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FIELD SCREENING OF RECOMBINANT INBRED LINES (RILS) OF QUINOA (CHENOPODIUM QUINOA WILD.) FOR RESISTANCE TO THE PESTS

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ABSTRACT

Quinoa (*Chenopodium quinoa* Wild.) cropping is very successful in Morocco. A breeding program was set up for 22 years ago to develop adapted, productive germplasm. Success of quinoa cultivation requires not only suitable germplasm but also sound production practices, including control of harmful pests that are likely to cause yield losses. The aims of current study were to (i) establish an inventory of quinoa pest's species in five contrasting agroclimatic sites; (ii) assess the severity of pest damage; and (iii) screen 156 RILs for resistance to the predominant pests. Two trials were carried at Berrechid and Tiflet in 2017-2018, with three others at Bouchane, Meknes, and El Kebab in 2018-2019. The experimental design was a randomized complete block with four replicates. The insects were collected and inventoried during both experimental years. The pest impact was assessed through the percentage of injured leaf area at three heights of plant foliage, apical, median, and basal. The inventoried insects belonged to eight species and six orders. *Aphis fabae*, *Myzus persicae* (Homoptera) and *Nezara viridula* (Hemiptera), were classified as the most frequent species. *Utetheisapulchella* and *Spodopetraeridani* (Lepidoptera), were the least abundant. Tiflet and Berrechid recorded occurrences averaging 19.64 and 15.73%, respectively. Bouchane, Meknes, and El Kebab expressed an outbreak rate of 5%. ANOVA revealed very significant differences among sites, lines and, their interaction. The two-dimensional PCA graph identified four clusters. The PC1 axis gathers 64.34% of the total variability while the PC2 axis explains an additional 24.01%.

The AMMI analysis revealed highly significant effects of the site, the genotype, and their interaction. The G×E explained 46% of the total variation, while the experimental site accounted for 28% and the genotype 26%.The examined RILs showed segregation for resistance and ranked from the most sensitive to the most resistant, such the *C. berlandieri* parent.

Keywords: Chenopodium quinoa Wild., inventory, pests, resistance, RIL.

1. INTRODUCTION

The world population's continuous growth requires mobilizing resources to ensure future production of adequate amounts of highly nutritious food. To meet this increasing demand, three actions are recommended: (i) slowing down population growth; (ii) increasing agricultural production by increasing yields or expanding agricultural areas; and (iii) reducing pre- and post-harvest crop losses (1).

Cereals are the world's most important food resource for human consumption (2). It is therefore imperative to develop new cereal varieties and other alternative crops adapted to extreme climatic conditions to meet future global demands and ensure food security for the population (3). The FAO is actively involved in promoting and evaluating the cultivation of quinoa in 26 countries outside the Andean region, with the aim of enhancing food security and nutrition. Indeed, the proclamation of 2013 as the International Year of Quinoa has given a boost not only to consumption, but also to global production (4).

Quinoa (*Chenopodium quinoa* Wild) is a pseudo cereal having potential to increase global food security. It is a species with high nutritional value (5), its primary attribute being its near-ideal essential amino-acid composition, while also having an excellent balance of carbohydrates, fats, and proteins for human consumption (6). Additionally, quinoa has the ability to thrive in dry, nutrient-deficient, and saline soils (7,8). However, quinoa quality can suffer through attack by biotic agents and these have been shown to reduce grain yield by 8-40% (9). The main pest problems in the Salars and Altiplano areas, where more than 80% of the world's quinoa is produced, are related to noctuid complexes and moth larvae (10). (11) described several species of quinoa-dependent pests in South American production environments. All plant parts are subjected to insect injury throughout the crop cycle.(12)observed that because of the rapid expansion of quinoa production into diverse regions of Peru, a phytosanitary hitch has arisen, resulting in the emergence of pests that were not previously known to infest quinoa. This is not unexpected, since two of quinoa's close relatives, *C. album* L. and *Chenopodiastrum murale* (L.) S. Fuentes, Uotila & Borsch are common worldwide weeds in temperate and subtropical lowland

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environments and are hosts to many of the same pests and diseases as quinoa (13,14,15 and 16).

To protect crops from pests, an essential initial step for a new crop is to make an inventory of harmful insects within the targeted production region. Also, the use of resistant varieties constitutes a major tool of control and an important alternative to the use of pesticides whose residues are harmful to the health of consumers, producers, and also the environment (17). As quinoa is a new crop introduced in Morocco, no pest inventory has been carried out so far. It is with this in mind that our objectives have been stated and aimed to:

i) Inventory and identify the quinoa- associated pests at five contrasting agro-climatic sites;
ii) Assess severity of damage caused by insect herbivory to a population of highly diverse recombinant inbred lines (RILs) at the different sites; and
iii) Evaluate the differential sensitivity of the recombinant lines.

2. MATERIAL AND METHODS

The current study was carried out at the following five agro-ecological sites: Berrechid and Tiflet during the 2017-2018 summer season; and Bouchane, Meknes, and El Kebab in 2018-2019. The sites were geographically separated from each other and each has unique soil and climatic conditions (Table 1).

Tiflet and Meknes have lowland Mediterranean climates; El Kebab has a highland Mediterranean climate (1503 m elevation); and Berrechid and Bouchane are semi-arid with continental environmental influences. The Berrechid, Tiflet, and Bouchane experimental sites are at ~300 m above sea level (Table1). At Bouchane in the Rehamna Province of southern Morocco, temperatures reach as high as 40°C at end of June and average 23°C during the winter-spring cropping season. Meknes and El Kebab experience milder temperatures throughout the crop cycle, 20°C on average; these are combined with higher rainfall, around 500 mm annually, as compared with the drier Tiflet (~340 mm), Berrechid (~250 mm) and Bouchane (~300 mm) sites.

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Table 1: Agro-pedoclimatic characteristics of the experimental sites

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

The plant material consisted of 156 F_{2-5} of quinoa and derived from a cross between the NL-6 line of Chilean origin and WM11-54, an isolate derived from passive intercrossing between quinoa Co407' and possibly 'Blanca' with native strain(s) of *Chenopodium berlandieri* Moq., at approximately 2300 m elevation in the San Luis Valley quinoa production area near Alamosa, Colorado (McCamant J., personal communication). The RILs represented lines that had been selfedfor at least five generations to achieve near-complete homozygosity.

The adopted experimental design was a randomized complete block (RCB) with four repetitions, one row per accession of 1 m length and 0.5 m inter-rows. Sowing was carried out on June 27 and July 12, 2018, successively in Berrechid and Tiflet; and on February 20, March 28, and April 30, 2019, in Bouchane, Meknes, and El Kebab, respectively. The trials received irrigation twice a week and hand-weeding every other week.

Insect specimens were collected in traps placed between plots 15 days after sowing and left throughout the crop cycle. The traps were yellow pots of 18 cm diameter and 14 cm height, filled to 2/3 of their capacity with 100 g/l salt solution where 4-5 drops of detergent were added. Every two weeks, the captured insects were saved by emptying the traps using a sieve (mesh diameter: 2 mm) and the saline solution was renewed. Once sieved, the insects were conserved in 70% ethanol until their counting and identification. The classification was carried out under the supervision of specialist from the plant protection department of the Institute Agronomic and Veterinary Hassan II.

To assess pest damage on the RILs, four plants per line per plot were examined. The injury intensity (DI) was determined by evaluating the damaged leaf area at three plant heights: apical, median, and basal; a binocular loupe was used when needed. The injury evaluation took into account devoured, shredded, and/or mined leaf tissue, stains, insect bites, leaf rot, and buckle. A

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0 to 5 scale was used, with a score of 0 for a total lack of symptoms; 1 for ≤ 10% damaged leaf area; 2 for ≤ 25%; 3 for ≤50%; 4 for ≤ 75%; and 5 for more than 75% damage.

At maturity, the aboveground plant dry weight (AGW), root dry matter (RDM), and grain yield (GY) were assessed after 48 hours of drying at 70°C for dry matter and 35°C for grains.

The database was examined using variance (ANOVA), principal components (PCA), and additive main effect and multiplicative interaction (AMMI) analyses. Significant tests were further subjected to the mean comparison Student-Newman test. AMMI analysis evaluated the main components of the genotype × environment (G×E) interaction (18). All statistical tests were performed using R software version 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria).

3. RESULTS

Six orders of pests were identified among the captured insects: Homoptera, Hemiptera, Diptera, Coleoptera, Pulmonated, and Lepidoptera (Table 2).

Significant variation for pests and their abundance was observed among sites (Table 2). In the 1st cropping season, a slight difference in pests' occurrence was recorded between Berrechid (98) and Tiflet (84). In the 2nd year, invertebrate incidence was much higher when compared to that of the 1st year. Meknes counted a vast number of invertebrates trapped (300), followed by El Kebab (237), then Bouchane (84). Clearly, the vast differences in edapho-climatic settings of the experimental stations and these species' ability to adapt to these conditions accounted for differential pest abundance among sites. In fact, for late sowing in Tiflet and Berrechid during the first season of screening, insects were confronted with stressful and lethal temperatures.

Table 2: Number (N) and relative abundance (A) of pest species per site

	Berrechid	Tiflet	Bouchane	Meknes	El Kebab	Total
	N (A %)	N (A %)	N (A %)	N (A %)	N (A %)	
Homoptera : *Aphis fabae*	20 (20.4%)	15 (17.8%)	40 (47.6%)	100 (33%)	80 (33.7%)	255 (31.76%)
Homoptera : *Myzus persicae*	15 (15.3%)	10 (11.9%)	10 (11.9%)	80 (27%)	45 (18.9%)	160 (19.93%)
Hemiptera : *Nezara viridula*	20 (20.4%)	15 (17.8%)	10 (11.9%)	40 (13%)	60 (25.3%)	145 (18.05%)
Coleoptera: *Hycleus duodecimpunctatus*	10 (10.2%)	06 (07.1%)	20 (23.8%)	50 (17%)	30 (12.6%)	116 (14.45%)
Mollusca: Snails	08 (08.1%)	12 (14.2%)	04 (04.7%)	10 (03%)	12 (05.0%)	46 (05.73%)
Lepidoptera *Spodopetra eridania*	04 (04.0%)	01 (01.1%)	00 (00.0%)	00 (00%)	00 (00.0%)	5 (00.62%)
Lepidoptera*: Utetheisa pulchella*	01 (01.0%)	01 (01.1%)	00 (00.0%)	00 (00%)	00 (00.0%)	2 (00.25%)
Diptera: *Pegomyia betae*	20 (20.4%)	24 (28.5%)	00 (00.0%)	20 (07%)	10 (04.2%)	74 (09.22%)
Total	98 (12.2%)	84 (10.4%)	84 (10.4%)	300 (37.3%)	237 (29.5%)	803

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Three species, *Aphis fabae* and *Myzus persicae* (Homoptera) and *Nezara viridula*, belonging to the Hemiptera order, were the most common (Table 2). *Hycleus duodecimpunctatus* (Coleoptera) and *Pegomyia betae* (Diptera) came next with an abundance of 14.45 and 9.22%, respectively. Thepulmonate mollusks represented 5.73%. Lepidoptera (*Spodopetra eridania* and *Utetheisa pulchella*) had the lowest abundance at 0.62 and 0.25%, respectively.

3.1 Damage Intensity

The damage caused to the quinoa IRL-s by the borers recorded in the 5 experimental stations was mainly on the leaves. Differences in damage intensities between localities concerned the species abundance listed and their activity; The latter behaved as defoliating pests by feeding on leaf parenchyma, rolling leaves and tender shoots and destroying young inflorescence. The sensitive lines then showed a decrease in photosynthesis which thus impacted their growth. To remedy the problems caused by these and prevent the damage from spreading in quinoa production, crop rotation is recommended to break the continuity of the pest food chain.

A clear variation in the damage intensity (DI) was observed among sites (Table 3). The highest coefficients of variation (CV) were recorded at El Kebab (96.60%) and Bouchane (91.47%). Pest impacts were most pronounced in Tiflet (DI=19.37%) and Berrechid (DI=15.73%). Bouchane, Meknes, and El Kebab experienced the least amount of pest damage, around 5%. Late sowing in Tiflet and Berrechid during the first season of screening would have exposed the quinoa plants to the most active phases of the pests' life cycles.

Table 3: Descriptive statistics and ANOVA of damage intensity (DI)

	Minimum	Maximum	Average	CV (%)	F
Berrechid	0	60.00	15.73	76.99	53.612***
Tiflet	0	71.67	19.37	71.55	30.323***
Bouchane	0	36.67	5.39	91.47	8.062***
Meknès	0	28.33	5.28	86.93	4.514***
El Kebab	0	36.66	5.98	96.60	6.203***

ANOVA also displayed highly significant differences in injury intensity among the quinoa RILs (Table 3). In Berrechid, the means comparison test identified twenty-eight homogeneous groups. The sensitivity varied from 0% for the most resistant cluster, which consisted of eight RILs, to 30.83% for the most sensitive group composed of five lines. In Tiflet, five RILs were totally unscathed, showing 0% damage, comparable to the WM11-54 parent. At the opposite end of the spectrum, RIL 16-333 presented a maximum DI equal to 40%.

The means comparison at Bouchane identified 22 groups, ranging from 0% damage in three

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RILs to a high of 25% in three others. In Meknes, nine homogeneous groups were identified: the first group included two unharmed RILs (DI=0%): L88-251 and L44-142. On the opposite side, the maximum DI equal to 20.55% was that of L25-354. At El Kebab, five lines were completely spotless, while three others had the highest DI of 21.66%. The RILs demonstrated clear genetic segregation for pest resistance as their *C. berlandieri*-containingWM11-54 parent was resistant.

3.2 Multivariate analysis

Two-way ANOVA, PCA, and AMMI analyses were performed on Berrechid, Tiflet, Bouchane, and El Kebab data sets. However, the Meknes data were discarded as only 52 of the 156 RILs survived to the reproductive stage and produced seed.

The two-way ANOVA revealed very highly significant differences among sites, lines, and their interaction for the four assessed traits (Table 4). Thus, the differences among RILs resulted mainly from the genetic factor and its interaction with the surrounding environment.

Table 4: Two-way ANOVA of the site, and line effects, and their interaction.

Variable	Source of variation	F value	Variable	Source of variation	F value
RDM (g)	Line	6.672***	GY(g)	Line	6.967***
	Site	235.588***		Site	384.400***
	Line × site	3.737***		Line × site	3.564***
AGW (g)	Line	9.992***	DI (%)	Line	53.985***
	Site	194.062***		Site	2608.011***
	Line × site	4.767***		Line × site	34.491***

RDM: root dry weight; AGW: Dry weight of the aerial part; GY: Grain yield; DI: Damage Intensity.

The Pearson correlation matrix displayed significant correlations among the four variables. Grain yield (GY) exhibited the strongest positive and significant correlations (R=0.8**) with aboveground dry matter (AGW) and root dry matter (RDW). On the other hand, the injury intensity (DI) displayed a low negative correlation with GY, AGW, and RDW.

According to the PCA, the first axis explained 64.34% of the total variation and the second axis an additional 25.01% (Table 5). The first axis had the highest positive correlations with GY, AGW, and RDW. The second main axis had a positive and significant relationship with DI.

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Table 5: Contribution of variables to the 1st and 2nd main axes

	Dim 1	Dim 2	Var. (%)
GY	93.8	2.4	
AGW	91.4	7.3	
RDM	92.3	3.4	
DI	7.6	99.7	
PC1	-	-	64.34
PC2	-	-	25.01

The two first axes biplot of the RILs accounted for 89.35% of the variance, highlighting the extreme variation for pest damage among the segregating RILs (Figure 1). Four clusters were identifiable: the first one (in black) brought 78 lines together; these were less productive and among the 12% most resistant lines. The second group (in red) consisted of 37 RILs that had low GY and were highly sensitive to pests. The third group (in green) comprised 29 RILs that together were rather productive but susceptible to pests. The fourth group (in blue) was made up of the 12 RILs that were both highly productive and insect tolerant.

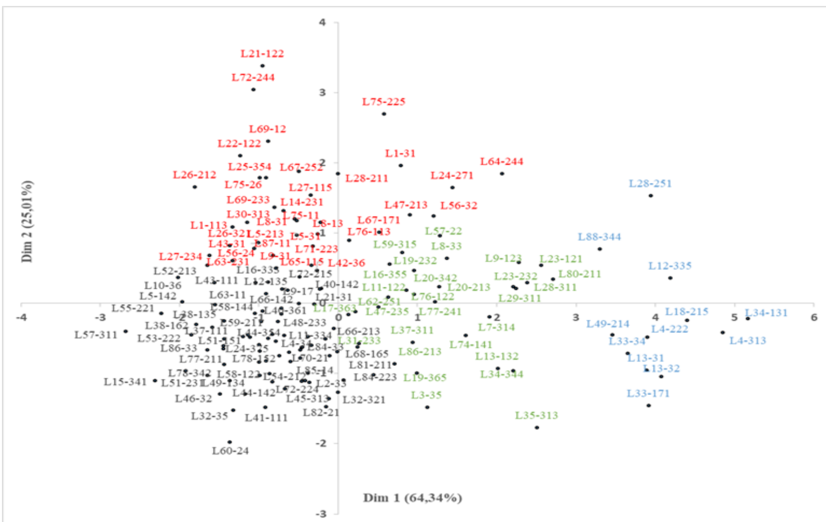

Figure 1: Hierarchical ascending classification of RILs

The multi-site analysis highlighted the genotype by environment (G×E) interactions. The AMMI analysis revealed highly significant effects of the site, the lineage, and their interaction (Table 6). The G×E explained 46% of the total variation, while the experimental site accounted for 28% and the genotype 26%. The environment at each site had significant effects on the RILs' behavior in the face of the pest infestation. On the other hand, the first main interaction axis (IPCA1) explained 54% of the total variation and the second an additional 41.3%.

Table 6: Analysis of variance for AMMI

	DF	Sum of squares	Mean of squares	F value	Pr. (>F)	Var. (%)
ENV	3	79664	26554.8	343.7	2.41e-13***	28
REP(ENV)	14	1082	77.3	5.4	2.52e-10***	-
GEN	156	72777	466.5	32.9	<2.2e-16***	26
GEN : ENV	405	130848	323.1	22.79	<2.2e-16***	46
Residuals	1600	22674	14.2	-	-	-
PC1	157	95806.61	606.37	42.79	0	54.0
PC2	155	73168.35	469.02	33.10	0	41.3

Figure 2 shows the IPCA1 scores of the RILs and those of the experimental sites according to the intensity of the damage (DI). The Tiflet site emerged at the negative side of PC1 and was opposite the other three sites. This site showed the highest damage intensity (DI=19.17%). Four lines, on the left side of the graph in orange, were among the most resistant lines (L60-24, L82-21, L35-313, and L33-171).Their DI scores ranged between 0.08 and 1.93.On the right side of the figure, six RILs in blue were the most susceptible; their DI scores ranged between 24.08 and 34.74%.

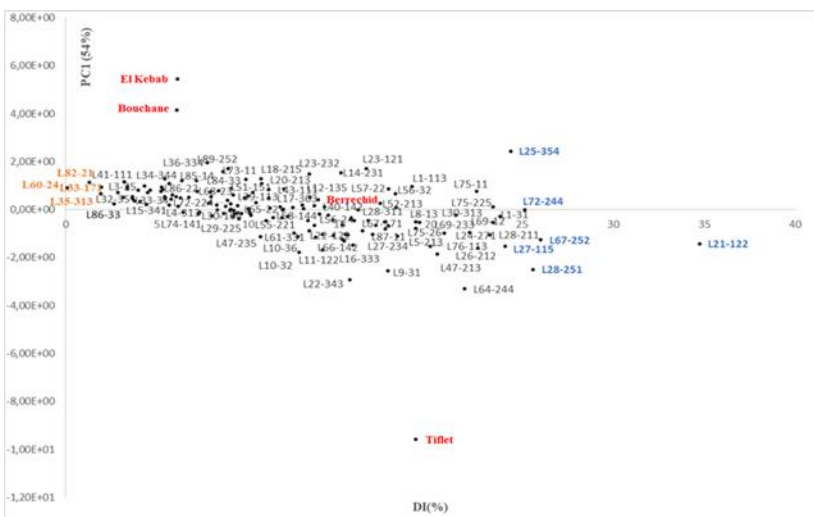

Figure 2: Biplot of the 1st principal component and the pests' injury intensity

4. DISCUSSION

The entomo-fauna inventory on the 156 quinoa RILs at diverse experimental sites in Morocco revealed a diversity of pest species throughout the crop cycle. The captured pests during the two

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testing seasons fit into six orders and seven identifiable species, not including the mollusks. Homoptera (*Aphis fabae* and *Myzus persicae*) was the most predominant and represented 51.69%of the total pests collected. Hemiptera (*Nezaraviridula*) was ranked second with 18.06% and beetles (*Hycleus duodecim punctatus*) third with 14.45%. Diptera, represented by *Pegomyia betae*, was thenext most abundant at 9.22%; the pulmonate mollusks showed an abundance of 5.72%. The last two species of Lepidoptera, *Utetheisa Pulchella* and *Spodopetra eridania*, were the least abundant.

The pest inventory that was established as part of this study matched closely the investigations of (19) who reported that the most abundant quinoa pests in the Himalayas are aphids (*Aphis sp*.), caterpillar (Pachyzanda), defoliating insects (Epicanta), and sucking insects (*Myzus persicae*). (20) and (21) compiled a list of 56 species of phytophagous insects associated with quinoa cultivation, of which 24 belonged to the order Lepidoptera, 15 to Coleoptera, 10 to Homoptera, and three to Hemiptera, in addition to two Thysanopterans, one Dipteran and one Orthopteran. According to (22), quinoa pests in new production areas include *Eurysacca melanocampta*, *Macrosiphum euphorbiae* (Thomas) aphid, *Frankliniella occidentalis* Pergande thrips, leafminer *Liriomyza huidobrensis* (Blanchard) and the harmful *Hemipterans Nysius* simulans Stål and *Liorhyssus hyalinus* (Fabricius). On the coast and in the Peruvian Maritime Yunga, new quinoa-growing areas have been affected by a greater number of pests corresponding to species of the genera *Spodoptera*, *Chloridea*, *Spoladea*and *Herpetogramma* (23). In 2021, a stem-boring fly, Amauromyza karli Hendel (Diptera: Agromyzidae), was reported in quinoa grown in Colorado's San Luis Valley and abruptly halted expansion of this climate-resilient crop. This new agromyzid pest has caused complete yield loss in some instances and contributed to substantial declines in quinoa acreage from 3,000 acres in 2021 to 900 acres in 2022 (24).At the national level within Morocco, our observation is that, the main quinoa pests are aphids, stink green bugs, ants, and sparrows (personal communication, O. Benlhabib).

Differences among the localities were evident for all species and their abundance was affected by weather conditions, sowing dates, and the cropping season at the various experimental sites. According to (25), abiotic factors have a decisive effect on the pest population's development; temperatures, atmospheric humidity, and precipitation shape the expansion of the pest populations and their interactions with host plants. (26) emphasized the effect of climate, especially temperature, on the pests' fecundity and growth rates. The optimum temperatures for the development of *Aphis fabae* and *Myzus persicae* range from 20-25°C, with a minimum of 4°C and the limiting upper range between 25-30°C (27). For the Hemiptera, the optimal development takes place at 30°C (28). The rate of development for *Pegomyia betae* depends on the ambient temperature; the higher it is, the faster the expansion of the pest (29). Thepulmonated mollusks require specific atmospheric humidity and temperatures above 10°C to

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develop (30).

The abundance of aphid species *Aphis fabae* and *Myzuspersicae* in the first year was moderate; their ratio in Berrechid was 35% and Tiflet 30%. They were the dominant group in the second year, representing 60% of the pests in both Meknes and Bouchane and 52% at El Kebab.

For the stink bug *Nezara viridula*, 35 individuals were collected in the first year with an increase to 110 specimens in the second. Among the second year's collections, El Kebab was ranked first with 25%, followed by Meknes, then Bouchane with less than 12%.

Hycleus duodecim punctatus represented 14% of the pests collected in the five environments; at Bouchane they were most abundant at 23.8%, followed by Meknes (17%), El Kebab (12%), Berrechid (10.2%), and Tiflet (7%).

Lesser quinoa pests included a small number of pulmonate mollusks identified at El Kebab (5%), Bouchane (4%), and Meknes (3%). *Pegomyiabetae* was present in four localities: Tiflet (28%), Berrechid (20.4%), Meknes (7%), and El Kebab (4%). Lepidopterans *Utetheisa pulchella*, and *Spodopetraeridania* were quite rare and only found in the first year, representing 0.62 and 0.25% of the total, respectively.

Collected pest populations were more abundant in the second than the first season. Such disparity is the consequence of the sowing dates and resulting environmental conditions, congruent with what was reported by (31)and (32). In the first year (2018), the two trials took place late in the cropping season, between June and September for Berrechid and between July and October for Tiflet. The mean temperatures during the cropping cycle were 33°C and 36°C for Berrechid and Tiflet, respectively; atmospheric humidity was low, and rainfall was less than 6 mm.

In the second year (2019), a large number of insects were collected at early sowing dates; the climatic conditions were auspicious for crop growth (33) and proliferation of the pests. The Bouchane trial took place between February and May, Meknes's between March and June, and El Kebab's between April and July 2019. The average humidity equaled 58, 68, and 65%; the rainfall 68,160, and 70 mm; and the average temperatures were roughly 20°C at these three sites. The relatively low pest populations in the first year were caused by the growing period's high temperatures, low humidity, and very low precipitation. Such a relationship is consistent with that of (34), who reported the dependency of the pests' expansion on seasonal factors. Other research in Peru reported by (35)showed that temperatures between 14.4 and 15.3°C reduced *Eurysacca melanocampta* development, while higher temperatures between 19.4 to 21.6°C, led to more than one generation of the insect per growing season. The climate patterns during the crop growth cycle, relative humidity, average temperature, and rainfall thus have decisive effects on the expansion of quinoa pests and concomitantly reduce productivity of the crop.

The pest populations caused significant damage mainly on the leaves at the experimental sites. The damage intensity varied among sites; the highest injury rate of 19.64% was recorded in Tiflet, followed by Berrechid (15.73%); the lowest of 5% was that of Meknes. The Mediterranean climate of Tiflet combined with the sandy-loamy soil, late sowing, and high temperatures of July, exposed the quinoa lines to the most active phases of the pests' life cycles, especially under organic farm management wherein no chemical treatment was applied. In fact, the most infested sites were sowed late in June-July. This finding agrees with those reported by (36)and (37) that late sowing exposes crops more to predation by boring insects.

The assessed traits as part of this study have well differentiated the 156 quinoa lines as reported by (38,39). The RILs' differences were clearly highlighted by the PCA biplot. They were structured into four clusters: the most resistant group; the very sensitive lines; those RILs that were moderately sensitive; and finally, those lines that were most productive and pest resistant. Based on their damage intensity, lines L35-313, L60-24, and L33-171 were ranked as most resistant. On the other side, over 50% of the RILs had moderate sensitivity ranging from 10 to 34%. (40) reported observing differential infestations of aphid pests on leaves and stems of quinoa in comparison with *C. berlandieri*. The variation for pest resistance among RILs in this study, being derived from crosses of quinoa with *C. berlandieri*-containing WM11-54, illustrates the potential value of wild pit seed goosefoot in quinoa breeding.

According to (41), *Chenopodium* species and its closely related genera produce defensive compounds that have toxic effects on harmful organisms such as insects. Saponin-containing powder from *Dysphania ambrosioides* (formerly *Chenopodium*) applied to *Vigna subterranean* plants infested with *Callosobruchus maculatus* caused a reduction of 18.75% after three days of treatment for a dose of 2.5% to 69.64% after six days of treatment for a dose of 7.5% (42). In quinoa, the presence of saponins in semi-sweet varieties suggested that the saponin-mediated component is part of a polygenic defensive system (43).

5. CONCLUSION

This study investigated the main pest species of quinoa in Moroccan cropping environments. The data assessment revealed significant variation among pests for both their distribution and abundance at the different localities and in two different cropping seasons. We identified eight pest species belonging to six orders; among the most prevalent were the aphids *Aphis fabae* and *Myzus persicae*, as well as the stink bug *Nezara viridula*. These findings will assist quinoa producers in selecting optimal pest control and crop management practices. As for the RIL

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breeding lines themselves, a pest severity scoring system was developed, with four lines exhibiting good behavior against the entomofauna and over 50% having moderate sensitivity (≥10%). In addition, our findings confirm the genetic control of pest resistance in quinoa. More investigations are required on pests' impact and their interaction with the environment under semi-controlled conditions. Research to identify genetic markers linked closely with resistance through skim-sequencing of RILs is another topic to investigate for the future application of marker-assisted selection, enabling the rapid accumulation of several resistance genes within high-performance lines for more effective resistance.

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