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RESEARCH ARTICLE

Evaluation of *Chenopodium quinoa* × *C. berlandieri* recombinant inbred lines (RILs) for heat tolerance

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ABSTRACT

There is growing interest in expanding quinoa (*Chenopodium quinoa* Willd.) cultivation; however, the main limitation is its sensitivity to temperatures above 32-35 °C. *Chenopodium berlandieri* Moq., the wild-weedy North American ancestor of quinoa, was employed as an exotic source of drought, heat, and salinity tolerance to enlarge genetic variation in cultivated quinoa. The present work aims to assess the influence of high temperatures on seed setting rate and production potential of two quinoa recombinant inbred lines (RILs), RIL2-4 and RIL2-5. Thus, trials were carried out in Morocco at Berrechid and Tiflet in 2017-2018 and Bouchane, Meknès, and El Kebab in 2018-2019. The field experiment design was a randomized complete block with four replicates. Ten quantitative traits were measured. The ANOVA showed significant variability for all the evaluated parameters. The seed setting rate had widely different degrees of variation among experimental sites, exceeding 120% at Berrechid and Tiflet while approaching 98% at Bouchane. Tiflet expressed the lowest seed setting at 11.26%. Pearson's correlation matrix analysis showed a significant positive correlation between plant size at maturity and inflorescence length. An analysis of mixed model interactions (AMMI) revealed the first interaction; principal component axis explained 53.3% of the total variation while the second axis described 46.7%. Four homogeneous groups emerged. The recombinant inbred lines have segregated for tolerance to high temperatures as evidenced by their variable seed setting rates and grain and DM yields.

Key words: Diversity, heat tolerance, high temperatures, RILs, seed setting, site effect.

INTRODUCTION

Heat stress from rising ambient temperatures and frequent hot weather episodes has become an agricultural constraint in many parts of the world (Wahid et al., 2007). When combined with unpredictable rainfall patterns, these phenomena are critically impacting crop growth and development (Sehgal et al., 2017). Globally temperatures have increased since the late 19th century, and each of the past three decades has been successively warmer than all previous decades (IPCC, 2021). In the past few decades, crop damage caused by heat stress had occurred more frequently (Huang et al., 2016).

Cereals are the most important food resource in the world, both for direct human consumption and, indirectly, as inputs for animal production (FAO, 2022). Constituting the basic diet of the majority of the world's population, cereals are therefore at the center of any reform of world food production. Hence, it is imperative to develop new cereal varieties and other alternative crops adapted to higher temperatures to meet future global demands (Challinor et al., 2014) and ensure food security for the population.

While not a true cereal, the highly nutritious broadleaf pseudocereal quinoa (*Chenopodium quinoa* Willd.) (Ruiz et al., 2014) from the High Andes and Chilean coastal zone currently includes cultivated varieties adapted to a 9-30 °C production temperature range (Bhargava et al., 2007). It has wide tolerance to abiotic stresses, except for flooding and heat, especially during flowering and seed setting. The elevated resistance of quinoa to non-thermal abiotic stresses results from a high degree of genetic diversity and unfavorable environmental conditions prevalent within the crop's center of origin (Sanchez et al., 2003). Currently, there is a growing interest in expanding globally quinoa cultivation (Maliro et al., 2017), due to its excellent nutritional properties (Wu et al., 2020) and its ability to grow on poor soils (Jacobsen, 2003). However, heat stress is a major constraint to the expansion of quinoa cultivation (Hinojosa et al., 2018); as, quinoa seed yield can be significantly impacted when temperatures during the flowering period exceed 32 °C (Hinojosa et al., 2018). However, quinoa's free-living sister species, South American *C. hircinum* Schrad. (avian goosefoot) and North American *C. berlandieri* Moq. (pit-seed goosefoot) include genotypes native to the lowland subtropics where temperatures often exceed 35 °C during flowering and they are entirely cross-compatible with quinoa (Wilson and Manhart, 1993). Jellen et al. (2019) described successful efforts to produce heat-tolerant, interspecific quinoa × pit-seed goosefoot breeding populations.

Quinoa cultivation was introduced to Morocco in 1999 with the main objective to diversify the cultivated crops and to fight against malnutrition in a rural community of the Middle Atlas Mountains. Two factors that hinder the development of the agricultural sector and the well-being of local populations were addressed. Among the possible solutions to meet this goal, a quinoa breeding program was set up in 2000 (Benlhabib, 2005) to develop varieties adapted to local conditions and to promote quinoa as alternative crop, alongside cereal crops. However, the main limitation is its sensitivity to high temperatures. It is within this framework that the present project aims to screen an interspecific quinoa population in segregation for tolerance to high temperatures through seed formation and yield potential evaluation in five contrasting agroclimatic sites.

MATERIALS AND METHODS

The multi-site screening trials included a set of 156 F_5 recombinant inbred lines (RILs) of which 39 were F_4 RILs (where insufficient F_5 seeds were obtained). The breeding population was developed from an interspecific cross between lowland quinoa line NL-6 of Chilean origin, and heterogeneous accession WM11-54, which arose through mass selection following years of passive exposure of quinoa (*Chenopodium quinoa* Willd.) 'Co407' to pollen of native *C. berlandieri* Moq. in the San Luis Valley of southern Colorado, USA (J. McCamant, personal communication).

The fieldwork encompassed five trials: Two in 2017-2018 at Berrechid and Tiflet and three in 2018-2019 at Bouchane, Meknes, and El Kebab.

The Berrechid experimental commercial farm lies at the interior edge of the semi-arid central coastal plain on fine-textured, water-holding Vertisols (Table 1). In Tiflet, the experiment was conducted at a previously abandoned small farm on well-drained Fluvisols on the edge of the Bou Regreg river watershed.

Table 1. Experimental sites and their agroclimatic characteristics.

	Geographic position			Temperature					
Location	Latitude	Longitude	Altitude	Min	Max	Mean	Rainfall	Humidity	Soil type
			m		°C		mm	%	
Berrechid	33.18	-7.49	309	8	33	18.92	246	69.52	Clayey-Silty
Tiflet	33.89	-6.30	340	7	35	19.00	337	70.58	Sandy-Silty
Bouchane	32.33	-8.36	334	11	39	23.00	310	47.08	Sandy
Meknes	33.85	-5.55	592	8	36	19.92	528	60.33	Clayey
El Kebab	32.71	-5.19	1503	9	35	20.67	470	59.67	Sandy-Silty

The Bouchane experimental site, a cooperative semi-commercial farm in southwestern Morocco, lies in the semi-arid Phosphate Plateau on medium-textured, non-water-retaining Cambisols (Table 1) (Jellen et al., 2021). The Meknes trial was conducted at a commercial farm under a continental climate with rainy winters and hot summers and on fertile, water-holding Cambisols. The El Kebab experiment was conducted on a subsistence farm within the foothills of the Middle Atlas Mountains at 1540 m elevation, under semi-arid climate with rainy winters, relatively cool temperatures and on stony Fluvisols.

The experimental sites' climate type varied from the warm Mediterranean at Tiflet, Meknes, and El Kebab; continental semi-arid at Berrechid; to semi-arid at Bouchane (Table 1).

The trials were sown late in the season so that pollination and seed setting would coincide with summer to early autumn heat stress, on 27 June and 12 July, 2017-2018 for Berrechid and Tiflet, respectively, and on 20

February, 28 March and 30 April, 2018-2019, at Bouchane, Meknes, and El Kebab, respectively. The sowing was performed by hand at a rate of 0.5 g seeds m^{-1}. The experimental design followed a randomized complete block with four replicates, one row per accession of 1 m length and 0.5 m inter-rows. The trials received irrigation twice a week and hand-weeding every other week.

At maturity, four individual plants per accession were randomly sampled and brought back to the laboratory for their evaluation. Data were collected for 10 agromorphological characters assessment: Stem height or plant stature (PS), stem diameter (SD), inflorescence length (IL) and width (IW), root depth (RD) and DM (RDM), grain yield (GY), aboveground DM weight (ADM), harvest index (HI), and seed setting rate (SSR).

Plant height (PS), root depth (DR), length (IL) and panicle width (IW) were measured using a graduated ruler. Stem diameter was measured with a caliper.

The DM and grains of each plant were weighed after being incubated for 48 h at 70 and 35 °C, respectively. The harvest index (HI) was calculated using the following formula:

$$HI = (GY)/(ADM + RDM + GY)$$

The SSR was assessed by counting the number of grains on three samples of 30 glomeruli collected at the apical, median and basal part of the panicles. Use of a binocular magnifying glass was necessary. The seed setting data were calculated as percentages; therefore, we applied the 2Arc sin $\sqrt{x}$ angular transformations to meet distribution normality and equality of the variance.

The statistical analyses focused on descriptive and ANOVA with two factors, genotype (RILs) and site. The evaluation of the G×E interaction was assessed using additive main effects and multiplicative interaction (AMMI) to assess the effects of genotype and experimental site on RIL grain yield and heat tolerance. Principal components analysis (PCA) was carried out in order to identify the best-performing individuals taking into account the traits that explained the most variability between lines.

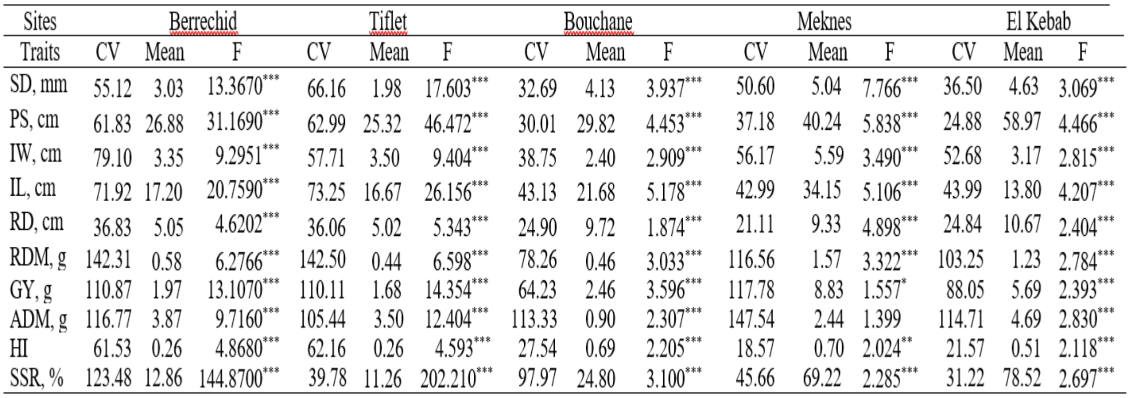
The AMMI model provided the Genotype × Environment (GE) principal components (Zobel et al., 1998) and the productivity and stability potential of the genotypes. The calculation of the stability value (ASV) of the RILs was performed according to Purchase (1997). This parameter reveals the genotype's stability based on the interaction principal components of the two first axes. For all the data analyses we used R software version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Analysis of intra and inter-locality variability

The coefficients of variation (CV) estimate the trait variability. The comparison of these coefficients in Table 2 shows notable variations between the trait's assessments.

Table 2. An ANOVA of agromorphological traits at the five experimental sites. SD: Stem diameter; PS: plant height at maturity; IW: inflorescence width; IL: inflorescence length; RD: root depth; RDM: root DM; GY: grain yield; ADM: aboveground DM; HI: harvest index; SSR: seed setting rate.



Sites	Berrechid			Tiflet			Bouchane			Meknes			El Kebab		
Traits	CV	Mean	F	CV	Mean	F	CV	Mean	F	CV	Mean	F	CV	Mean	F
SD, mm	55.12	3.03	13.3670***	66.16	1.98	17.603***	32.69	4.13	3.937***	50.60	5.04	7.766***	36.50	4.63	3.069***
PS, cm	61.83	26.88	31.1690***	62.99	25.32	46.472***	30.01	29.82	4.453***	37.18	40.24	5.838***	24.88	58.97	4.466***
IW, cm	79.10	3.35	9.2951***	57.71	3.50	9.404***	38.75	2.40	2.909***	56.17	5.59	3.490***	52.68	3.17	2.815***
IL, cm	71.92	17.20	20.7590***	73.25	16.67	26.156***	43.13	21.68	5.178***	42.99	34.15	5.106***	43.99	13.80	4.207***
RD, cm	36.83	5.05	4.6202***	36.06	5.02	5.343***	24.90	9.72	1.874***	21.11	9.33	4.898***	24.84	10.67	2.404***
RDM, g	142.31	0.58	6.2766***	142.50	0.44	6.598***	78.26	0.46	3.033***	116.56	1.57	3.322***	103.25	1.23	2.784***
GY, g	110.87	1.97	13.1070***	110.11	1.68	14.354***	64.23	2.46	3.596***	117.78	8.83	1.557*	88.05	5.69	2.393***
ADM, g	116.77	3.87	9.7160***	105.44	3.50	12.404***	113.33	0.90	2.307***	147.54	2.44	1.399	114.71	4.69	2.830***
HI	61.53	0.26	4.8680***	62.16	0.26	4.593***	27.54	0.69	2.205***	18.57	0.70	2.024**	21.57	0.51	2.118***
SSR, %	123.48	12.86	144.8700***	39.78	11.26	202.210***	97.97	24.80	3.100***	45.66	69.22	2.285***	31.22	78.52	2.697***

The seed setting rate (SSR) varied widely among experimental sites, exceeding 120% at Berrechid and Tiflet while it approached 98% at Bouchane (Table 2). The highest CV for SSR was registered at Tiflet (139.78%). On the other hand, Meknes and El Kebab recorded the lowest variability; their CVs reached 45.66% and 31.22%, respectively. These two sites recorded also the highest SSR average values, 69.22% in Meknes and 78.52% at El Kebab. The low variability observed at Meknes, would be due to the low number of measured samples because the late sowing did not allow 64% of RILs to show their potential, and therefore only 52 lines of a total of 156 were evaluated. In El Kebab, the agroclimatic conditions were quite auspicious for seed filling for all the RILs, the reason why the lowest coefficient of variation of SSR was recorded on this site. Indeed, the different lines expressed their seed production potential.

Total DM varied between 1.36 and 5.92 g plant^{-1} at Bouchane and El Kebab, respectively, while grain yield per plant ranged from 1.68 to 8.83 g in Tiflet and Meknes, respectively. Data analysis also showed that the harvest index (HI) fluctuated significantly between sites from 0.26 in Berrechid to 0.70 in Meknes.

The ANOVA detected highly significant differences across the five sites for the 10 traits except for the grain yield (GY) and the above-ground DM (ADM) at Meknes (Table 2).

The RILs' SSR means comparison identified 37 homogeneous groups in Berrechid. This test clusters L61-331 and L59-211 lines in a first group, with SSRs of 70.55% and 60.66% respectively. At Tiflet, the analysis revealed also 37 homogeneous classes, L31-233 and L73-11 were ranked in the most fertile group with SSRs 52.77% and 50.55% respectively. At Bouchane, SSRs means comparison revealed nine homogeneous classes and three lines (L76-122, L20-222, and L52-213) recorded the highest values close to 60%. The lowest SSR group gathered six lines, L13-32, L23-121, L45-313, L46-32, L62-212, and L83-112. In the Meknes site, 11 groups were identified, the first group was made of the two totally fertile accessions (SSR equal to 100%), L22-122 and L59-315. There, the most heat susceptible line, accession L44-354, has an SSR equal to 0.55. At El Kebab, the first group includes three lines, L3-35, L34-344, and L8-31, with an SSR equal to 100%. The lowest SSR value 24.81% was noted with L55-221.

Multivariate analyses

The ANOVA, principal component analysis (PCA), and AMMI analyses focused on Berrechid, Tiflet, and Bouchane data since Meknes and El Kebab sites did not discriminate between the genotypes. The two factor ANOVA revealed highly significant effects of the site, genotype, and their interaction (Table 3). A significant correlation of (R = 0.8**) was found between grain yield and root DM, and between above ground DM and plant size.

Table 3. Three-factor ANOVA for agromorphological traits. SD: Stem diameter; PS: plant height at maturity; IW: inflorescence width; IL: inflorescence length; RD: root depth; RDM: root DM; GY: grain yield; ADM: aboveground DM; HI: harvest index; SSR: seed setting rate.

Variable	Source of variation	F value	Variable	Source of variation	F value
SD; mm	Line	31.717***	RDM; g	Line	13.702***
	Site	1318.300***		Site	21.779***
	Line×Site	8.375***		Line×Site	3.191***
PS; cm	Line	100.287***	GY; g	Line	49.300***
	Site	221.486***		Site	109.632***
	Line×Site	22.239***		Line×Site	10.645***
IW; cm	Line	49.546***	ADM; g	Line	76.314***
	Site	254.864***		Site	1525.135***
	Line×Site	13.102***		Line×Site	19.660***
IL; cm	Line	58.487***	HI	Line	8.587***
	Site	216.446***		Site	1972.077***
	Line×Site	13.425***		Line×Site	2.471***
RD; cm	Line	10.799***	SSR; %	Line	11.539***
	Site	1618.786***		Site	163.323***
	Line×Site	2.518***		Line×Site	8.348***

The SSR correlates positively with root DM. These results validate the relevance of SSR, grain yield, root DM and above ground DM on highly heat stress-tolerant genotypes selection under field conditions.

Principal component analysis (PCA) of agromorphological traits data across the three experimental sites is presented in Figure 1. The PC1 axis gathers 52.66% of the total variability while the PC2 axis explains an additional 14.75%. The PC1 showed a strong and positive correlations for root DM, grain yield, stem diameter, and inflorescence length. The second principal axis displayed a significantly positive relationship with SSR and HI and a negative correlation with inflorescence width.

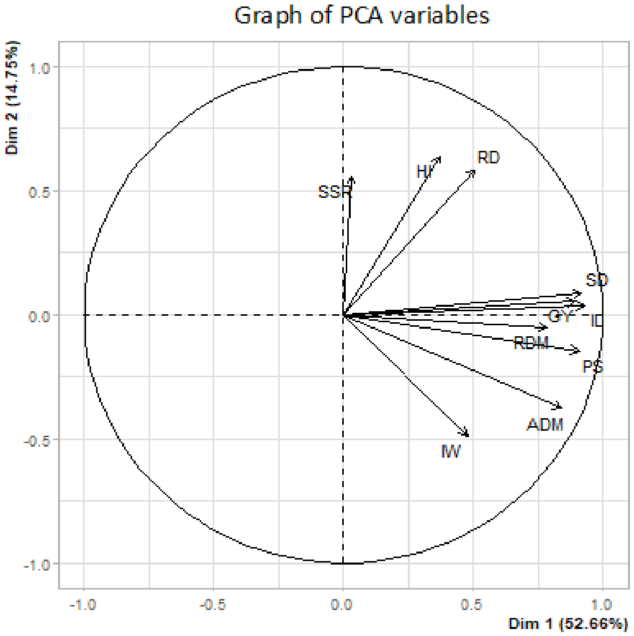


Figure 1. Two-dimensional PCA plot for agromorphological traits. SD: Stem diameter; PS: plant height at maturity; IW: inflorescence width; IL: inflorescence length; RD: root depth; RDM: root DM; GY: grain yield; ADM: aboveground DM; HI: harvest index; SSR: seed setting rate.

The two-dimensional PCA graph (Figure 2) identified four clusters. The first cluster (in black) brought together 71 lines that shared GY and SSR close to 1.04 g plant^{-1} and 15%, respectively. The second cluster (in red) grouped 33 heat-sensitive and low-yielding genotypes; their mean SSR across the three experimental sites was as low as 9.14%. The third cluster (in green) grouped 43 moderately performing lines; their GY was approximately 3.1 g plant^{-1} and their SSR approached 19%. The fourth cluster (in blue) gathered nine lines all with relatively high GY averaging 7 g plant^{-1} and an SSR approaching 13%.

The AMMI analysis of SSR identified the most productive and stable quinoa lines (Table 4). The multi-site interaction analysis revealed highly significant differences for sites, genotypes, and their interactions. The AMMI principal interaction axis IPCA1 explained 53.3% of the total variation; the second axis accounted for an additional 46.7%.

Figure 3 displays the genotypes and environments' IPCA1 scores plotted against the SSR. Berrechid and Tiflet sites emerged on the negative side of PC1 and were opposite to Bouchane site. The highest SSR average of 24.8% was that of the Bouchane site. In contrast, the lowest SSR average of 11.26% was observed at Tiflet. Tiflet has a lowland Mediterranean climate combined with loam sandy soil; there, July late sowing exposed the quinoa plants to the high temperatures and drought stress which reduced the number of seeds per panicle and the production potential of the RILs.

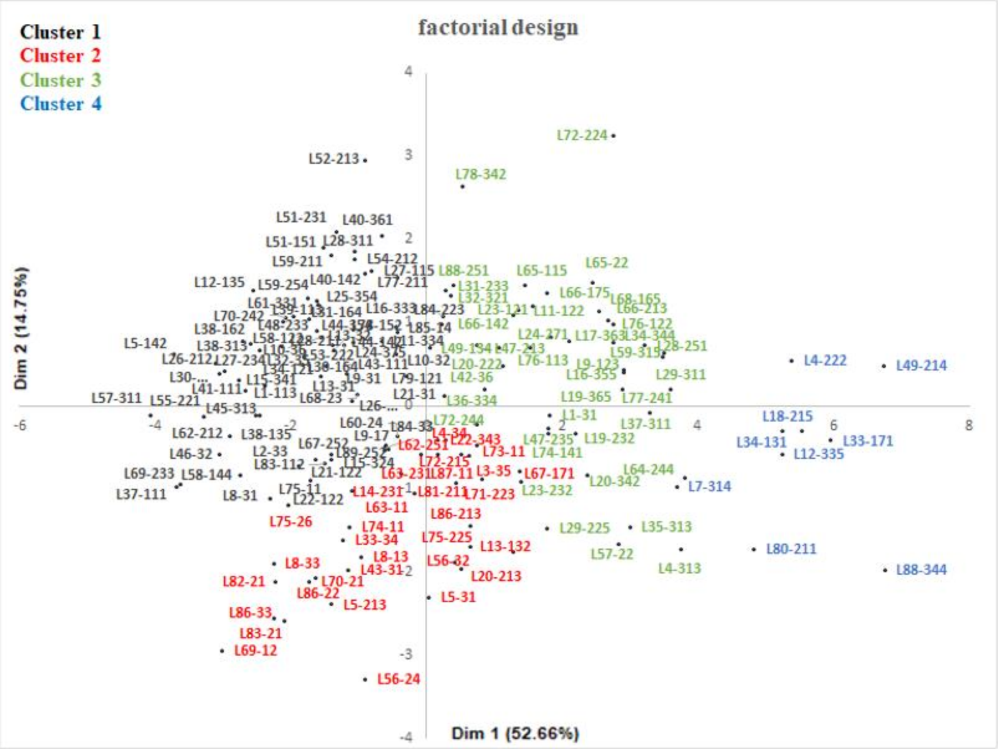


Figure 2. Two-dimensional PCA plot for genotypes recombinant inbred lines (RILs).

Table 4. AMMI analysis of variance for seed setting rate of recombinant inbred lines (RILs) at three locations.

	D	Sum of squares	Mean of squares	F value	Pr. (> F)	Var. (%)
ENV	2	50836	25418.1	34.659	3.19e-02***	-
REP(ENV)	10	7334	733.4	8.035	1.29e-09***	-
GEN	156	178732	1145.7	12.553	< 2.2e-16***	-
GEN: ENV	267	246727	924.1	10.124	< 2.2e-16***	-
Residuals	1139	103960	91.3	-	-	-
PC1	157	179667.8	1144.382	12.54	0	**53.3**
PC2	155	157562.0	1016.529	11.14	0	**46.7**

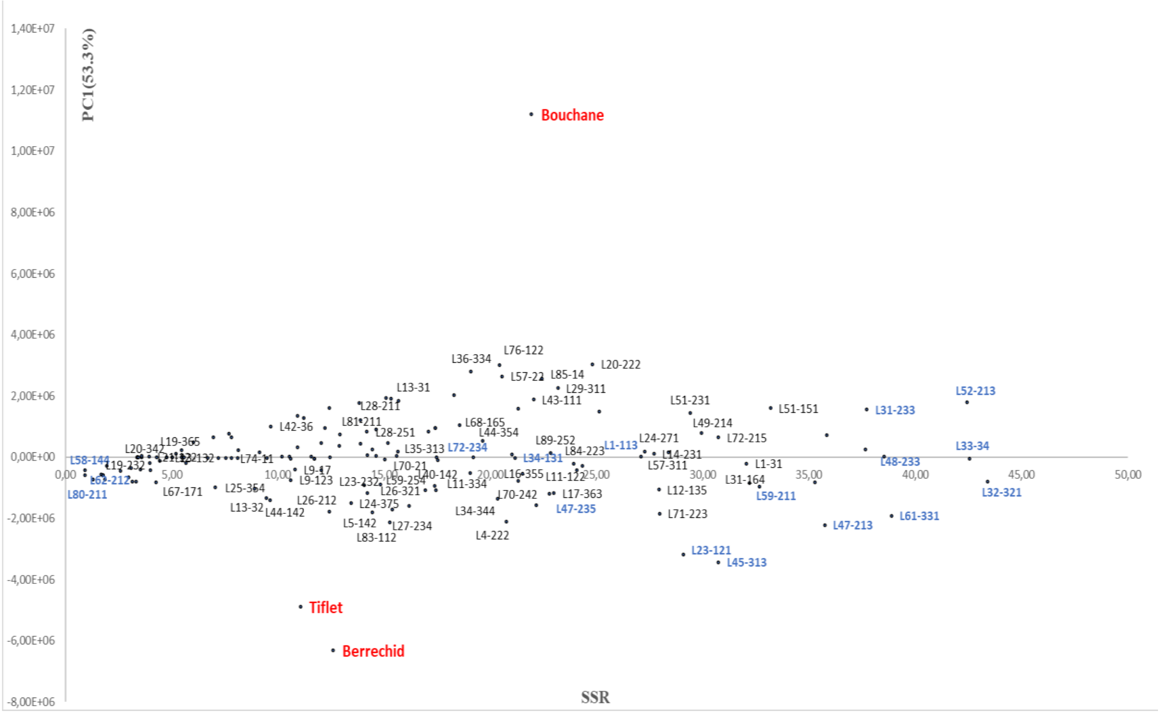


Figure 3. Factorial plan of the first principal component (PC1) and seed setting rate (SSR) of the lines.

In this study, 42.94% of the RILs surpassed the overall mean SSR of 15.20%; among this group, 11 lines were identified, L32-321, L33-34, L52-213, L61-331, L48-233, L31-233, L59-211, L23-121, L45-313, L1-113, and L47-235 (Figure 3). These were the most fertile lines, recording significant SSRs ranging from 20% to 43%. The lines L32-321, L1-113, and L47-235 reveal also high stability, they were plotted close to the center of the IPCA1-IPCA2 biplot (Figure 4). The rest of the performing lines reveals low stability; they are placed away from the IPCA1-IPCA2 biplot center. The lowest SSRs were those of L58-144 (0.93%), L80-211 (0.93%), and L62-212 (1.30%) (Figure 3); their stability scores were intermediate, equal to 5.01, 6.65, and 8.26, respectively.

The AMMI biplot depicted the relationship between IPCA1 and IPCA2 (Figure 4), which can be used to evaluate the genotypes and environments interaction (Rashidi et al., 2013). According to Li et al. (2003), discriminating environments can be detected through their vector length in the AMMI biplot. Based on the vectors' length, the best discriminating environments for the RILs are Bouchane and Berrechid as they presented the longest vectors. The IPCA1 and IPCA2 biplot indicated also the genotypes' relationship through their position and closeness to the graph center. Lines L31-233, L20-222, L59-211, L45-313, and L23-121 are unstable. Lines L19-232, L19-365, L13-132, L21-122, L1-113, L32-321, and L 67-171 as positioned closer to the biplot origin, have better stability across the environments. The genotypes' orthogonal projections on the environmental vectors indicate their relative performance at a given site. The greater the genotype projection is in the positive direction of a specific vector, the better is its performance in that environment. Thus, L61-331 and L59-211 are better adapted to Berrechid, L52-213 performed better at Bouchane, and L31-233 adapts more to Tiflet; their seed-setting rates were respectively equal to 70.56%, 66.67%, 68.44%, and 52.78% in the corresponding locations.

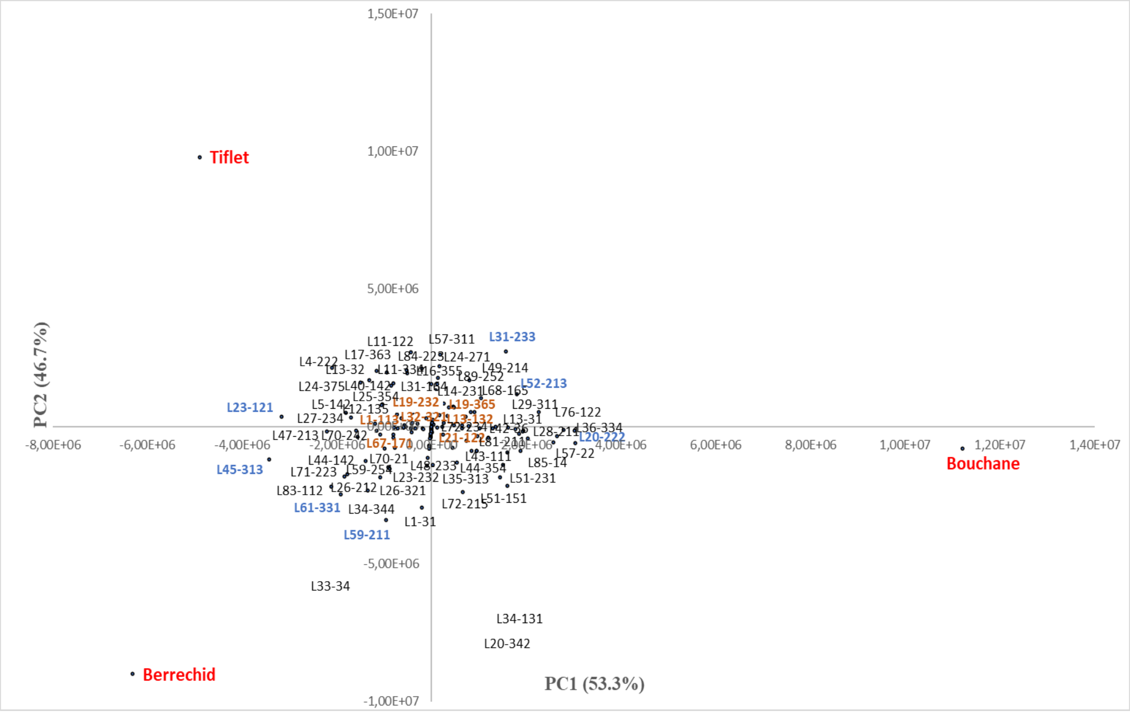


Figure 4. Biplot of the second interaction principal component axis (IPCA2) against the first interaction principal component axis (IPCA1).

The AMMI stability value (ASV) calculation uses the genotype's IPCA1 and IPCA2 scores. Genotypes having low ASV values are the most stable. Based on the ASV values, the RILs were gathered into four groups. The first group made of 72 lines is the most stable, with ASV values ranging from 0.60 to 0.99 and SSR value from 0.93% to 43.41%. Lines L33-34 and L32-321 were the most fertile (Figure 3) and their SSR sized above the mean (15.20%). The second group included 42 accessions whose stability values fluctuate between 1.02 and 1.97 and SSR percentages between 3.33% to 38.52%. Line L48-233 was the most fertile accession with an SSR equal to 38.52% (Figure 3). The third group made of 31 RILs was relatively unstable as its ASV values ranged from 2.01 to 2.99 and its SSR values from 9.44% to 42.44%. Within this 3rd cluster, 78% of the lines were among the most productive; their SSRs were above the mean. The fourth group gathered 11 unstable RILs, whose ASV values fluctuate between 3.04 and 4.09.

DISCUSSION

The data analyses show clearly the specificities of the different sites. The tested quinoa RILs were significantly influenced by the contrasting climates of the five experimental sites, from the warm Mediterranean to semi-arid and the altitudes ranges. The most relevant differences between sites were observed on root DM, grain yield, aboveground DM, and SSR. They showed the greatest variability across the sites.

The variability of grain yield per plant exceeded 100% in Berrechid, Tiflet, and Meknes when it approached 60% in Bouchane. Meknes site recorded the highest average yield per plant (8.83 g), followed by El Kebab and Bouchane with average yields per plant of 5.69 and 2.46 g, respectively. Berrechid and Tiflet sites recorded the lowest yields (1.1 g). This varied between 0 and 15 g plant^{-1} in Berrechid, between 0 and 14 g plant^{-1} in Tiflet, between 0.22 and 10.4 g plant^{-1} in Bouchane, and between 0.4 and 60 g plant^{-1} in Meknes and El Kebab. This yield variation between sites is well explained with the edaphoclimatic conditions and the growing season. According to Bertero et al. (1999), environmental factors, namely temperature and photoperiod, have significant effects on the plant growth and development. February, March and April sowing in Bouchane, Meknes, and El Kebab respectively, allowed better plant development. In Berrechid and Tiflet, the summer growing conditions impacted the RILs' flowering, seed filling, plant development, and yield elaboration because of the elevated temperatures of June and July.

Regarding the SSR, there were significant variations for heat tolerance among RILs derived from the quinoa population carrying genes from its wild North American sister-species, pit-seed goosefoot (*C. berlandieri*). Three of five experimental environments (Berrechid, Bouchane and Tiflet) discriminated between the lines through their seed setting potential.

The SSR depends on the genotype and the agroclimatic environment such as temperature, aerial humidity and crop management.

El Kebab site recorded the highest SSR (78.5%), followed by Meknes and Bouchane with average 69.2% and 24.8% respectively. Berrechid and Tiflet recorded the lowest average seed setting, around 12%. The significant reduction in the seed setting is generally linked to the high temperatures at the flowering and pollination stages combined with the soil texture and humidity shortage. Indeed, at Berrechid and Tiflet, the flowering and seed filling stages concurred with an average temperature of 33 and 36 °C respectively; such heat causes quinoa seed abortion. On the other side, spring sowing in Bouchane, Meknes and El Kebab sites, the pollination stage took place while the temperatures were still auspicious to plants growth and development. According to Bertero et al. (1999) quinoa is a susceptible species to temperatures over 30 °C.

Oilseeds, cereals and pulses crops are defenseless to hot temperatures in the field. Reproductive growth is most sensitive to heat, especially at temperatures around 30 °C (Bueckert et al., 2015), resulting in poor pollination, flower abortion, seed loss and lower yield. Therefore, knowing the role of temperature in the development of the seeds is an important step according to the results obtained by Lesjak and Calderini (2017), who found significant unfavorable differences in quinoa in terms of growth, grain production and their protein content, when the temperature increases during the flowering phase.

According to Hinojosa et al. (2018), it has been recognized that the temperature increase during the quinoa growth phase, is considered one of the most relevant abiotic stresses; it has consequences on its vegetative and reproductive growth, modifications in its physiological functions, and alterations on its productive parameters such as yield and quality of the production. This has been recognized that exposing quinoa plant to heat stress, generates changes in the water flow at the vascular level (Bosque-Sanchez et al., 2003), alterations of the crop phenological cycles (Bois et al., 2016), changes in the physiological activities (Becker et al., 2017), as well as modifications in the CO_2 assimilation activity, and its conversion into the grain storage product (Bunce, 2017). In effect, temperatures above 35 °C tend to induce plant dormancy or pollen sterility in many Latin American quinoa varieties, as was demonstrated under arid production conditions in trials in central Greece (Qin et al., 2017). At the flowering and seed filling stages, temperatures above 35 °C were associated with significant quinoa yield reduction experimental plots near Pullman, Washington (Walters et al., 2016). Other studies in growth chambers and under field conditions showed that temperatures above 32 °C reduced yields and induced physiological and phenotypic changes in quinoa (Hinojosa et al., 2018). Peterson and Murphy (2015) reported a heat tolerance variation among quinoa varieties, with Chilean sea level '407D', 'QQ74', and 'Kaslaea' exhibiting the highest tolerance degree to heat compared to other varieties under field conditions at Pullman.

In Berrechid, the temperatures generally fluctuated between 12 and 33 °C during flowering in August but exceeded 38 °C on several days. The highest SSR in Berrechid reached 70%, with an overall SSR average of 12.86% and with 17.94% of the lines being fully sterile. There, lines L61-331, L59-211, L1-31, and L45-313 had the best performance.

In Tiflet, during July-August, when the quinoa plants were in their most sensitive flowering and post-flowering stages (Lesjak and Calderini, 2017), temperatures fluctuate between 23 and 36 °C, while the photoperiod was at its maximum and atmospheric humidity at its minimum. The seed setting inhibition is quite common among highland-tropical quinoa ecotypes when they grow under long photoperiods and high temperatures (Bertero et al., 1999). The highest SSR of 52% occurred in just two RILs. Some lines showed significant susceptibility to the heat and a lack of adaptation to late-spring/early-summer sowing, indeed 53% of RILs did not produce any seed, though they flowered and developed panicles. The Tiflet site expressed 12%, the lowest performance against the heat.

At Bouchane some RILs exhibited poor panicle development because of hydric and thermal stress. There, L52-213 exhibited the highest SSR at 68.44%. Several lines produced few seeds and the overall SSR across the RIL population reached only 24.8%.

In Meknes, where temperatures fluctuated between 10 and 36 °C, two lines recorded complete fertility. In contrast, L44-354 accession did not produce any seed. There, the late sowing did not allow 64% of the RILs to express their optimal productivity potential.

At El Kebab at 1540 m elevation, the RIL performance was significantly higher; 95% of lines recorded more than 50% glomerular fertility. The highest SSR of 100% occurred in three accessions, while the lowest of 24.81% was observed in L55-221.

In Meknes and El Kebab, the average temperature during the cropping season is typically close to 20 °C. Their respective average annual rainfalls are 528 and 470 mm, which is an advantageous climate for seed production if sowing is timed to coincide with the onset of the autumn rainy season, which was not the case in this experiment. However, under the Meknes and El Kebab, cropping conditions and with sowing timed toward the middle-end of the winter-spring precipitation period, the adverse impact of heat on the quinoa lines' seed yield was relatively limited. The effects of temperature on quinoa reproductive organ development are dependent on genotype, ecotype, and cropping environment (Hedhly et al., 2009); the sea level ecotype 'NL-6' recorded its highest SSR at 30 °C (Isobe et al., 2012). The other parent of the original cross from which these RILs were derived, *C. berlandieri*, is known for its tolerance to heat, which explains the genetic segregation in SSR among the interspecific-cross offspring (Jellen et al., 2019). In this case, the interspecific quinoa derivative WM 11-54 possesses genes from *C. berlandieri* native to the San Luis Valley of southern Colorado, an area of relatively mild average summer high temperatures of 20-28 °C but where mean maximums can exceed 32 °C. For this experiment, under the relatively mild conditions at El Kebab and Meknes seed-set rates of 78.52% and 69.22%, respectively, were observed. Lines L3-35, L34-344, and L8-31 in El Kebab and L22-22 and L59-315 in Meknes presented achieved full seed set of 100%.

The two most important determinants of grain yield are the accumulation of the biomass and its distribution in the storage organ, which is evaluated through the harvest index (HI). The HI represents the capacity for photosynthesis and translocation to seeds. For Rojas (2003), the quinoa HI fluctuated from 0.06 to 0.87. It is sensitive to agronomic practices and environmental conditions (Bertero and Ruiz, 2010). The results obtained for this study, revealed that line L59-315 had the best HI of 0.66 and 0.63 at Berrechid and Tiflet respectively. As for the Bouchane station, the highest HI of 0.97 comes from L30-313. Indeed, the February sowing at Bouchane was favorable enough for a good grain production. In general, the RILs HI were ranged between 0.01 and 0.97 for respectively L45-313 at Berrechid and Tiflet and L30-313 at Bouchane. The low yields obtained at Berrechid and Tiflet could be explained by high temperatures that occurred at the plants flowering stage which usually affect quinoa seed production.

Under different stress conditions, the best genotype in terms of yield will exhibit stable production and significant yield at a particular cultivation area (Kadi, 2012). Therefore, RILs L37-311 and L80-211 were most suited to the Berrechid and Tiflet sites and L42-36 to Bouchane, with all of these lines producing significant yield in the corresponding sites. Based on the AMMI analysis, the significant G×E interaction allowed us to classify the SSR and yield stability across the three sites. Bouchane and Tiflet experienced strong G×E interaction as shown through their high IPCA1 scores. According to Crossa et al. (1990), high values of genotype scores (positive or negative) indicate specific adaptation to environments close to the position of the genotype under consideration. Therefore, RILs L61-331 and L59-211 were better adapted to the Berrechid site, L52-213 and L20-222 were performing better at Bouchane, L31-233 and L73-11 at Tiflet. Their respective SSR were 70.56%, 66.67%, 68.44%, 66.11%, 52.78%, and 50.56% at their most favorable locations. According to the ASV scores, the highest stability line L72-244 presented an above-average SSR of 17.47%.

CONCLUSIONS

With greater knowledge regarding effective adaptational traits, we can develop relevant crop management and breeding strategies to fight against climate change. The present study revealed the major criteria that are powerful in resilient lines selection to changing environments. This study also revealed the effect of high temperatures and, to a lesser extent, water stress, on quinoa's reproductive potential. Indeed, high temperatures during the flowering and seed-filling stages have affected significantly the production potential of several recombinant inbred lines (RILs) to the point they presented total empty-seeds inflorescences. The tested RILs were ranked from most susceptible to most resistant to a degree comparable to the *berlandieri* parent. The significant variation found among the RILs in this experiment is the result of plant genes expression and edaphoclimatic factors' effects during the crop cycle; on the other side, it is a confirmation of the genetic control of heat tolerance in quinoa and it also underscores the value of pit-seed goosefoot (*Chenopodium berlandieri*) as a source of heat-tolerance genes for quinoa improvement.

Author contributions

Conceptualization: A.A., O.B., E.J. Methodology: A.A., O.B, E.J. Software: E.T., A.A. Validation: A.A., O.B. Formal analysis: A.A., E.T., O.B. Investigation: A.A., E.T. Resources: O.B. Data curation: A.A., O.B., E.J. Writing-original draft: A.A Writing-review & editing: A.A., O.B., E.J. Visualization: A.A., O.B., E.J. Supervision: O.B., E.J. Project administration: O.B. Funding acquisition: O.B., E.J. All co-authors reviewed the final version and approved the manuscript before submission.

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