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Adaptation of Quinoa (*Chenopodium quinoa* Willd.) to Australian Environments

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Abstract: Quinoa is being evaluated in cropping systems in many countries outside of its natural range of South America. Very few attempts have been made by farmers or researchers to grow or evaluate quinoa under Australian environments. Given the growing popularity of quinoa with consumers, new commercial opportunities for farmers and international interest in the crop, it was timely to undertake a comprehensive evaluation of the potential of quinoa in Australia. Two advanced selections and nine germplasm lines (six of Chilean and three of Bolivian origin) identified in an earlier project were tested in 23 field trials at 14 locations on mainland Australia. Targets included irrigated sites in tropical, Mediterranean, semi-arid and desert climates, and rain-fed sites of south-western Australia with a Mediterranean climate. The field experiments were either a randomised complete block design (RBCD) or a split plot/factorial design with 2–4 replicates, and a linear mixed model was used to compare the treatment lines. Seed yield of quinoa was highest when grown in winter and spring under rain-fed conditions in Geraldton, in spring and summer under irrigation at Bool Lagoon, and summer, autumn and winter under irrigation at Leeton. The highest seed yield achieved was 3 t/ha for a germplasm line from Chile, while the highest yield for a germplasm line from Bolivia was 2.6 t/ha. Advanced selections from Australia yielded well in comparison at most trial sites. Declining seed yield was associated with mean daily temperatures during seed development increasing above 17 °C, mean daily temperatures during flowering declining below 15 °C, and rainfall during seed development under rain-fed conditions falling below 50 mm. Seed produced at Bool Lagoon was the closest in colour to white quinoa imported from Peru; however, it was more than noticeably different. Seed produced at Geraldton and Leeton was significantly larger than from other field sites; however, none were larger than 2 mm in diameter as found in Royal white quinoa from Bolivia. Superior seed colour and seed size were associated with dry conditions at maturity and cool conditions during seed development, respectively. We conclude that quinoa can become a potential crop option for Australian agriculture by exploiting genetic diversity and supplementing with suitable management practices matched to agro-climatic environments. There are reasonable prospects to raise the seed yield potential in areas in all states, especially in the regions where quinoa grew well in our experiments.

Keywords: quinoa; germplasm; adaptation; climate; yield; quality

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1. Introduction

Most of the world production of quinoa (*Chenopodium quinoa* Willd.) takes place at high altitudes in Peru and Bolivia, with smaller production in neighbouring countries including Ecuador, Chile and Argentina. Countries outside of South America also have

smaller production including USA, Canada, France, Spain and China [1]. By 2015, ninety-five countries are stated as having grown or tested quinoa, including Australia [2]. In Australia, quinoa was first grown commercially in northern Tasmania where it continues to be supplied to select retail outlets [3]. In 2015, a larger scale of production was undertaken under rain-fed conditions near Narrogin in Western Australia [4]. Production levels dropped soon after due partly to unfavourable weather conditions. Just prior to this time a small research project was undertaken to evaluate the potential of quinoa in the Western Australian wheatbelt [5]. In 2015, quinoa was grown under irrigation at Kununurra in the far north of Western Australia where plant selection and seed multiplication were undertaken. Results of the initial project were the catalyst to further funding the development of quinoa for Australian environments [6]. Activities included developing quality specifications for quinoa produced in Australia, investigating alternative methods of saponin removal, developing a rapid test for measurement of saponin [7], field testing quinoa germplasm at multiple sites across Australia and commercialisation of advanced selections made in the earlier project.

The production of quinoa outside of its countries of origin has increased in the past 10 to 15 years, particularly in Europe, Asia and North America. Most of this quinoa is grown with irrigation while the success of rain-fed quinoa has been limited to far fewer environments. Testing the suitability of quinoa to new environments has been undertaken at different levels of sophistication. Most have been restricted in terms of number of test sites and germplasm lines. Some genotype × environment studies at both a national and international level have been effective in identification of superior germplasm lines of quinoa to new environments [8–10].

This work aims to report on the first widespread field testing of a range of landrace germplasm and advanced selections of quinoa in Australia. Field testing sites were at a range of latitudes from the northern tropics to the southern Mediterranean regions, and both irrigated and rain-fed farming systems. Specifically, an attempt was made to explain the performance of quinoa, in terms of both seed yield and seed quality, as affected by temperature and rainfall with the aim of identifying regions in Australia where quinoa is best adapted for commercial production.

2. Materials and Methods

2.1. Germplasm

Six accessions originating from coastal regions of Chile south of Santiago and two accessions originating from the highlands of Bolivia identified in earlier research as having the best potential [5] were sourced from the United States National Plant Germplasm System (NPGS) [11] (Table 1). Seed was multiplied at the Department of Primary Industries and Regional Development (DPIRD) Manjimup Research Station between January and May 2016 for use in field trials. Two advanced lines (referred to here as 'varieties') originating from the same earlier research project in Western Australia were used as control varieties in field trials. Their origin is uncertain. However, BEW was an undocumented selection from a bulk planting of 30 germplasm lines originating from NPGS, and JC1 originated from an unconfirmed source from within Australia. They were multiplied at the DPIRD Kununurra Research Station in 2014. BEW was renamed variety 'Kruso White Þ' in 2019. The origin of PI 433232 is documented as Groben, Chile but is likely to be a misspelling of Gorbea, Chile.

Table 1. Origin of two varieties and eight germplasm lines (accessions) used in field trials.

Name	Accession Name	Origin	Latitude, Longitude (dec.deg)	Altitude (m a.s.l.)
BEW	DQS2	Unknown		
JC1	DQS1	Unknown		
Chile				
PI 433232	AusTRCF 132975	Groben, Chile (possibly Gorbea, south of Temuco)	−39.10, −72.67	100
PI 614885	QQ57	Faro Ranch, Chillan	−36.60, −72.12	120
PI 614888	QQ61	Curanilahue	−37.48, −73.34	50
PI 634918	Baer	Cajon, near Temuco	−38.68, −72.51	100
PI 634919	Pichaman	Coast near Constitucion	−35.33, −72.42	100
PI 634925	UDEC-3	Llico, north of Constitucion	−34.76, −72.08	50
Bolivia				
PI 470932	Pasankalla (Pasan Ralle)	Belen Research Station	−16.04, −68.69	3800
PI 665278	Ames 13220	Patacamaya	−17.23, −67.92	3700

2.2. Trial Site Information

Sites for field trials were selected from a broad range of climates from the northern tropics to the southern temperate regions (Table 2, Figure 1). Both irrigated and rain-fed systems were targeted and systems that suit autumn, winter and spring plantings. Soil preference for quinoa is not well documented, hence a broad range of soil types were selected across the trial sites (Table 3).

Table 2. Description and location of quinoa trial sites in Australia.

Trial Grouping	Rain-Fed or Irrigated	Growing Season	Climate Classification [1]	Trial Location
Northern tropics	Irrigated	Winter	Hot semi-arid	Kununurra
			Tropical savanna	Katherine, Walkamin
Mediterranean	Rain-fed	Winter, spring	Hot-summer Mediterranean	Geraldton, Eradu, Mingenew, Cunderdin, Northam
			Warm-summer Mediterranean	Esperance, Katanning
	Irrigated and/or rain-fed	Spring, summer	Warm-summer Mediterranean	Manjimup, Bool Lagoon
Semi-arid	Irrigated	Variable	Cold semi-arid	Leeton
Desert	Irrigated	Variable	Hot desert	Alice Springs

[1] Climates according to Koppen–Geiger Classification [12].

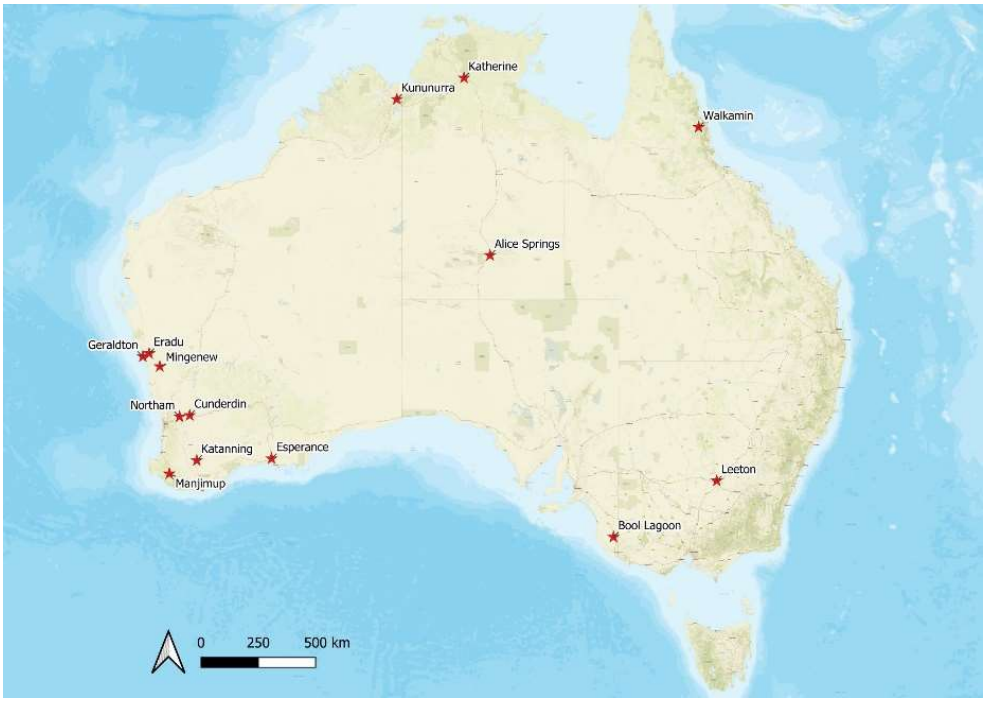


Figure 1. Map of trial sites.

Table 3. Latitude, longitude, altitude and soil texture at trial sites.

Trial Location	Latitude, Longitude (dec.deg)	Altitude (m a.s.l.)	Soil Texture
Kununurra	−15.652, 128.719	35	Grey self-mulching clay
Katherine	−14.513, 132.438	200	Red Kandasol
Walkamin	−17.134, 145.427	600	Brown clay
Mingenew	−29.260, 115.598	210	Red clayey sand
Cunderdin	−31.590, 117.255	225	Grey sandy loam
Geraldton	−28.779, 114.658	26	Red clayey sandy loam
Eradu	−28.634, 115.009	200	Course yellow sand
Northam	−31.653, 116.693	160	Grey/brown hard setting clay
Esperance	−33.604, 121.783	160	Loamy sand
Katanning	−33.688, 117.644	290	Grey sandy loam
Manjimup	−34.298, 116.125	260	Red/brown sandy loam
Bool Lagoon	−37.137, 140.714	50	Self-mulching black cracking clays (Rendzina)
Leeton	−34.621, 146.427	150	Grey self-mulching soils
Alice Springs	−23.767, 133.879	560	Clay loam

2.3. Weather Data for Field Trials

Temperature and rainfall estimates for trial sites are based on data from selected weather stations at the Australian Bureau of Meteorology (BOM) [13] or from a DPIRD or Natural Resource Management (NRM) weather station. The distances between weather stations and trial sites are detailed below.

Kununurra—Aero, BOM weather station 002056, 14.2 km from trial site;
Katherine—Tindal RAAF, BOM weather station 014962, 10.0 km from trial site;

Walkamin—Research Station, BOM weather station 031108, at trial site;
Mingenew—Yandanooka, BOM weather station 008143, 4.7 km from trial site for rainfall; DPIRD weather station 'Dudawa' 12.5 km from trial site for temperature;
Cunderdin—Airfield, BOM weather station 010286, 4.5 km from trial site;
Geraldton—Airport, BOM weather station 008315, 4.7 km from trial site;
Eradu—DPIRD weather station 'Eradu' 7.0 km from trial site;
Esperance—Aero, BOM weather station 009542, 9.5 km from trial site for temperature; Research Station at trial site for rainfall;
Manjimup—BOM weather station 009573, 4.2 km from trial site;
Bool Lagoon—NRM weather station 'Robertson' at trial site;
Leeton—Yanco Agricultural Institute, BOM weather station 074037, at trial site.

2.4. Field Trials

Kununurra—Trial 1 (2015) was a randomized complete block design (RCBD) but with 2 incomplete blocks or columns per replicate with 3 replicates, 5 varieties by 3 sowing rates. Seed was planted on 15 April 2015 and plants harvested on 25 July 2015. Data are presented for varieties BEW and JC1 sown at 3 kg/ha. Plant establishment was assessed at harvest time by counting all plants in plots from 1 replicate only. Trial 2 (2016) was a 2-dimensional or latinized RCBD with 4 replicates, planted on 25 May 2016 at a sowing rate of 2.5 kg/ha and harvested on 29 August 2016. Trial plots were sown using a gravity-fed cone-seeder into four 21 m long rows at 22 cm row spacings on 90 cm wide raised beds. DAP (N 18%, P 20%, S 1.6%) at 320 kg/ha plus Zinc 10% was drilled into the sides of the bed at a depth of around 50 mm. The sites were irrigated using flood irrigation immediately after sowing with further irrigation applied fortnightly. Plots were harvested with an experimental small-plot harvester at maturity (plant senescence). Seed was cleaned and weighed to assess plot seed yield.

Katherine—Trial 1 (2016) was a split-plot design with 2 sowing dates as the main-plots, and 3 sowing rates by 2 nitrogen rates by 2 varieties as sub-plots partially randomized in 3 replicates. Data are presented for the planting date of 20 May 2016 and sowing rate of 8 kg/ha with data for nitrogen levels combined. Plant establishment was assessed on 3 June and seed harvested on 29 August. Trial 2 (2017) was a split-plot design with 2 sowing dates as main-plots and 12 varieties/lines as sub-plots fully randomized in 3 replicates. Data are presented for the planting date of 12 May 2017 and 10 varieties/lines. Plots were sown at a rate of 8 kg/ha. Plant establishment was assessed on 23 May and seed harvested from 4 September to 3 October based on maturity of lines. Plots were 10 m $\times$ 1.5 m in trial 1 and 10 m $\times$ 2.8 m in trial 2 and sown with a disc-seeder. Fertilisers applied were DAP at 120 kg/ha and muriate of potash (K 50%) at 100 kg/ha just after sowing and nitrogen was applied as urea (N 40%) top-dressed in late June. Plots were irrigated using a lateral move overhead irrigator delivering 640 mm during the growing period of 2016. Plant establishment was assessed using three quadrats per plot each of size 0.35 m^2. Plots were harvested with an experimental small-plot harvester at maturity. Seed was cleaned, dried and weighed to assess plot seed yield.

Walkamin (2018)—The trial used a split-plot design where the main-plots were 2 times of sowing, and the subplots were 2 varieties and 3 sowing rates. Data are presented for variety BEW, the planting date of 22 June 2018 and the sowing rate of 4 kg/ha. DAP at 250 kg/ha was spread on 18 June 2018 then incorporated and rolled with a multi-weeder to provide the final seedbed. Seed was sown using a Jang-seeder about 20 mm deep and each plot was 10 m $\times$ 2.75 m wide sown as seven rows at 25 cm row spacing. Overhead irrigation with sprinklers was applied at weekly intervals. The trial was machine harvested on 17 October 2018 and seed cleaned and weighed to assess plot seed yield.

Mingenew, Cunderdin, Geraldton, Eradu and Esperance—The trials were sown using air-cone-seeders fitted with knife points and press wheels at approximately 1 cm depth (soil coverage). Each trial plot consisted of seven 10-metre-long rows that were 22.5–27 cm apart, with 40 cm buffers. Plots were fertilised according to the recommendations for

canola (Agras (N 16%, P 9%, S 14%) 80 kg/ha at Cunderdin; Agstar Extra (N 14%, P 14%, S 9%) 80 kg/ha at Mingenew, Geraldton and Eradu; Agras No1 100 kg/ha at Esperance). Grass weeds were controlled using pre-emergent s-metolachlor (e.g., Dual® Gold) and post-emergent grass selective herbicides like clethodim. Broad-leaved weeds were controlled with post-emergent diflufenican (e.g., Brodal®) and/or by manual weeding. Plant density was assessed at or close to maturity using a range in number and size of quadrats as follows: 6 quadrats per plot of 0.2 m × 1.6 m at Mingenew and Cunderdin, 4 quadrats per plot of 0.21 m × 1.15 m at Geraldton (2 at anthesis and 2 at maturity), 6 quadrats per plot of 0.22 m × 1.6 m at Eradu and 3 quadrats per plot of 0.45 m × 1 m at Esperance. All plants in the plots were harvested using experimental small-plot harvesters using a low drum speed setting. Some trials were desiccated before harvest if required. The harvested seed was threshed and then aspirated for cleaning before weighing.

Mingenew (2016)—The trial was a split-plot design with the main-plots as 3 times of sowing and the sub-plots with 4 sowing rates by 3 nitrogen levels by 3 varieties. Plot size was 10 m × 1.8 m. Data for control varieties are presented for the planting date of 9 June 2016 and 8 kg/ha sowing rate with data for nitrogen levels combined (data from means of 6 replicates in each of 2 blocks). Nine germplasm lines were randomly arranged in the borders of main trial plots in 1 replicate only. Plant establishment was visually assessed in only 1 block. Control varieties were machine harvested and germplasm lines were hand harvested on 6 December 2016.

Cunderdin (2016)—The trial was a split-plot design with 3 times of sowing as the main-plots, and the sub-plots of 4 sowing rates by 3 nitrogen levels by 3 varieties. Plot size was 10 m × 1.8 m. Data for control varieties are presented for the planting dates of 3 May, 25 May and 14 June 2016 and 8 kg/ha sowing rate with data for nitrogen levels combined (data from means of 6 replicates in each of 2 blocks). Nine germplasm lines were randomly arranged in the borders of main trial plots in 3 replicates of the 14 June planting time. Plant establishment was visually assessed in the 3 replicates. Control varieties were machine harvested and germplasm lines were hand harvested on 15 December 2016.

Geraldton (2017)—The trial was a proportionally balanced-RCBD with 2 sowing rates blocked as replicates, 8 germplasm lines, 5 control varieties replicated one, two or three times, and planted on 1 June 2017. Data are presented for 2 of the 5 control varieties. Data for the 2 sowing rates were combined and treated as replicates in the analysis. Plots were harvested on 20 October 2017.

Eradu (2017)—The trial was a proportionally balanced-RCBD with 2 sowing rates blocked as replicates, 8 germplasm lines, 5 control varieties replicated one, two or three times, and planted 23 May 2017. Data are presented for 2 of the 5 control varieties. Data for the 2 sowing rates were combined. Plots were harvested on 13 November 2017.

Esperance (2017)—The trial was a proportionally balanced-RCBD with 2 sowing rates blocked as replicates, 8 germplasm lines, 5 control varieties replicated one, two or three times, and planted on 26 June 2017. Data are presented for 2 of the 5 control varieties. Data for the 2 sowing rates were combined. Plots with very poor plant establishment (<5 plant/m^2) were eliminated from the analysis (6 entries with 2 reps and 4 entries with 1 rep were analysed). Plots were harvested in January 2018.

Manjimup (2016–2017)—The trial was a RCBD with 2 sowing rates, 5 control varieties and 9 germplasm lines, 2 replicates, and sown on 4 October 2016. Data for the 2 sowing rates were combined. Plots consisted of eight rows 10 metres long with each row 17 cm apart. Seed was sown using a gravity-fed pasture cone-seeder (super seeder points without press wheels). Agstar at 100 kg/ha was applied at sowing. The trial site was irrigated based on evapotranspiration using overhead impulse sprinklers from the middle of December to the end of January. Hand harvesting and plant counts were undertaken on four 1.6 m × 0.2 m quadrats per plot on 26 April 2017. Whole plots (less quadrat cuts) were machine harvested on 22 March 2017 and seed cleaned and weighed to assess yield.

Bool Lagoon—Plots were 8 m × 2.2 m with 8 rows 15 cm apart and sown with a cone-seeder with disc and knife points. Plots were fertilised at 140 kg/ha with nitrogen (18%),

phosphorus (13%) and sulphur (10%) and 400 mL/ha Impact™ at sowing and 100 kg/ha nitrogen as urea top-dressed in early summer. During 2015 and 2016 plant establishment was assessed using three 30 cm × 50 cm quadrats per plot at the seedling stage. At or close to maturity plots were swathed and allowed to dry before harvesting with an experimental small-plot harvester. Seed was cleaned and weighed to assess plot seed yield.

Trial 1 (2015–2016) was a split-plot design with 4 times of sowing as the main-plots and 2 varieties by 3 sowing rates as sub-plots, in 4 replicates. Buffer plots were sown on each side of the main plots. Data are presented for the planting time of 30 October 2015 and sowing rate of 3 kg/ha. Overhead sprinkler irrigation was applied as required. The trial was harvested between 2 and 22 March 2016.

Trial 2 (2016–2017) was a RCBD in 4 replicates (lines in replicate 1 were arranged systematically and lines in replicates 2–4 were fully randomized). There were 5 varieties, 9 germplasm lines and 11 selections. Data are presented for 2 varieties and 9 germplasm lines. Only replicates 2–4 were analysed. The trial was sown on 10 November 2016 at 4 kg/ha, and harvested on 8 March 2017. No irrigation was applied.

Trial 3 (2017–2018) was a RCBD with 4 varieties, 4 germplasm lines and 4 selections in 4 replicates. Data are presented for 2 varieties and 4 germplasm lines. Seed was sown at 4 kg/ha on 9 October 2017. No irrigation was applied. Plots were hand harvested between 2 March to 3 April 2018 using 1 quadrat (4 rows × 21 cm) per plot.

Leeton—Trials were sown with a precision cone-seeder in four rows with 30 cm centres on 1.8 m raised beds. Plot length was 8.5 m. The seed was dropped on the soil surface and then covered with trailing chain and press wheels. After sowing, the trials were watered using furrow irrigation to wet up the whole bed. The timing of subsequent irrigation was determined using evapotranspiration measurements, occurring approximately every two weeks. Based on soil test results, fertiliser was applied pre-sowing at 110 kg/ha of Granulock Z (N 11%, P 22.8%, S 4.0% and Zn 2.0%) for all trials. Weed control was achieved using pre- and post-emergent herbicides. Glyphosate 360 was used pre-sowing and metolachlor (e.g., Dual Gold®) as the post sowing pre-emergent. Additional grass weed control was achieved using post-emergent haloxyfop (e.g., Verdict 520) at label rates. Flea beetles were controlled with Maldison 500® applied post-seeding pre-emergent. Establishment counts and hand harvest of seed were carried out on a 1 m × 4 row pegged area of each plot. Establishment counts were assessed at the seedling stage. An experimental harvester was used to harvest seed of the whole of plots. Quadrat seed yields are presented for Trials 1, 3 and 4 and machine harvested seed yields presented for Trial 2 (machine harvested yields were generally unreliable due to poor suitability of harvester for small seeded crops).

Trial 1 (2017) was a RCBD with 3 replicates, 4 varieties and 8 germplasm lines. Data are presented for only two of the varieties. Seed was sown at 4 kg/ha on 16 March 2017 and plots harvested from quadrats on 27 July 2017. Plots were machine harvested on 24–25 August 2017.

Trials 2–4 (2017–2018) were RCBD with 3 replicates, 2 varieties and 4 germplasm lines. Data are presented for only one of the varieties. Seed at the three trials was sown on 27 October, 20 November and 19 December 2017 at 7 kg/ha and hand or machine harvested on 24 April, 26 April and 16 May 2018, respectively.

Trials not harvested—

Northam—Trial was a RCBD with four replicates, 10 varieties, 4 times of sowing and a plot size of 5 m × 0.5 m.

Katanning—Trial was a RCBD with two replicates, 13 varieties and a plot size of 10 m × 1.6 m.

Alice Springs—Two trials, autumn and winter/spring sown, were RCBD with four replicates, 12 varieties and a plot size of 10 m × 2 m.

2.5. Seed Quality

Four field replicates from the trial in Kununurra and two field replicates from trials in Katherine, Geraldton, Manjimup, Bool Lagoon and Leeton were sampled for seed quality.

Seed weight was assessed by counting and weighing 1 set of 500 seeds for Kununurra and 4 sets of 100 seeds for the remaining trials. Seed size and colour was assessed on one 50–100 g sample of seed that was washed and stirred vigorously with an egg beater three times each for 90 s each time with fresh water, drained and dried at 60 °C in an air forced oven. Dried samples were firstly assessed for colour using a Konica Minolta CR-310 colorimeter by testing one 30 g sample 5 times with mixing between times. Data are presented as ΔE*ab or colour difference index [14] which is a comparison between the sample colour and the colour of imported white quinoa from Peru (Ecoquinoa and Impulse Trading). Values for ΔE*ab and their interpretation are as follows: 0–0.5 trace difference; 0.5–1.5 slightly discernible (hard to detect with the human eye); 1.5–3.0 noticeable (detectable by trained people); 3.0–6.0 appreciable (detectable by ordinary people); 6.0–12.0 large (large difference in the same colour group) and greater than 12 extreme (another colour group) [15]. Seed samples were then assessed for size using Endecott sieves of size 1, 1.18, 1.4, 1.7 and 2.0 mm. Whole samples were passed through a combination of sieve sizes, portions weighed, mean seed size (width) calculated using proportions by weight and sieve mid-points, and standard deviation of seed size computed for the discrete distribution of each plot/sample.

Protein analysis was undertaken on washed seed samples using the Leco TruSpec CN Nitrogen analyser in a commercial laboratory (Chemistry Centre, Perth, Western Australia, Australia). In this method, protein was not analysed directly. Nitrogen, a constituent of proteins was measured by the combustion in-house Leco method and expressed as protein by multiplying it by a factor of 6.25 [16]. A restricted number of lines from five trial sites were analysed in order to make a preliminary observation of the variation in protein content.

2.6. Statistical Analysis

A linear mixed model was used to compare the mean effects (model means) of the treatments. Depending on the trial, this could be just varieties, varieties by seeding rate and/or nitrogen, or varieties by time of sowing). The responses analysed include the raw data (seed yield, density, days to first flower, the weighted mean seed size and standard deviation of the seed size distribution, colour ΔE*ab) and/or a transformation of these responses if necessary. By default, we used the square root (SQRT) transformation since the new data then stays positive.

The R Statistical System [17] denoted as R was used to fit the linear mixed model using the aov or lmer function to do this 'analysis of variance' for balanced and unbalanced data, respectively. The R function emmeans produces the model means and contrasts with degrees of freedom with standard errors (which are used to compute the least significant difference (LSD) at the 5% level of significance ($p = 0.05$).

The raw means are usually presented with the transformed model means in parentheses as required. The model means may be adjusted for the raw data if untransformed because of possible unbalance in the dataset under analysis.

The statistical designs were generated using the R package DiGGer (URL: http://nswdpibiom.org/austatgen/software (accessed on 18 July 2022)).

A multiple linear regression was used to relate the mean seed yield of BEW to the climatic variables at each site of mean daily temperature (minimum temperature + maximum temperature/2), maximum temperature and number of days with maximum temperature exceeding 34 °C. A step-wise multiple linear regression (forward-, backward- and both-) was used in R. The criterion to determine the stopping rule is the smallest AIC (Akaike Information criterion).

Linear regressions (intercept + a slope parameter β) were fitted to the data for seed yield and average daily mean temperature during the seed development stage (second half of the period between commencement of flowering and maturity) for each of the germplasm lines and varieties. The estimate of the slope parameter (β) is shown with a test of the coefficient (p-value shown to 2 significant digits). Similarly, linear regressions were

also fitted to the data for seed yield and average mean temperature during the first 30 days after commencement of flowering for varieties BEW and JC1.

Data for seed yield and rainfall during the seed development stage (second half of the period between commencement of flowering and maturity) for each of the germplasm lines and varieties were analysed as follows. A k-means clustering procedure was used to form the mean centres and then line segments drawn connecting the estimated cluster-means in the graphs for the 3 groups of varieties/lines (Variety, Bolivian and Chilean). Line segments through clustered means were concatenated to approximate the logistic curve. Dashed lines indicate the prediction beyond the maximum observed yield. The natural response of biological processes follows the cumulative normal distribution or the S-shaped logistic curve. The genotype by environment (G × E) ANOVA was conducted to estimate the variation of the two components, genotype and environment.

3. Results

3.1. Germplasm Description

Most lines had green, yellow or red coloured stems and flowers, and white coloured seed when the surface saponin layer was removed (Table 4, Figure 2). Line PI 665278 from Bolivia was distinct in having flowers that had a purple appearance and yellow/orange coloured surface saponin layer covering the seed. Germplasm accession PI 470932 from Bolivia had a mixture of two distinct types, one with a red coloured saponin surface layer covering large seeds (PI 470932), and one much taller with a brown coloured saponin surface layer covering smaller seeds (PI 470932T).

Table 4. Plant description of varieties and germplasm lines used in field trials (arrow indicates colour change as plants mature).

Name	Stem and Flower Colour	Seed Characteristics
Varieties		
BEW	green → light brown	white
JC1	green → red → brown	white
Germplasm (Chile)		
PI 433232	green → yellow → light brown	white
PI 614885	green → red	white
PI 614888	green → intense red	white
PI 634918	green, yellow, orange and red	white
PI 634919	green or green → red	white
PI 634925	green/yellow → brown	red/brown and white seed
Germplasm (Bolivia)		
PI 470932	green → red → light brown	red saponin covering white seed
PI 470932T	green → light brown	small size, brown saponin covering white seed
PI 665278	stems green → pink, flowers green & pink (purple)	yellow/orange saponin covering white seed

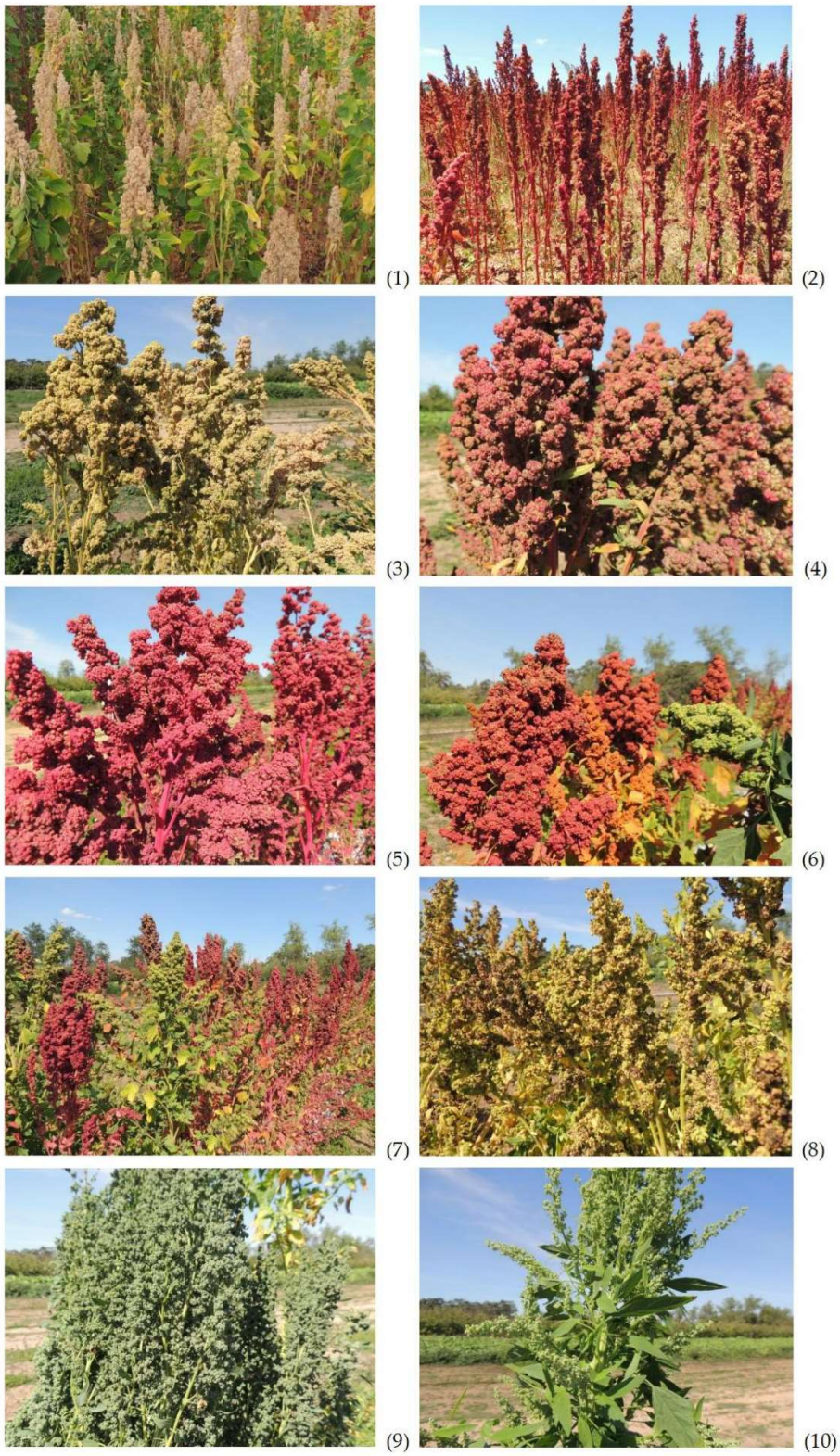


Figure 2. *Cont*.


(11)

Figure 2. Illustration of varieties and germplasm lines used in field trials (1—BEW, 2—JC1, 3—PI 433232, 4—PI 614885, 5—PI 614888, 6—PI 634918, 7—PI 634919, 8—PI 634925, 9—PI 470932, 10—PI 470932T, 11—PI 665278).

3.2. Field Trials

3.2.1. Flowering

Flowering time ranged from 43 days in germplasm line PI 614888 at Katherine to over 95 days in germplasm line PI 470932T at Bool Lagoon (Table 5). The variation in flowering time between trial sites was greater than within sites for varieties and germplasm lines from Chile. Flowering time of germplasm lines from Bolivia was highly variable.

Table 5. Flowering time (days from planting to flowering) of varieties and germplasm lines at selected trial sites.

Site	Katherine	Geraldton	Bool Lagoon	Leeton
Planting date	12-May-17	2-June-17	10-November-16	16-March-17
Variety				
BEW	51	71	72	48
JC1	49	67	66	53
Germplasm (Chile)				
PI 433232	50	75	81	57
PI 614885	49	68	67	53
PI 614888	43	67	68	58
PI 634918	51	78	73	60
PI 634919	50	68	71	53
PI 634925	50	67	76	60
Germplasm (Bolivia)				
PI 470932	-	-	90	55
PI 470932T	49	89	>95	>60
PI 665278	49	71	95	46
LSD (p = 0.05)	*3*	*5*	*5*	*4*

Heatsums (sum of daily mean temperatures above 0 °C) for flowering were between 1000 and 1200 for most varieties and germplasm lines from Chile but much higher in germplasm lines from Bolivia when grown at Bool Lagoon in spring-summer conditions (Table 6). Daylength between planting and flowering was less than 12 h and 14 min at Katherine, Geraldton and Leeton, and more than 13 h and 26 min at Bool Lagoon.

Table 6. Heatsums (sum of daily mean temperatures) from planting to commencement of flowering of varieties and germplasm lines and daylength (hours:minutes) between planting and flowering at selected trial sites (arrows indicate if daylength is increasing or decreasing).

Site	Katherine	Geraldton	Bool Lagoon	Leeton
Planting date	12-May-17	2-June-17	10-November-16	16-March-17
Variety				
BEW	1207	1112	1224	956
JC1	1161	1051	1104	1018
Germplasm (Chile)				
PI 433232	1184	1174	1398	1066
PI 614885	1161	1064	1117	1018
PI 614888	1023	1051	1133	1081
PI 634918	1207	1208	1241	1113
PI 634919	1184	1064	1201	1018
PI 634925	1184	1051	1301	1113
Germplasm (Bolivia)				
PI 470932	-	-	1559	1046
PI 470932T	1161	1366	-	-
PI 665278	1161	1104	1672	925
Daylength (hours:minutes)				
Planting	11:25↓	10:22↓	13:55↑	12:14↓
Shortest or longest day (planting to flowering)	11:13↑	10:15↑	14:39↓	-
Flowering	11:13–11:15	10:52–11.26	14:23–13:26	10:40–10:16

3.2.2. Plant Establishment

The number of plants established ranged greatly between trial sites and varieties or germplasm lines (Table 7). In only two cases did planting result in zero plant density at maturity (germplasm lines PI 470932T and PI 665278 at Esperance). By calculation, between approximately 70 and 400 viable seeds (excluding line PI 470932T) were sown in each square metre depending on variety and sowing rate at each trial site. The low plant establishment of some germplasm lines at several trial sites were associated with various factors. At Eradu it was with the coarse textured, non-wetting sandy soil at the site in combination with low rainfall prior to and immediately following planting, at Esperance with waterlogging early in the growing season, at Manjimup with poor textured loam soil in combination with absence of press wheels on the seeder, and in the March sown trial at Leeton with high temperatures at planting and seedling stages.

Table 7. Plant density at selected trials assessed at maturity or at establishment (plants/m^2).

Site	Kununurra 2015 [b]	Katherine 2016 [a]	Katherine 2017 [a]	Mingenew [bc]	Cunderdin 14 June [bc]	Geraldton [b]	Eradu [b]	Esperance [b]	Manjimup [b]	Bool Lagoon 2015 [a]	Bool Lagoon 2016 [a]	Leeton [a] March	Leeton [a] October	Leeton [a] November	Leeton [a] December
Sowing rate (kg/ha)	3	8	8	8	8	2.5/5	2.5/5	5	5/10	3	4	4	7	7	7
Varieties															
BEW	75	49	72	29	17	64	15	5	15	36	119	20	51	78	61
JC1	71	44	54	38	16	95	15	15	18	39	106	35	-	-	-
Germplasm (Chile)															
PI 433232	-	-	167	low	low	105	27	28	41	-	372	24	-	-	-
PI 614885	-	-	44	low	low	51	13	49	57	-	149	23	-	-	-
PI 614888	-	-	82	medium	low	78	15	16	25	-	106	42	134	90	61
PI 634918	-	-	52	medium	low	52	14	20	27	-	92	3	-	-	-
PI 634919	-	-	70	medium	medium	81	17	21	26	-	107	19	123	100	93
PI 634925	-	-	41	medium	medium	63	11	27	29	-	109	21	78	85	72
Germplasm (Bolivia)															
PI 470932	-	-		high	high	-	-	-	31	-	97	44	24	68	78
PI 470932T	-	-	64	high	high	26	9	0	-	-	96	25	-	-	-
PI 665278	-	-	42	high	high	52	10	0	31	-	87	16	-	-	-
LSD (p = 0.05)	-	*35*	*40*	*16*	*10*	*37*	*6*	*150*	*19*	*14*	*49*	*28*	*47*	*58*	*43*

[a] Assessed at the seedling stage. [b] Assessed at maturity. [c] Plant density of germplasm lines assessed visually.

3.2.3. Seed Yield

- Northern tropics/Irrigated

Seed yields of varieties BEW and JC1 at Kununurra in 2015 were 2.1 and 1.4 t/ha, respectively (Table 8). Seed yields of varieties and germplasm lines from Chile at Kununurra in 2016 were lower ranging from 0.4 to 1.3 t/ha. Germplasm lines originating from Bolivia did not produce seed due either to lower tolerance to high temperatures or inadequate irrigation. Observed plant density across all varieties and lines was not considered a factor in these results. Seed yields at Katherine were generally low with few lines exceeding 1 t/ha. The highest yielding lines in the 2017 trial were PI 433232 from Chile yielding 1.4 t/ha and PI 470932T from Bolivia yielding 1.1 t/ha ($p < 0.05$). Plant density exceeded 40 plants/m^2 and was unlikely to be a factor influencing seed yield (Table 7). Variety BEW yielded 1.4 t/ha at Walkamin in 2018. Plant establishment was not recorded; however, observations suggest this was not a factor in seed yield.

Table 8. Seed yield (t/ha) of quinoa varieties and germplasm at irrigated trial sites with a hot semi-arid climate (Kununurra) and a tropical savanna climate (Katherine, Walkamin).

Site	**Kununurra**		**Katherine**		**Walkamin**
Sowing date	15-April-15	25-May-16	20-May-16	12-May-17	22-June-18
Varieties					
BEW	2.10	1.26	1.07	0.50	1.38
JC1	1.39	0.94	1.07	0.32	-
Germplasm (Chile)					
PI 433232	-	0.51	-	1.43	-
PI 614885	-	1.27	-	1.00	-
PI 614888	-	0.44	-	0.84	-
PI 634918	-	1.14	-	0.54	-
PI 634919	-	1.09	-	0.81	-
PI 634925	-	0.87	-	0.38	-
Germplasm (Bolivia)					
PI 470932T	-	0	-	1.11	-
PI 665278	-	0	-	0.59	-
LSD (p = 0.05)	*0.35*	*0.26*	*0.38*	*0.31*	*0.46*

- Mediterranean/Winter & Spring grown/Rain-fed

Seed yields at Mingenew were very low with the best line (PI 634919 from Chile) yielding 116 kg/ha (Table 9). Seed yields at Cunderdin were also very low ranging from zero in germplasm lines from Bolivia to 24 kg/ha in variety BEW. Low plant establishment did not explain the very low seed yields (Table 7). Varieties at Geraldton yielded close to 3 t/ha, the yield of one germplasm line from Chile exceeded 3 t/ha and germplasm lines from Bolivia yielded between 0.85 and 1.7 t/ha. Plant density at Eradu was less than 20 plants/m^2 for most lines (Table 7). This may account for the low seed yields of between approximately 300 and 800 kg/ha for all lines except PI 470932T. Seed yields at Esperance were generally poor with germplasm lines yielding between 110 and 680 kg/ha and JC1 yielding 200 kg/ha. A low plant density for BEW of 5 plants/m^2 may have contributed to its low seed yield (Table 7); however, plant establishment of most other lines was unlikely to limit seed production. Very poor plant establishment and plant growth at the Northam trial site was associated with poorly structured, rapidly drying, clay soil. There was no seed production of lines tested. Plant establishment was very low (between 0.1 and 1.0 plants/m^2) at the Katanning trial site (data not presented). Surviving plants

grew well and flowered; however, harvesting was not undertaken due to the very low plant density.

Table 9. Seed yield (t/ha) of quinoa varieties and germplasm lines at rain-fed trial sites with a hot-summer Mediterranean climate (Mingenew, Cunderdin, Geraldton, Eradu) and a warm-summer Mediterranean climate (Esperance). (Figures in brackets are square roots of yield).

Site	Mingenew	Cunderdin	Geraldton	Eradu	Esperance
Sowing date	9-June-16	14-June-16	2-June-17	23-May-17	26-June-17
Varieties					
BEW	0.057	0.024	2.91	0.57	0.06 (0.22)
JC1	0.056	0.018	2.63	0.65	0.20 (0.47)
Germplasm (Chile)					
PI 433232	0.005	0.012	1.62	0.29	0.40 (0.62)
PI 614885	0.006	0.006	2.73	0.69	0.68 (0.81)
PI 614888	0.027	0.019	1.95	0.37	0.19 (0.41)
PI 634918	0.038	0.013	3.04	0.53	0.11 (0.39)
PI 634919	0.116	0.015	2.65	0.58	0.53 (0.72)
PI 634925	0.043	0.008	2.55	0.83	0.48 (0.67)
Germplasm (Bolivia)					
PI 470932	0.035	0	-	-	-
PI 470932T	0	0	0.85	0.05	-
PI 665278	0	0	1.71	0.39	-
LSD (p = 0.05)	*0.069*	*0.026*	*0.49*	*0.24*	*(0.35)*

- Mediterranean/Spring & Summer grown/Rain-fed +/− Supplementary Irrigation

Several lines at Manjimup gave hand harvested yields of approximately 1.5 t/ha with supplementary irrigation during the flowering and seed development stages (Table 10). Yields of some lines may not have reached potential due to relatively low plant density (Table 7). Machine harvested seed yields were assessed one month later during which 35 mm of rain fell over a 6-day period with a mean maximum temperature of 20 °C. Seed yields were similar or lower than the earlier hand harvest yields. Seed yields at Bool Lagoon in the 2015/6 and 2016/7 growing seasons were high approaching 2.8 t/ha. However, germplasm lines from Bolivia did not yield any seed in 2016 most likely due to flowering much later (Table 5) at a time when soil moisture was limited. Plant density can be ruled out as a factor affecting seed production (Table 7). Seed yields in 2017 were much lower, averaging 1.6 t/ha.

Table 10. Seed yield (t/ha) of quinoa varieties and germplasm at irrigated and/or rain-fed trial sites with a warm-summer Mediterranean climate.

Site	Manjimup [1]	Manjimup [2]	Bool Lagoon [2]	Bool Lagoon [2]	Bool Lagoon [1]
Sowing date	4-October-16	4-October-16	30-October-15	10-November-16	9-October-17
Varieties					
BEW	0.92	0.53	1.10	1.83	1.43
JC1	1.58	1.30	2.76	2.70	1.73

Table 10. *Cont.*

Site	Manjimup [1]	Manjimup [2]	Bool Lagoon [2]	Bool Lagoon [2]	Bool Lagoon [1]
Germplasm (Chile)					
PI 433232	0.76	0.77	-	2.16	-
PI 614885	1.65	1.60	-	2.63	1.55
PI 614888	1.46	1.03	-	1.77	-
PI 634918	1.49	1.20	-	2.28	1.68
PI 634919	1.54	1.46	-	2.54	1.59
PI 634925	1.76	1.27	-	2.68	1.65
Germplasm (Bolivia)					
PI 470932	0.04	0.05	-	0	-
PI 470932T	-	0.01	-	0	-
PI 665278	0.09	0.15	-	0	-
LSD (p = 0.05)	*0.53*	*0.25*	*0.52*	*0.63*	*0.38*

[1] Hand harvested, [2] Machine harvested.

- Semi-arid/Grown all seasons/Irrigated

Seed yield of variety BEW approached 2 t/ha at Leeton when sown in March or December; however, yield of many lines was not significantly lower ($p > 0.05$) (Table 11). Only germplasm line PI 470932 from Bolivia performed better at all four sowing dates (significant at $p < 0.05$ for October and November). Low plant density of 3 plants/m^2 for germplasm line PI 634918 likely explained its low seed yield of 0.34 t/ha (Table 7). Plant density for October, November and December sowing dates was much higher than for the March sowing and was not a factor in determining seed yields. Germplasm line PI 470932T yielded very little most likely because it flowered later in June when frosts occurred (Table 5) [13].

Table 11. Seed yield (t/ha) of quinoa varieties and germplasm at irrigated trial sites with a cold semi-arid climate.

Site	Leeton [1]	Leeton [2]	Leeton [1]	Leeton [1]
Sowing date	16-March-17	27-October-17	20-November-17	19-December-17
Varieties				
BEW	1.81	0.60	0.68	1.98
JC1	1.64	-	-	-
Germplasm (Chile)				
PI 433232	0.39	-	-	-
PI 614885	1.77	-	-	-
PI 614888	1.45	0.40	0.17	0.96
PI 634918	0.34	-	-	-
PI 634919	1.48	0.42	0.32	1.69
PI 634925	1.52	0.51	0.30	1.31

Table 11. *Cont.*

Site	Leeton [1]	Leeton [2]	Leeton [1]	Leeton [1]
Germplasm (Bolivia)				
PI 470932	2.15	1.01	2.48	2.57
PI 470932T	0.03	-	-	-
PI 665278	0.75	-	-	-
LSD (p = 0.05)	*0.97*	*0.20*	*0.78*	*0.74*

[1] Hand harvested, [2] Machine harvested.

- Desert/Autumn and spring grown/Irrigated

Plant establishment was very low at the Alice Springs trial sites, and consequently plot seed yields were not measured.

3.2.4. Seed Weight

Individual seed weight ranged from 1 mg in line PI 470932T at Manjimup to 3.9 mg in variety JC1 at Geraldton (Table 12). For trials with a range of germplasm Geraldton had the highest seed weights followed by Leeton, Bool Lagoon, Katherine, and Manjimup with the lowest. Mean seed weights across the trial sites were 2.5 mg for BEW, 2.9 mg for JC1, and ranged from 1.9 mg in PI 433232 to 2.9 mg in PI 634925 for germplasm lines from Chile.

Table 12. Seed weight at selected field trials (mg/seed).

Trial	Kununurra	Katherine	Geraldton	Manjimup	Bool Lagoon	Bool Lagoon	Leeton
Planting date	15-April-15	24-May-17	2-June-17	4-October-16	30-October-15	10-November-16	16-March-17
Varieties							
BEW	2.70	2.06	3.53	1.97	1.81	2.28	3.17
JC1	2.94	2.50	3.86	2.57	2.17	2.78	3.20
Germplasm (Chile)							
PI 433232	-	1.66	2.61	1.50	-	1.77	1.71
PI 614885	-	2.12	3.30	2.06	-	2.13	2.97
PI 614888	-	1.81	2.58	1.68	-	1.56	2.06
PI 634918	-	2.15	3.17	2.09	-	2.27	2.45
PI 634919	-	2.20	3.29	2.05	-	2.40	3.02
PI 634925	-	2.45	3.70	2.37	-	2.84	3.05
Germplasm (Bolivia)							
PI 470932	-	-	-	2.35	-	-	2.80
PI 470932T	-	1.39	1.18	0.99	-	-	-
PI 665278	-	2.09	2.87	2.32	-	-	2.72
LSD (p = 0.05)	*0.20*	*0.30*	*0.27*	*0.35*	*0.26*	*0.33*	*0.29*

3.2.5. Seed Quality

Estimates for the weighted mean seed size across all lines were 1.51, 1.52, 1.52, 1.61 and 1.63 mm wide at Bool Lagoon, Manjimup, Katherine, Geraldton and Leeton, respectively (Table 13). The highest mean seed size across all trial sites was 1.65 mm wide for variety JC1 and the lowest mean was 1.27 mm wide for germplasm line PI 470932T from Bolivia. The highest individual mean seed size was 1.7 mm wide for several germplasm lines and

varieties at both Geraldton and Leeton. Seed of germplasm lines PI 433232 and PI 614888 were consistently smaller across the five trial sites than the other four germplasm lines originating from Chile. The independence bi-variate analysis (G × E) showed that the variety term accounted for 92.4% of the variation in weighted mean seed size. There was very little Variety × Site interaction. The variety term accounted for 54.8% of the variation in SD (standard deviation of the discrete distribution of seed size) while the Variety × Site term accounted for 45.2%.

Table 13. Estimated mean weighted seed size-width (mm) and standard deviation (SD) of the discrete distribution at selected trial sites. LSD (p = 0.05) is 0.064 for Size; LSD (p = 0.05) is 0.020 for SD.

Trial	**Katherine**		**Geraldton**		**Manjimup**		**Bool Lagoon**		**Leeton**	
Planting date	24-May-17		2-June-17		4-October-16		10-November-16		16-March-17	
Trait	Size	SD	Size	SD	Size	SD	Size	SD	Size	SD
Variety										
BEW	1.55	0.14	1.69	0.17	1.55	0.16	1.55	0.15	1.67	0.17
JC1	1.60	0.17	1.74	0.16	1.60	0.16	1.60	0.16	1.70	0.17
Germplasm (Chile)										
PI 433232	1.48	0.14	1.53	0.14	1.50	0.13	1.40	0.13	1.50	0.15
PI 614885	1.56	0.16	1.67	0.17	1.56	0.16	1.52	0.11	1.69	0.17
PI 614888	1.50	0.16	1.54	0.15	1.43	0.14	1.40	0.13	1.50	0.14
PI 634918	1.52	0.15	1.63	0.16	1.54	0.14	1.52	0.13	1.59	0.17
PI 634919	1.53	0.15	1.67	0.16	1.56	0.15	1.53	0.13	1.65	0.18
PI 634925	1.57	0.15	1.68	0.17	1.62	0.16	1.58	0.14	1.67	0.18
Germplasm (Bolivia)										
PI 470932					1.58	0.14			1.66	0.16
PI 470932T	1.33	0.15	1.27	0.12	1.20	0.12				
PI 665278	1.54	0.15	1.67	0.16	1.53	0.15			1.68	0.17

A ΔE*ab score of <6.0 ('nil to noticeable difference in colour' compared with imported white quinoa seed from Peru) was not observed in seed from any trial site (Table 14). There were several results showing a 'large difference in the same colour group' (ΔE*ab score 6.0–12.0) including variety BEW and line PI 433232 at Katherine, line PI 614888 at most sites and most lines at Bool Lagoon. Most other results show an extreme difference in colour with Peru white quinoa. Variety BEW and lines PI 614888 and PI 665278 had in general the whitest coloured seed.

Protein content in seed of varieties BEW and JC1 ranged from 11.7 to 15.5% (Table 15). Protein content in seed of most germplasm lines was significantly higher than in variety BEW ($p < 0.05$). Seed grown at Leeton had the highest protein content reaching over 18%.

3.3. High Temperatures during the Reproductive Stage

High temperatures during the seed development stage (second half of the period between commencement of flowering and maturity) were associated with low seed yields in variety BEW at trial sites where temperatures were optimal or higher and end of season water was presumed not to be limiting (Table 16). R and p-values showed stronger correlations between temperatures during seed development and seed yield of variety BEW than temperatures during flowering (first half of the period between the commencement of flowering and maturity).

Table 14. Colour difference index or comparison of the sample colour with the colour of imported white quinoa from Peru (ΔE*ab). LSD (p = 0.05) = 6.1 (varieties), 5.4 (varieties × sites).

Trial	Katherine	Geraldton	Manjimup	Bool Lagoon	Leeton
Planting date	24-May-17	2-June-17	4-October-16	10-November-16	16-March-17
Variety					
BEW	10.6	13.3	35.0	9.3	14.0
JC1	21.1	22.3	25.1	12.9	25.0
Germplasm (Chile)					
PI 433232	11.2	13.4	17.4	10.5	24.3
PI 614885	15.2	14.9	23.1	10.0	22.1
PI 614888	11.5	11.2	14.1	6.0	11.9
PI 634918	16.8	16.3	17.4	11.2	25.1
PI 634919	15.8	14.7	19.9	11.9	23.0
PI 634925	24.1	22.9	27.9	19.3	28.6
Germplasm (Bolivia)					
PI 470932	-	-	40.2	-	13.3
PI 470932T	28.6	30.3	24.0	-	-
PI 665278	11.2	13.6	17.1	-	9.6

Table 15. Seed protein % at selected field trials.

Trial	Katherine	Geraldton	Manjimup	Bool Lagoon	Leeton
Planting date	24-May-17	2-June-17	4-October-16	10-November-16	16-March-17
Variety					
BEW	11.7	12.9	14.0	13.6	14.8
JC1	11.8	13.2	14.0	13.5	15.5
Germplasm (Chile)					
PI 433232	-	14.5	-	-	18.4
PI 614885	-	13.5	-	-	15.3
PI 614888	-	15.3	-	-	17.9
PI 634918	-	15.4	-	-	16.3
PI 634919	-	14.5	-	-	15.8
PI 634925	-	14.7	-	-	15.7
Germplasm (Bolivia)					
PI 470932T	-	14.1	-	-	-
PI 665278	-	13	-	-	15.9
LSD (p = 0.05)	*NS*	*0.9*	*NS*	*NS*	*0.7*

Table 16. Average mean daily temperatures (°C), average maximum daily temperatures (°C) and number of days greater than 34 °C during the first (flowering) and second (seed development) halves of the period between commencement of flowering and harvest for variety BEW (Multiple correlation coefficient R and probability *p*-value are presented for regressions with seed yield of BEW from Tables 8–11).

Trial	Mean Temp 1st Half	Mean Temp 2nd Half	Max Temp 1st Half	Max Temp 2nd Half	Days >34 °C 1st Half	Days > 34 °C 2nd Half
Trial sites where end of season water was presumed not to be limiting and temperatures were optimal or higher						
Kununurra—2015	24.8	21.7	33.0	30.0	7	0
Kununurra—2016	23.4	24.0	31.5	33.1	4	10
Katherine—2016	22.6	24.0	31.1	32.8	1	5
Katherine—2017	24.4	25.0	33.0	35.0	1	26
Walkamin	19.5	21.8	26.5	28.5	0	0
Geraldton	15.1	17.2	21.5	24.1	0	0
Manjimup	19.7	19.3	27.3	27.4	4	4
Bool Lagoon—2015/6	21.5	20.3	30.8	29.6	9	6
Bool Lagoon—2016/7	19.5	19.1	28.0	27.9	2	3
Bool Lagoon—2017/8	19.8	20.3	29.0	29.5	7	11
Leeton—October	27.7	27.8	35.4	35.0	15	15
Leeton—November	27.9	25.9	35.3	33.5	16	10
Leeton—December	26.1	22.9	33.8	30.6	12	3
R (Multiple correlation coefficient)	0.55	0.69	0.60	0.76	0.31	0.70
p-value	0.05	0.0084	0.031	0.0027	0.3	0.0073
Trial sites where end of season water was presumed to be limiting or temperatures were lower than optimal						
Leeton—March	11.8	9.8	18.4	15.9	0	0
Mingenew	14.5	21.1	22.3	30.9	1	10
Cunderdin—14 June	14.7	20.9	24.5	30.9	1	9
Eradu	15.2	19.8	21.4	27.6	0	9
Esperance	18.3	20.2	24.7	27.2	1	4

Yield responses for individual varieties and germplasm lines to average mean daily temperatures during the seed development stage are presented for trial sites where water was presumed not to be a limitation for seed production (Figure 3). These include all irrigated trial sites (except Leeton sown in March when temperatures at flowering and seed development were low) including all trials at Bool Lagoon and Geraldton. There was a clear trend down in seed yield with increasing average mean daily temperatures during the seed development stage for most varieties and germplasm lines. For every 1 °C increase in temperature above 15 °C seed yield declined by an average across all lines of 215 kg/ha. Maximum seed yields appeared to correspond to average mean daily temperatures below approximately 20 °C at the seed development stage for most varieties and germplasm lines.

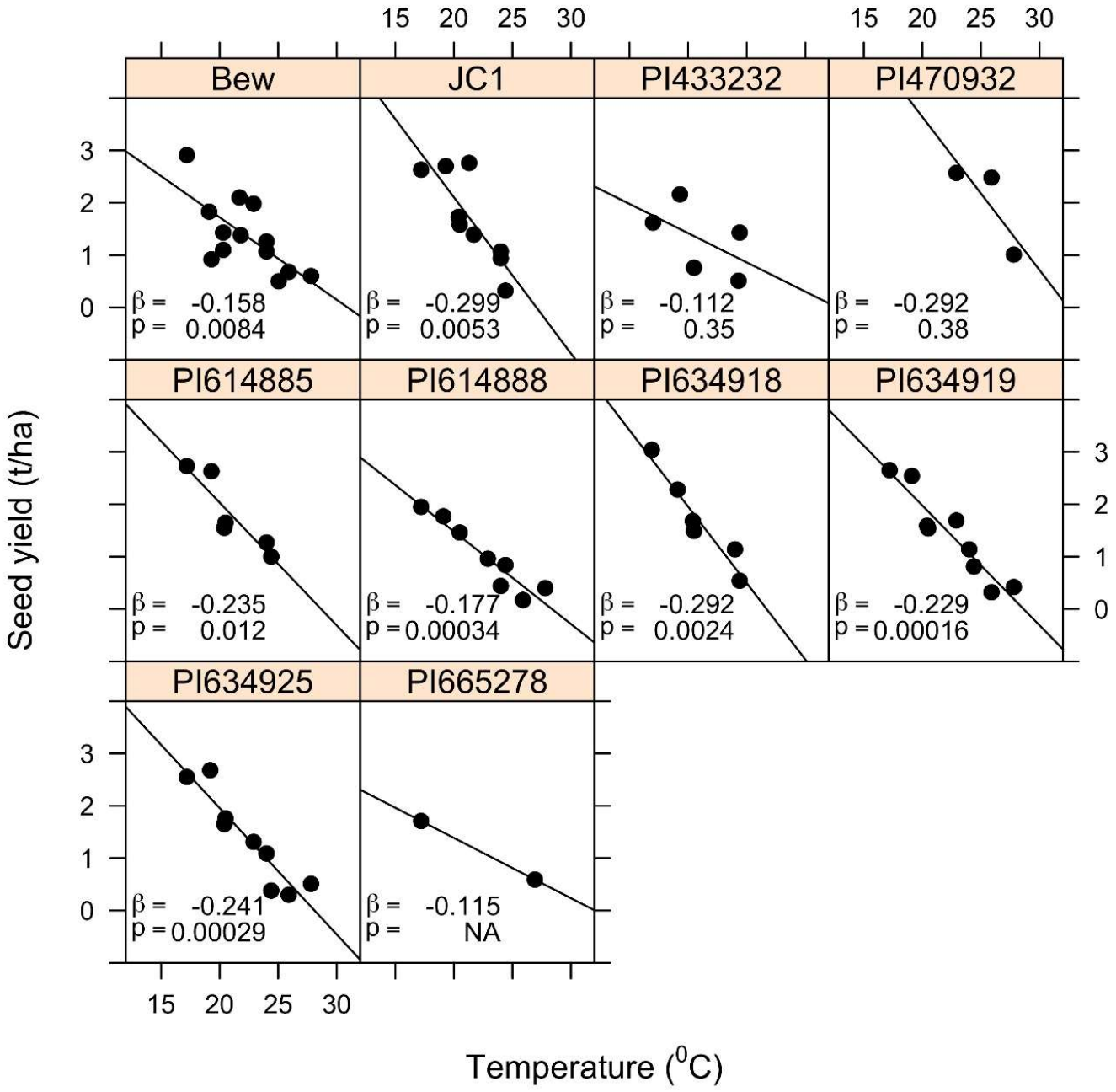


Figure 3. Relationship between seed yield (t/ha) and average mean daily temperature (°C) during the seed development stage for selected field trials where water was presumed not to be limiting production (i.e., all irrigated field trials, excluding Leeton—March, and including the rain-fed site at Geraldton).

3.4. Low Temperatures during Flowering

There was a clear decreasing trend in seed yield with decreasing average mean daily temperatures during the flowering period of varieties BEW and JC1 (Figure 4). For every 1 °C decrease in temperature below 15 °C seed yield declined by 690 kg/ha. Average mean daily temperatures below 11 °C and frequent days with freezing conditions were associated with near zero seed yields at Cunderdin in 2016 (Table 17). Temperature and rainfall (or irrigation in the case of Leeton) during the seed development stage at these sites were not considered to be a limitation for seed production.

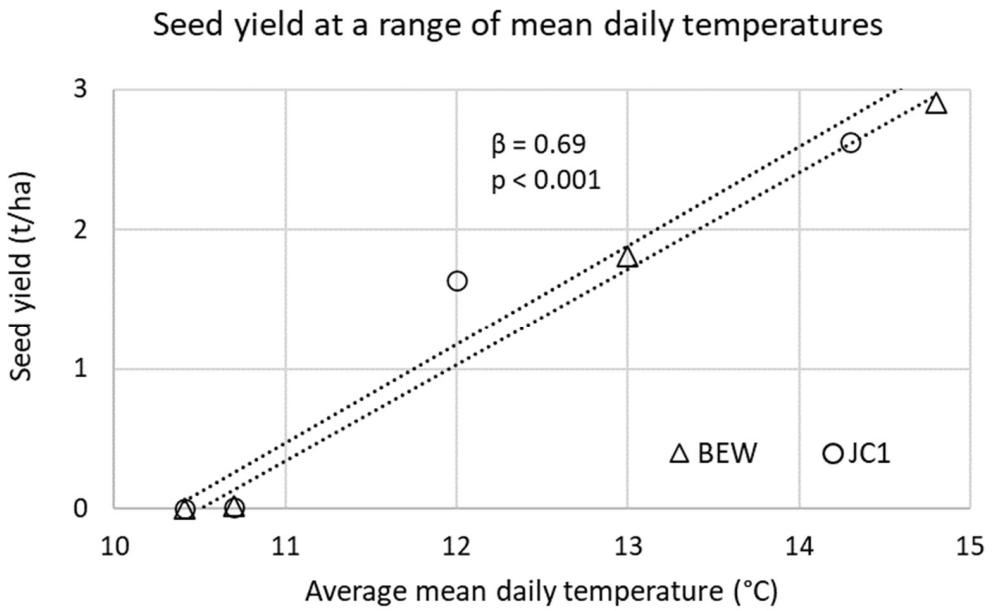


Figure 4. Relationship between average mean daily temperature for the 30-day period after commencement of flowering and seed yield for varieties BEW and JC1 at Cunderdin, Leeton and Geraldton. LSD (p = 0.05) = 0.57.

Table 17. Weather records at Cunderdin, Leeton and Geraldton field trial sites for the 30-day period after commencement of flowering of varieties BEW and JC1 (figures separated by a comma are data for BEW followed by data for JC1).

Field Trial	Cunderdin	Cunderdin	Leeton	Geraldton
Planting date	03-May-16	25-May-16	16-March-17	02-June-16
Average mean temperature (°C)	10.3	10.7	13.0, 12.0	14.8, 14.3
Average minimum temperature (°C)	4.0	3.6	6.7, 5.6	8.5, 8.1
Number of days below 0 °C	7	6	1, 2	0
Lowest minimum temperature (°C)	−1.6	−2.3	−1.6	2.6

3.5. End of Season Rainfall

Low rainfall during the seed development stage at Cunderdin, Mingenew, Esperance and Eradu was associated with severe seed yield penalties (Figure 5). Mean temperatures for the 30 days after the commencement of flowering at these trial sites were unlikely to have caused a significant yield decline (Table 18, Figure 4). Mean temperatures during the second half of the period from flowering to harvest might have reduced seed yield by between 20–30% (Table 18, Figure 3). Therefore, most of the large declines in seed yield at Cunderdin, Mingenew, Esperance and Eradu may have been attributed to low rainfall during the seed development stage. The ANOVA analysis (G × E) showed that the site term accounted for 85.7% of the variation in seed yield.

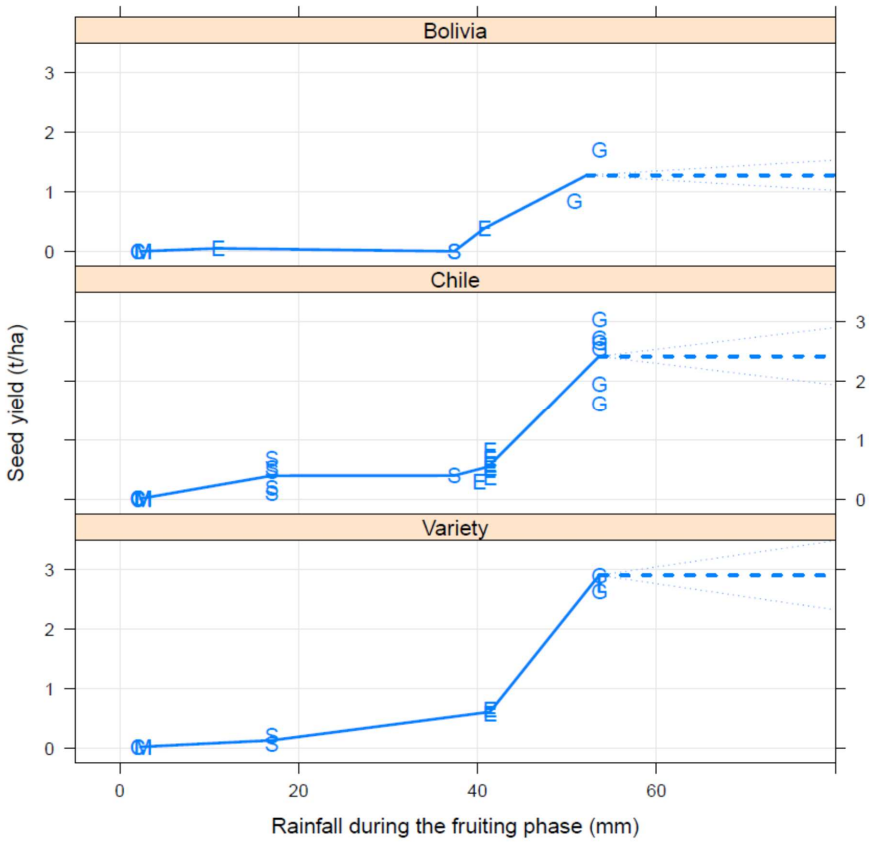


Figure 5. Relationship between rainfall during the seed development stage and seed yield of germplasm from Bolivia, germplasm from Chile and varieties (BEW and JC1) at rain-fed sites in Western Australia (C-Cunderdin, M-Mingenew, G-Geraldton, E-Eradu and S-Esperance).

Table 18. Average mean daily temperature and rainfall during the seed development stage, average mean daily temperature during the first 30 days after commencement of flowering, long term average rainfall during the seed development stage and long-term growing season rainfall for a range of varieties and germplasm lines tested at rain-fed sites in Western Australia (a range is listed where flowering times for individual varieties and germplasm lines varied).

Trial Site	Mingenew	Cunderdin	Geraldton	Eradu	Esperance
Sowing date	9-June-2016	14-June-2016	2-June-2017	23-May-2017	26-June-2017
Mean Temperature (°C) (first 30 days of flowering)	13.5	14.7	14.3–16.5	13.9–16.5	17.9–19.5
Mean Temperature (°C) (seed development)	21.1	20.9	16.9–17.2	16.8–17.8	19.9–20.6
Rainfall (mm) (seed development)	2.6	2	51–54	11–41	17–37
20-year average rainfall (mm) (seed development)	15.4	14.6	17–26 *	-	21–38
Growing season rainfall (mm)	224	150	236	220	278

* 10-year average.

4. Discussion

4.1. Temperature and Rainfall Effects on Seed Yield

There are several references to the impact of high temperature during the reproductive phase on seed yield of quinoa. Oelke et al., (1992) [18] quotes research in Colorado where

yield was reduced with temperatures at flowering >95 °F (35 °C) caused by plant dormancy or pollen sterility. Fuentes and Bhargava (2011) [19] point to high temperatures at flowering resulting in seed yields of between 21 and 993 kg/ha in highland Chilean quinoa germplasm grown at low altitude desert conditions under irrigation. During flowering maximum temperature ranged from 31 to 35 °C and mean temperature ranged from 18 to 23 °C; however during seed development in January mean temperature was high at approximately 22–23 °C. Mean daily temperatures of approximately 25 °C during seed development were associated with lower seed yields of northern European varieties when grown over two years under irrigation in south-western Spain [20]. In a remarkable result Hinojosa-Sanchez et al., (2018) [21] showed increasing the day/night temperature from 25 °C/16 °C to 40 °C/24 °C for the first 15 days of flowering did not reduce seed yield. Additionally, under controlled conditions, seed yield of variety Titicaca declined significantly when temperature was raised from 34 °C to 38 °C for the first 10 days of flowering [22]. Mean temperatures during the seed development stage throughout the main quinoa growing areas in highland Bolivia and coastal Chile are 10–12 °C and 18–19 °C, respectively [23,24]. Considering these inconsistencies and the clear results of this work we suggest that temperatures during seed development are a better predictor of seed yield than temperatures at flowering time. This has also been suggested by Peterson and Murphy (2015) [25]. However, in a few cases quinoa yields remained high despite high mean temperatures close to 25 °C towards the end of the growing season [26,27]. It is possible that abundant irrigation during seed development can offset the negative effects of high temperature at this time. Anecdotal evidence from Perth, Western Australia in December 2021 and January 2022 showed some quinoa yielded the equivalent of 1 t/ha when supplied with water daily. This was despite plants being exposed to an average mean daily temperature of 27 °C between flowering and harvest and 19 days out of 31 with maximum temperatures exceeding 34 °C during seed development. Quoted preliminary work in eastern Washington State, USA also suggests that irrigation may ameliorate the effects of heat stress [25].

There is likely to be variation in yield responses to temperature across the wide diversity of germplasm lines of quinoa. In this study line PI 470932 from Bolivia yielded well under high temperatures when grown under irrigation at Leeton. This may be explained by its distinct floral architecture which displays a greater degree of branching (third and fourth order) and a more horizontal orientation of branches that may facilitate more ventilation and cooling. Bazile et al., (2016) [1] makes reference to flower type (dense or open) in relation to ventilation and its possible impact on drying times after late season rain.

High individual seed weights most likely contributed to high seed yields at Geraldton and Leeton and conversely low individual seed weights likely contributed to low seed yields at Katherine. It is not possible to allocate to seed yield the two components, seed number and individual seed weight. High temperatures during seed filling have been reported to reduce individual seed weight [28]. Seed weight was higher (3–3.5 mg/seed) when mean temperatures during seed development were 19.6 °C and lower (2–2.5 kg/seed) when temperatures were 25.6 °C for irrigated coastal varieties. This is in line with results from Geraldton, Bool Lagoon and Katherine in this study.

Very cold conditions including several frosts during the flowering period at the Cunderdin field site were associated with near zero seed yields. Several reports point to the negative effects of low temperature at flowering time on seed yield of quinoa. A University of Western Australia study recorded 63–82% yield loss in four quinoa lines/varieties, including BEW, when plants were exposed to mean chilling temperatures of 6–12 °C compared to 22 °C at flowering [29]. Exposing quinoa cultivars Quillahuaman and Witulla to 4 h at −2 °C and −4 °C at anthesis resulted in yield declines of 19% and 66%, respectively compared with plants grown at a continuous temperature of 19 °C [30,31]. Similarly, Oelke et al., (1992) [18] quotes Johnson and Croissant (1990) [32] stating a 60–70% loss in seed yield was observed with temperatures below 28 °F (−2.2 °C) in Colorado. Bhargava and Srivastava (2013) [33] quote similar findings by Tapia et al., (1979) [34] and Limache (1992) [35] that support the susceptibility of quinoa to frost at anthesis. There are no re-

ports of low temperature effects at flowering time on seed yield in Chile. Spring sown quinoa in coastal Chile is unlikely to experience frosts during flowering in the months of December and January [23]. Given the published evidence it is reasonable to conclude that the cold conditions experienced at Cunderdin were directly responsible for the very poor seed yields.

There is repeated reference in the literature to the drought tolerance of quinoa. In this, there is little evidence of adaptation of quinoa to moisture deficit or stress during flowering or seed development in an environment of increasing temperatures. These conditions occur with spring sown quinoa in coastal regions of Chile and with winter sown crops in the dry Mediterranean regions of southern Australia. Soil type and depth and the corresponding capacity to store moisture, and the ability of quinoa to grow deep roots on these soils, combine to influence the potential seed production. The very sandy soil at Eradu in this work likely contributed to low seed yields of germplasm lines from Chile despite receiving >40 mm rainfall during the seed development stage. There are many references to quinoa possessing a deep and extensive root system; however, observations in this work do not support this. If quinoa in Chile was tolerant of low end of season rainfall through development of a deep root system yields greater than 0.37 to 1.2 t/ha reported in the Maule and O'Higgins regions would be expected [23]. Nevertheless, given an ideal soil such as a deep self-mulching clay quinoa may develop deep roots and offset dry and warming end of season conditions given sufficient growing season rainfall.

4.2. Weather Effects on Seed Quality

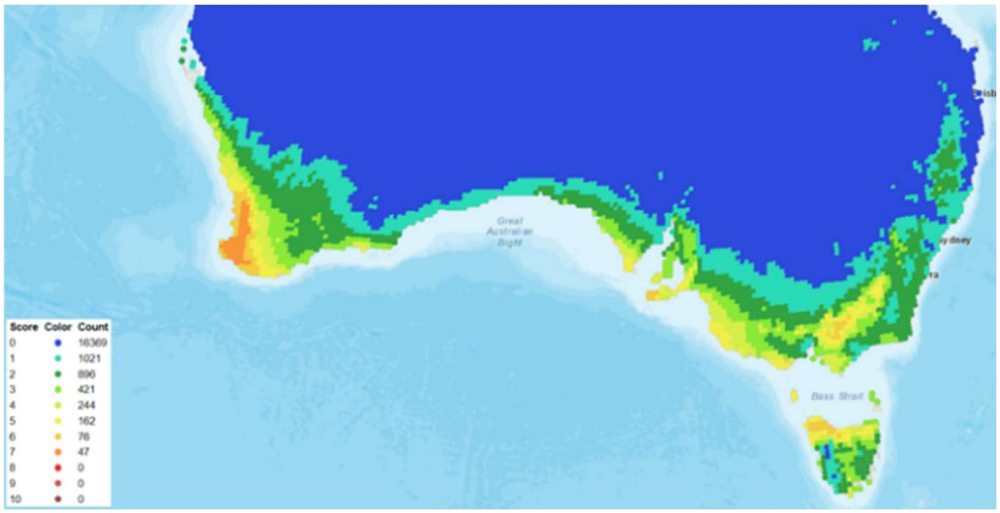
Seed size greater than 2 mm wide was not encountered in any of the field trials in this study. However, seed size was the highest at Geraldton and Leeton where quinoa appears to be well suited and where seed development took place at lower temperatures and shorter daylengths. This is supported by work of Bertero et al., (1999a) [36] who showed that increasing temperature and daylength after anthesis was correlated with decreasing seed size, and later by Bhargava et al., (2007) [37] who found that increasing days to maturity (and by association increasing temperature and daylength after anthesis) was also correlated with decreasing seed size. Seed size at Bool Lagoon was low possibly due to plants being swathed too early. The limited genetic diversity available for this work likely contributed to the absence of seed exceeding 2 mm wide found in some varieties of quinoa from the highlands of Peru and Bolivia [38].

High ΔE*ab values at Manjimup are consistent with discolouration of seed most likely caused by one significant wet event prior to harvest (35 mm over a 6-day period) [13]. Likewise high values at Leeton are also consistent with discolouration most likely caused by cool and wet conditions over an extended pre-harvest period (20–30 mm over a 20-day period) [13]. Values at Geraldton are also generally high and may be a reflection of the relatively cool and humid period approaching harvest. Wieme et al., (2020) [39] suggest darker seeds result from secondary fungi associated with a delayed harvest. However, their L, a and b values translate to ΔE*ab values increasing only from 32 to 35. Results for line PI 634925 are not relevant since seeds are a mixture of white and red/brown and could potentially serve a different market. Overall, there would appear to be limited potential to compete with Royal White Quinoa produced in Bolivia or Peru in terms of 'whiteness' of seed, despite some good results for variety BEW and lines PI 614888 and PI 665278 in this study. Cool and dry conditions at the end of the quinoa growing season in the highlands of Bolivia and Peru are unlikely to be regularly encountered in any part of Australia where there is potential to grow the crop.

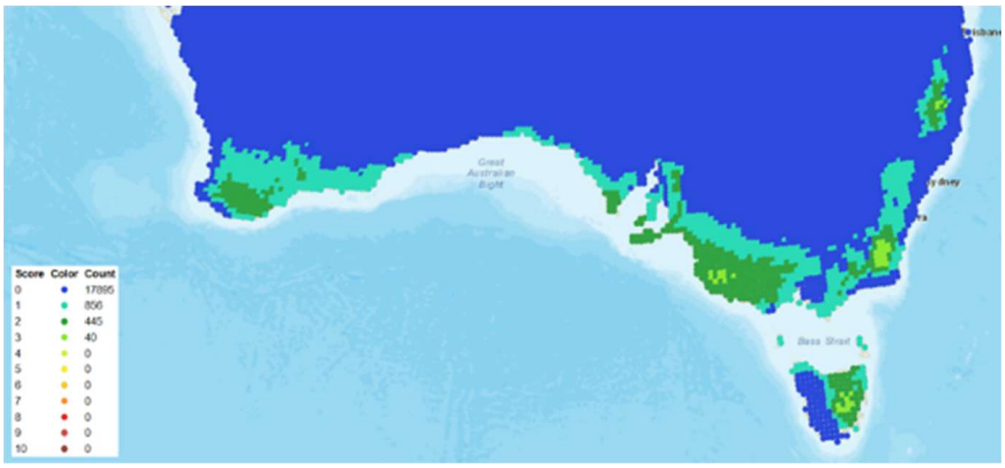
4.3. Adaptation to Australian Environments

Predictions of quinoa's area of adaptation to rain-fed environments in Australia using CLIMATCH [40] shows a good climate match with south-western Australia for Chilean germplasm (score 6–7) and a poor climate match for germplasm from Bolivia (score 2–3 for south coastal regions of the mainland and eastern Tasmania) (Figure 6a,b). However,

CLIMATCH lacks sensitivity for climatic conditions in spring when winter grown quinoa in southern Australia is undergoing the critical phases of flowering, seed development and seed maturation. A closer analysis of temperature and rainfall across the regions is required as well as an understanding of how flowering is affected across the range of latitudes and the range of planting times.



(**a**)



(**b**)

Figure 6. (**a**). Climate match for germplasm origin sites in Chile with Australia. (**b**). Climate match for germplasm origin sites in Bolivia with Australia.

Flowering in quinoa is controlled by both temperature and daylength [41]. Temperature rather than daylength is the main driver of flowering in most of the Chilean germplasm tested in this work and is consistent with earlier observations [42]. Heat sums of between approximately 1000 and 1200 degree days corresponded with the commencement of flowering across a broad range in latitude from 15 °S to 37 °S. Only when day length between planting and flowering exceeded 13:26 h:min at Bool Lagoon was flowering in all of the Bolivian germplasm significantly delayed. Rapid flower initiation can limit the potential seed production of quinoa by insufficient resources being directed to plant growth during the vegetative stage. This is likely the case in the far north (e.g., Katherine, Kununurra and Walkamin) where short flowering times of <50 days were observed. Very slow flower initiation in southern regions caused by cold winter temperatures in rain-fed crops (in the case of germplasm from Chile) or long days in summer irrigated crops (in the case of germplasm from Bolivia) may also limit potential seed production. Knowing when flowering will occur makes it possible to predict climate conditions during the growing

season based on the preferred planting time. Quinoa is highly tolerant of abiotic stresses including cold and heat during the vegetative stage [30]. However, seed production in quinoa is strongly affected by cold and heat during the reproductive stage as indicated in this work. In addition, rainfall at maturity can severely affect seed quality as well as yield. Finding areas where temperature and rainfall conditions are suitable is a challenge on the Australian continent for both irrigated and rain-fed quinoa production.

Climate conditions in Geraldton appear to be ideal for optimal growth and seed production of rain-fed quinoa originating from Chile with seed yields as high as 3 t/ha. In particular, mild winter temperatures during vegetative growth, mild temperatures and moderate rainfall during flowering and seed development in early spring, and a rapid cut off in rainfall immediately prior to maturity appear to be important factors. Relatively calm conditions (low wind speed and low rainfall intensity) in Geraldton during the growing season compared with regions much further south are also likely to favour plant establishment and limit lodging later in the season (mean wind speed of 13–16 km/h c.f. 20 km/h) [13]. Results of a herbicide trial in 2018 at Dongara which experiences a very similar climate to Geraldton 60 km to the north where quinoa variety BEW yielded >3 t/ha supports these claims [43].

There are few regions around the globe with a similar climate where rain-fed quinoa yields similar or better than at Geraldton. The Humbolt County in northern California where the Lundberg Family Farms operate is likely to be one [44]; however, there are no published yield data. Other locations include Washington State, Colorado and south-eastern Idaho in the USA [45], Saskatchewan and Alberta, Canada [46], south-western Germany [47], Morocco [48] and Malawi [49] where yields of 1.7–2.0 t/ha, 1.0–2.0 t/ha, 1.7–2.4 t/ha, 0.93 t/ha and 1.5–2.0 t/ha are reported, respectively. Variety Regalona yielded over 3 t/ha under rain-fed conditions on the Volturno River plain, north of Naples, Italy despite mean temperatures of 22–24 °C and rainfall of 5–10 mm at the end of June during the seed development stage [50]. It is possible that the soils in this region have very good water holding capacity and nutrition. The climate in Cambridgeshire, UK appears to suit quinoa very well with yields up to 5 t/ha in Chilean varieties [8]. Quinoa growing areas in coastal Chile also have similar climatic conditions to Geraldton and yields up to 1.2 t/ha are reported [23]. In the highlands of Peru and Bolivia quinoa is grown under rain-fed conditions during the wet, warmer half of the year and allowed to mature under drying, cooling conditions. It is likely that quinoa varieties in Chile have retained the adaptation to these conditions and as such are intolerant to the end of season terminal drought experienced in the southern grain belts of Australia. However, rain-fed quinoa may be suited to coastal areas of southern Australia at lower latitudes. Attempts to extend the range of quinoa production in Peru into the inter-Andean valleys at lower altitudes have been faced with the negative impacts of heavy rainfall and hail storms [51]. This supports the view that optimal conditions are unlikely to be found in more southerly regions of Australia where winter and spring conditions are likely to be unfavourable for the production of rain-fed quinoa. Matching planting date with location to achieve ideal weather conditions at the critical times of plant establishment, flowering, seed development and harvest is problematic.

The success of quinoa under irrigation in Australian environments depends on suitable temperatures during the growing season and the scarcity of rainfall during the planting and harvesting periods. Suitable locations exist in the northern tropics where the rain season is well defined; however, winter temperatures may be excessive and in the eastern areas of the savanna, the rainfall season is less well-defined. For example, the Atherton Tablelands (including Walkamin) may offer a suitable environment; however, rainfall at harvest time in September could risk seed sprouting damage. The difference in seed yields observed in the two years of trials in Kununurra in this study can be explained by warmer temperatures as a result of delayed planting caused by late rains in April and May. Much of the east of Australia may be suitable; however, coastal regions often do not have a well-defined dry period and inland arid regions experience greater temperature extremes.

Good results at Leeton in the cold semi-arid region of southern New South Wales point to the importance of sowing time and the corresponding effect of high temperatures on seed yield and rainfall on seed quality of quinoa. South coastal regions often have highly variable weather conditions during summer when quinoa would be irrigated. Irrigation is available to supplement rainfall as spring and summer rainfall is often inadequate to sustain a crop. High temperatures during the seed development stage are a risk for spring sown crops in these regions. The good performance of quinoa at Bool Lagoon may be related to soil type as much as to climate. A deep, self-mulching clay soil with large water holding capacity will buffer against low rainfall to a significant extent. Coastal regions in southern Australia may have the potential for seed yields of 2.0 to 2.5 t/ha, if grown on suitable soil types and if high temperatures towards the end of the growing season and prolonged rainfall at maturity are avoided. The limited irrigation infrastructure will impact the adaptation of quinoa to vast areas with a suitable climate (for example central west coast of Western Australia—Carnarvon to Broome; south coast—Albany to Esperance WA and Eyre peninsula SA). There may be potential for quinoa in coastal Victoria where irrigation is available, and the potential in the north coast of Tasmania has already been demonstrated [3].

In the irrigated field trials of this study where end of season water was not limiting, germplasm from both Chile and Bolivia performed well with the best seed yields reaching between 2 and 3 t/ha. Irrigated quinoa in Peru, Bolivia, Ecuador, Columbia, Argentina and Brazil yielded between 1 and 2.5 t/ha [9]. At Ovalle and Coquimbo in the dry coastal area north of Santiago quinoa lines from coastal, lower latitudes yielded between 0.5 and 5.0 t/ha [52]. Quinoa has been field tested under irrigation in many countries outside of South America. Yields of a large range in germplasm ranged from 0.3 to 9.8 t/ha at Lucknow in sub-tropical north India [37]. Quinoa yielded 2–3 t/ha in Foggia, Italy [53], 3 t/ha in Bunda and Bembeke, Malawi [49], and 3 t/ha in Agadir, southern Morocco [54]. In Turkey, quinoa yielded an average of nearly 3 t/ha at Agdir on slightly saline soil [26], between approximately 1 and 3 t/ha in one advanced line at Izmir depending on N application [27] and over 6 t/ha in Titicaca with fresh irrigation water at Adana [55]. The best yields of 3 t/ha in this work compare reasonably well with yields from many of these reported field trial.

It is clear that successful quinoa production in Australia is critically dependent on a suitable climate. Given this, it is important to note that only a limited range of germplasm was evaluated in this study. Elevating the yield potential above 3 t/ha is possible through access to and testing of the large genetic diversity of quinoa landrace germplasm. Germplasm collections are extensive for Peru, Bolivia and Ecuador amounting to over 13,000 accessions; however ex situ collections of quinoa originating from coastal Chile total less than 300 accessions [23,56]. High altitude varieties may offer further yield improvements when grown under irrigation at latitudes ranging from 14 °S to 34 °S, given the good performance of lines from Bolivia at Katherine and Leeton in this study. Coastal varieties from Chile may offer further yield improvements when grown either under irrigation or in rain-fed environments, particularly in more southern latitudes.

5. Conclusions

Crops introduced into new regions often pose production risks similar to the domestication of species from the wild. Introducing quinoa to Australia, a large continent with a huge agroclimatic diversity, required ground truthing of its perceived potential. This study looked at the available germplasm diversity to test for plant and growth features. The results were encouraging and data show that quinoa can be successfully grown, albeit varieties and crop management practices need to match agroclimatic diversity. We found high temperatures and frost during the reproductive phase and end of season rainfall to be the critical limiting factors determining seed yield, while rainfall at maturity affected seed quality.

Author Contributions: R.S. and D.L.S. conceived the goals and aims. R.S. acquired funding for the project, coordinated the planning of the research activity and wrote the initial draft. R.S., D.L.S., M.W., D.T., A.P. and C.T. designed and conducted field trials. I.B. and H.S.D. undertook data preparation. H.S.D. coordinated the research activity in the final stage of the project. M.F.D. undertook formal data analysis. H.S.D. and M.F.D. provided critical review, commentary and revision of drafts. All authors provided commentary and review of drafts. All authors have read and agreed to the published version of the manuscript.

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