/a	n/a	84	63	134	92	50	49	81	90	100	6
Unikum	n/a	n/a	83	47	130	85	48	51	84	85	72	4
Sunup	n/a	n/a	90	68	132	106	54	52	88	98	61	5
Earlybird	n/a	n/a	79	60	128	94	58	52	91	91	49	12
Huntsman	n/a	n/a	75	53	127	83	66	62	95	101	53	11
Sunrise	n/a	n/a	79	60	119	91	59	56	91	93	31	5
Turghai	n/a	n/a	87	65	143	107	50	47	81	79	46	29
Minsum	n/a	n/a	85	62	140	98	53	53	85	86	76	50
TU-85-074-03	n/a	n/a	84	64	134	96	56	55	91	84	26	45
TU-85-087-01	n/a	n/a	86	66	136	91	52	51	91	85	25	9
Mean			83	61	134	94	56	55	90	92	55	18
LSD ( $p < 0.05$ )			2.5		5		4		8.1		11.5	
<b>2013</b>												
Dari	43	31.7	95	22	141	15	60	70	86	98	164	9
Tlicevskoje	83.3	45	78	23	157	37	76	n/a	114	125	238	1
GR 658	63.3	21.7	86	28	164	n/a	76	66	108	n/a	180	n/a
GR 664	83.3	50	92	28	168	49	74	76	108	121	279	5
GR 665	66.7	83.3	91	31	161	43	76	n/a	108	122	290	9
Bolgar 159	48.3	28.3	82	27	121	29	60	60	83	82	89	1
Kamusinszkoe 67	41.7	13.3	88	33	129	45	66	66	90	91	166	9
Komsomolskoe 996	66.7	43.3	92	30	137	37	66	73	91	105	271	8
Kazanskoe 176	60	45	92	34	125	32	60	60	83	83	184	2
Tuvinskoe	38.3	21.7	86	28	143	26	64	76	86	108	88	2
Veszelopodoljanskoe 403	66.7	50	92	32	136	37	64	73	91	99	198	3
Unikum	93.3	78.3	96	37	130	49	60	69	88	102	268	14
Sunup	28.3	30	71	22	151	33	74	n/a	108	117	344	1
Earlybird	40	30	83	24	146	36	72	n/a	108	117	365	8
Huntsman	11.7	15	64	n/a	140	n/a	76	n/a	108	n/a	155	n/a
Sunrise	35	25	79	n/a	155	n/a	74	n/a	108	n/a	336	n/a
Turghai	10	6.7	72	n/a	146	n/a	66	n/a	90	n/a	64	n/a
Minsum	30	53.3	79	27	152	35	69	n/a	101	117	227	3
TU-85-074-03	26.7	31.7	78	31	124	38	66	76	90	98	129	3
TU-85-087-01	21.7	8.3	93	33	121	n/a	64	66	87	n/a	97	n/a
Mean	48	35	84	29	142	36	68	69	97	106	206	5
LSD ( $p < 0.05$ )	25		3		8		4		3		43	
<b>2014</b>												
Dari	48	12	90	19	96	32	49	49	92	93	5	2
Tlicevskoje	83	n/a	75	n/a	75	n/a	74	n/a	113	n/a	19	n/a
GR 658	74	12	78	17	100	17	73	75	111	113	26	n/a
GR 664	85	3	81	n/a	94	n/a	79	80	113	113	28	n/a
GR 665	74	15	87	7	98	11	71	70	111	113	112	n/a
Bolgar 159	56	n/a	69	n/a	69	n/a	45	n/a	94	n/a	11	n/a
Kamusinszkoe 67	47	n/a	83	n/a	80	n/a	51	n/a	93	n/a	4	n/a
Komsomolskoe 996	66	12	77	5	91	17	51	51	92	113	8	1
Kazanskoe 176	73	8	92	28	91	31	48	48	86	103	6	n/a
Tuvinskoe	36	12	78	10	67	13	51	n/a	89	86	n/a	n/a
Veszelopodoljanskoe 403	65	18	90	19	74	16	51	51	93	97	12	n/a



Table 5. Cont.

Plant Name/Year	Emergence Rate (%)		PH1 (cm)		PH2 (cm)		DH (Day)		DM (Day)		Yield (g/Plot)	
	Ir.	N-Ir.	Ir.	N-Ir.	Ir.	N-Ir.	Ir.	N-Ir.	Ir.	N-Ir.	Ir.	N-Ir.
Unikum	96	15	94	16	69	14	49	49	92	93	16	n/a
Sunup	40	18	73	9	99	24	54	54	94	93	19	12
Earlybird	49	14	82	10	86	25	55	51	96	93	24	4
Huntsman	26	9	66	10	90	9	70	70	113	113	26	n/a
Sunrise	45	9	75	5	96	7	65	65	113	113	39	n/a
Turghai	17	14	80	13	113	60	51	51	94	93	26	12
Minsum	30	n/a	78	n/a	109	n/a	54	n/a	93	n/a	34	n/a
TU-85-074-03	48	n/a	75	n/a	47	n/a	54	n/a	96	n/a	4	n/a
TU-85-087-01	42	15	79	16	62	18	55	55	86	86	7	n/a
Mean	55	12.4	80	13	85	21	57	58	98	101	22	6.2
LSD ( $p < 0.05$ )	12		3		8		1		3		7	

Ir: irrigation (irrigated treatment); N-Ir: non-irrigation (non-irrigated treatment); n/a: not available (there was not data available). For the case of 2012 emergence rate data were not recorded. LSD: Least Significant Deference. LSD comparisons significant at the 0.05 level.

### 3.2. Emergence and Plant Height

Irrigation had a significant impact on plant emergence and stand establishment. Mean emergence rates were 52% in irrigated treatments and 24% in the non-irrigated treatments across all of the three growing seasons. In 2013, there was a significant interaction between irrigation and variety ( $p = 0.0039$ ) (Table 3). Irrigation significantly affected the emergence rates of the varieties Tlicevskoje, GR 658, GR 664, Kamusinszkoe 67, and Minsum (Table 4). Hommel-adjusted  $p$ -values were estimated ( $p > 0.05$ ) across all varieties except variety GR 658 ( $p = 0.0162$ ). In 2014, there was a significant interaction between irrigation and variety ( $p < 0.0001$ ); irrigation significantly affected the emergence rate of all the varieties ( $p < 0.0001$ ) except Turghai ( $p = 0.2071$ ) (Tables 3 and 4). Hommel-adjusted  $p$ -values were estimated ( $p < 0.05$ ) across all varieties except Turghai ( $p = 0.2071$ ). In 2013 and 2014, there was a significant interaction between variety and irrigation on emergence (Table 3).

Across all three years, the varieties with the highest emergence rates under irrigation were Unikum, GR 664, Tlicevskoje, GR 665, and GR 658, with 94%, 84%, 83%, 70%, and 68% emergence, respectively, whereas Turghai, Huntsman, Minsum, TU-85-087-01, and Sunup had 13%, 19%, 30%, 32%, and 34% emergence, respectively. In the non-irrigated treatment, Minsum, GR 665, Unikum, Tlicevskoje, and Veszolopodoljanskoe 403 had the highest emergence rates, with 53%, 49%, 46%, 45%, and 34%, respectively. Turghai, TU-85-087-01, Huntsman, Kamusinszkoe 67, and Tuvinskoe had low emergence rates with 10%, 11%, 12%, 13%, and 17% in the non-irrigated treatment, respectively (Table 5). Turghai, TU-85-087-01, and Huntsman showed the lowest emergence across irrigation treatment, whereas GR 665, Unikum, and Tlicevskoje had among the highest emergence across irrigation treatment. Minsum had 30% emergence in the irrigated treatment, and 53% emergence in the non-irrigated treatment.

In all three years, irrigation had a significant effect on PH1 and PH2 across all varieties ( $p < 0.0001$ ) (Table 4). A significant interaction between irrigation and variety was detected at PH2 in 2012 and 2013 (Table 3). No significant variety  $\times$  irrigation interaction was found at stem extension (PH1) in any of the three years tested (Table 3). Several varieties in the irrigated treatment lodged before harvest. In 2012, lodged varieties included Unikum, TU-85-074.03, and TU-85-087-01. In 2013 and 2014, varieties that lodged before harvest included Dari, Tuvinskoe, Veszolopodoljanskoe 403, Unikum, Minsum, and TU-85-074-03. Other varieties either remained completely upright or showed minor lodging. For all three years of the experiment the non-irrigated plots did not show any signs of lodging.

### 3.3. Days to Heading and Maturity

Irrigation did not have an effect on heading date in 2012 and 2014; however, in 2013, a significant variety  $\times$  irrigation interaction was observed (Table 3). In 2013, days to heading of varieties such as



Dari, GR 658, Komsomolskoe 996, Tuvinskoe, Veszelopodljanskoe 403, Unikum, and TU-85-074-03 were significantly affected by irrigation treatment ( $p < 0.05$ ) (Table 4).

Turghai, Bolgar 159, Kazanskoe 176, Dari, and Earlybird had the earliest heading dates in both irrigated and non-irrigated fields with 49, 50, 51, 52, and 52 days, respectively. GR 664, Tlicevskoje, GR 658, GR 665, and Huntsman had delayed heading dates in both irrigated and non-irrigated treatments at 75, 75, 73, 71, and 71 days, respectively (Table 5). Days to heading was strongly correlated to days to maturity ( $r = 0.76$ ;  $p < 0.0001$ ) and moderately correlated to grain yield ( $r = 0.43$ ;  $p < 0.0001$ ) (Table 6), but showed no relationship with other traits.

**Table 6.** Pearson correlation coefficient for percent emergence (PE), plant height (PH1 and PH2), days to heading (DH), days to maturity (DM), and yield, from 2012 to 2014.

	PH1	PH2	DH	DM
PH1				
PH2	0.86 ***			
DH	−0.12	0.09		
DM	−0.37 ***	−0.23 *	0.76 ***	
Yield	0.45 ***	0.65 ***	0.42 ***	0.11

\*  $p < 0.05$ , \*\*\*  $p < 0.001$ .

All varieties except Bolgar 159, Kamusinszkoe 67, Kazanskoe 176, and TU-85-074-03 were affected by irrigation (Table 4). In addition to those varieties that were not affected by irrigation, the Hommel-adjusted  $p$ -values indicated that maturity for Dari, Veszelopodljanskoe 403, Sunup, and Earlybird were also not affected by irrigation ( $p > 0.05$ ). Tlicevskoje, GR 664, GR 665, Komsomolskoe 996, Tuvinskoe, Unikum, and Minsum were significantly affected by irrigation ( $p < 0.05$ ).

In 2014, there was a highly significant irrigation  $\times$  variety interaction ( $p < 0.0001$ ) (Table 3). Irrigation had no effect on time to maturity for all varieties tested except Komsomolskoe 996 and Kazanskoe 176 ( $p < 0.0001$ ) (Table 4). Hommel-adjusted  $p$ -values were estimated ( $p < 0.0001$ ) for the same varieties (Komsomolskoe 996 and Kazanskoe 176) (Table 4). However, our data were not consistent with other reports on soybean [20], pea [21], and corn [22], where water deficit decreased seed-filling duration. Across all three years, the five varieties with the quickest maturity in both irrigated and non-irrigated fields were Bolgar 159, Kamusinszkoe 67, TU-85-087-01, Turghai, and Tuvinskoe with 82, 83, 85, 86, and 86 days, respectively. Tlicevskoje, GR 665, GR 664, GR 658, and Huntsman required more days to maturity in both irrigated and non-irrigated with 115, 115, 114, 110, and 107 days, respectively (Table 5). Days to maturity was negatively correlated with PH1 ( $r = -0.37$ ;  $p < 0.0001$ ) and PH2 ( $r = -0.23$ ;  $p = 0.017$ ) (Table 6).

## 4. Discussion

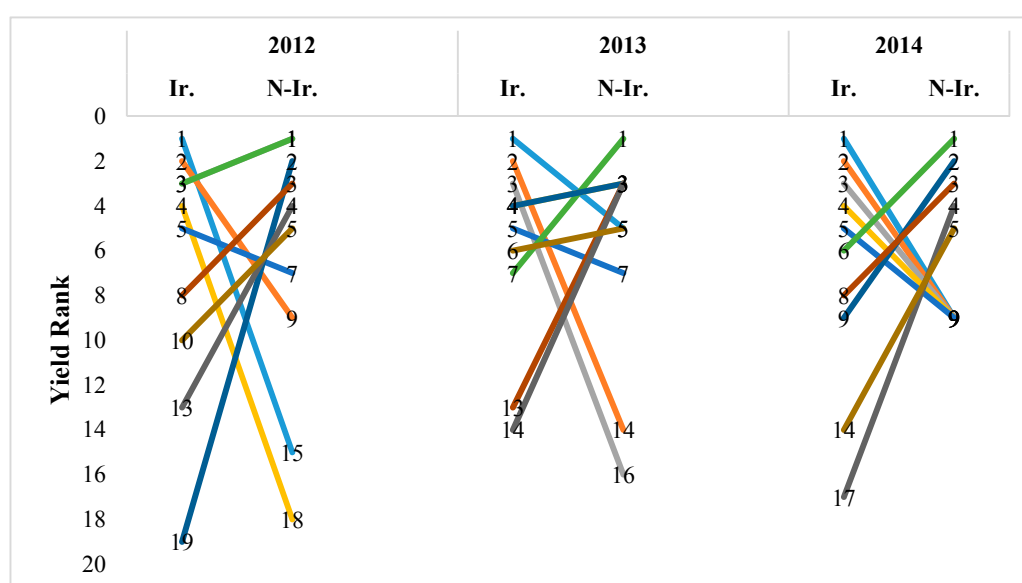
### 4.1. Yield

Selection of varieties with high grain yields in drought stressed conditions is a major goal in many cereal breeding programs [23]. Grain yield is the result of the expression and association of several plant growth components and conditions [24]. Abiotic stressors such as extreme temperature and low water availability are often the most important restricting factors in the growth and productivity of major cereal crop species [2,25]. As expected, irrigation increased grain yield of proso millet significantly in this present study (Table 4). Across all three years, grain yields were highest in irrigated treatments (Table 5) and the top five yielding varieties were GR 665, Earlybird, Sunup, Sunrise, and GR 664 with 166, 146, 141, 136, and 125 g/plot, respectively.

These results are consistent with previous reports which showed that water stress in millet reduced seed yield [26]. Yadav et al. [27] reported that grain yield decreased 5% to 19% in terminal drought stress environments compared to a fully irrigated treatment. In this study, we were able

to identify specific varieties which yielded well despite conditions of drought. GR 658, Minsum, TU-85-074-03, Turghai, and Tuvinskoe were high yielding in the non-irrigated treatments with 29, 27, 24, 21, and 20 g/plot, respectively. The first two weeks after planting are a critical period when determining the success of a proso millet crop in the Palouse. In 2012, even though drought was the most severe from July to September, not all varieties were responsive to irrigation due to the adequate precipitation received in June during the first two weeks after planting in 2012. This is in contrast to 2014, which had precipitation more evenly distributed over the growing season, but significantly less during the important weeks directly after planting. Reduction of grain yield under conditions of drought stress can be caused by several regulative mechanisms that plants use to withstand against water stress, such as reduction in number of tiller for millet and reduction in ear size for maize [26,28]. Hussain et al. [29] reported that drought causes impaired mitosis, cell elongation, and expansion, leading to the reduction of plant growth and yield traits.

Across all three years, grain yield was positively correlated with plant height ( $r = 0.45$ ,  $p < 0.0001$  for PH1;  $r = 0.65$ ,  $p < 0.0001$  for PH2) and days to heading ( $r = 0.42$ ,  $p < 0.0001$ ), but did not show any relationship with days to maturity (Table 6). Spearman's rank correlation coefficient ( $R_S$ ) for yield was non-significant ( $p < 0.05$ ) for varietal changes in rank between irrigated and non-irrigated treatments.  $R_S$  was 0.18, 0.40, and 0.29 in 2012, 2013, and 2014, respectively (Figure 1). Because there was no significant correlation in rank among the twenty varieties for yield between irrigated and non-irrigated treatments (Figure 1), we suggest that the varieties optimally adapted to dryland farming systems are not necessarily the same varieties best adapted to irrigated farming systems. To test for the optimal irrigation requirements of proso millet in the Palouse, a potential next step would be to choose a smaller set of varieties, and grow them in larger plots with more irrigation treatments.



**Figure 1.** Spearman's rank yield correlation irrigated vs non-irrigation treatments. The yield change in rank between Ir: irrigation (irrigated treatment) and N-Ir: non-irrigation (non-irrigated treatment) of proso millet varieties. The top five ranking varieties for yield in both irrigated and non-irrigated treatments were compared at each year.  $R_S$ : Spearman's rank correlation. Varieties are ranked from 1 = highest yield to 20 = lowest yield.  $R_S = 0.18$  (2012),  $R_S = 0.40$  (2013), and  $r = 0.29$  (2014). Spearman's rank correlation coefficient, tested ( $p < 0.05$ ).

#### 4.2. Emergence and Plant Height

Water deficit causes impaired germination and poor crop stand establishment [30], and water availability during the first two weeks after planting proso millet are the most critical periods

when growing proso millet; during this period even a light rain can be very helpful in boosting germination rates [14]. Irrigation had a significant impact on plant emergence and stand establishment. Mean emergence rates were 52% in irrigated treatments and 24% in the non-irrigated treatments across all of the three growing seasons. Similarly, in sunflower, Kaya [31] reported that water deficit severely reduced germination and seedling stand, and delayed germination by one to two days.

In the present study, the varieties Minsum, GR 665, Unikum, Tlicevskoje, and Veszelopodoljanskoe 403 had the highest emergence rates without supplemental irrigation, whereas Turghai, TU-85-087-01, Huntsman, Kamusinszkoe 67, and Tuvinskoe had the lowest emergence rates (Table 5). This information is particularly useful to farmers without access to irrigation. Interestingly, Minsum was the only variety to consistently have higher emergence in the non-irrigated treatment than in the irrigated treatment. Across years and treatments, emergence was positively correlated with plant height ( $r = 0.60$ ;  $p < 0.0001$  for PH1;  $r = 0.41$ ;  $p < 0.0001$  for PH2) but did not show any relationship with emergence, days to heading, or days to maturity (Table 6).

Across all three years, each variety in the irrigated treatment was taller than in the non-irrigated treatment at PH1 and ripening (PH2) (Table 5). Similarly, drought stress and water scarcity have been reported to reduce plant height on switchgrass (*Panicum virgatum*), channel millet (*Echinochloa turneriana*), barnyard millet (*Echinochloa crus-galli*), and pearl millet (*Pennisetum americanum*) [32,33]. Drought reduces leaf size, stem extension, and root proliferation, and this causes disruption of photosynthetic pigments and reduces the gas exchange leading to a reduction in plant growth and productivity [23,24]. Cell elongation of higher plants can be inhibited by interruption of water flow from xylem to the surrounding elongating cells under water deficit conditions [34].

#### 4.3. Days to Heading and Maturity

The appropriate matching of the pattern of inflorescence development and the time of flowering to the temporal variation in water availability is recognized as one of the most important traits conferring adaptation to drought [35,36].

The process of grain filling, the accumulation of reserve nutrients in the developing and maturing grain, is also sensitive to environmental conditions strongly affecting final yield [37]. Our study indicated that no irrigation effect was observed across varieties in 2012 for days to maturity. In 2013, there was a significant variety  $\times$  irrigation interaction ( $p = 0.0015$ ) (Table 3). Days to maturity was negatively correlated with PH1 ( $r = -0.37$ ;  $p < 0.0001$ ) and PH2 ( $r = -0.23$ ;  $p = 0.017$ ) (Table 6), indicating that the shorter plants tended to mature more quickly than the taller plants.

The most significant factors for heat stress-related yield loss in cereals include the high-temperature-induced shortening of development of vegetative phases, reduced light perception over the shortened life cycle, and perturbation of the processes associated with carbon assimilation (transpiration, photosynthesis, and respiration) [38]. It is critical to recognize how a particular crop shows signs of sensitivity to drought stress during floral initiation. Drought stress in barley was shown to be more sensitive during and just prior to spike emergence [39–41]. Water stress during flowering induction and inflorescence development was reported to cause a delay in flowering (anthesis) in pearl millet and sorghum [25]. Wopereis et al. [42] also reported a delay in flowering and maturity of two lowland rice cultivars caused by drought stress. However, the results from our study indicated that this does not necessarily apply to proso millet; with few exceptions, water stress did not affect flowering of proso millet.

## 5. Conclusions

Irrigation resulted in higher mean seed yield across all varieties, with 'GR 665' and 'Earlybird' performing best under irrigation, and 'GR 658' and 'Minsum' achieving the highest yields in the non-irrigated treatment. The Spearman's rank correlation coefficient for yield was non-significant across varieties between irrigated and non-irrigated treatments. Irrigation was shown to significantly

improve emergence and increase plant height at stem extension and ripening of all the varieties; whereas heading date was not affected by irrigation in two of the three years tested. Interestingly, Minsum was the only proso millet variety which achieved higher percent emergence under dryland conditions than under irrigation. This could be a useful trait to Palouse farmers, and future studies should explore and exploit possible mechanisms of, and explanations for, this important trait.

Our results indicate that: (1) the highest yielding varieties in irrigated systems are unlikely to be the highest yielding in dryland systems; and (2) in order to optimize yield of proso millet in dryland conditions, it is necessary to identify and utilize varieties adapted to low rainfall conditions. Our results further show that although irrigation results in higher yields compared to dryland production, the increased plant height due to irrigation also can result in lodging in certain varieties. Therefore, selection of millet varieties should be conducted with the production system of the target farmers in mind.

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**Conflicts of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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