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ARTICLE

Evaluation of Pre-Harvest Sprouting (PHS) Resistance and Screening of High-Quality Varieties from Thirty-Seven Quinoa (*Chenopodium quinoa* Willd.) Resources in Chengdu Plain

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ABSTRACT

Pre-harvest sprouting (PHS) will have a serious effect both on the yield and quality of quinoa (*Chenopodium quinoa* Willd.). It is crucial to select and breed quinoa varieties with PHS resistance and excellent agronomic traits for guidance production and utilization of quinoa. A comprehensive evaluation of the PHS resistance and agronomic traits of 37 species of quinoa resources was conducted in Chengdu Plain. The evaluation used various methods, including grain germination rate (GR), grain germination index (GI), total spike germination rate (SR), total grain germination index (SI), grey correlation analysis (GCA), cluster analysis and correlation analysis. Results showed significant differences in PHS resistance and agronomic traits amongst the 37 quinoa resources. CDU-23 was most resistant to PHS within 24 h, with a germination rate of 2.67% and 0% according to the GR and SR results, respectively. However, in the same time, CDU-31 showed the maximum susceptibility to PHS based on the SR of 31.07%, while CDU-34 was the most sensitive to PHS according to the GR of 100%. The comprehensive evaluation identified one and nine kinds of high resistance species for grain and whole spike germination, respectively. In both cases, the coefficients of variation (CV) for these parameters were 34.78% and 82.13%, respectively. GCA results showed that the magnitude of the association between each trait and yield in the thirty-seven quinoa resources was in the following order: thousand grain weight > seed length > seed area > seed width. Although the seed weight of CDU-18 reached 3.7010 g, the seed weight of CDU-5 was only 1.6030 g. However, the size of the seeds, their width and area did not correlate with their 1000-grain weight. There was a complex correlation between PHS resistance index and agronomic traits. Based on clustering analysis, thirty-seven quinoa resources were classified into three taxa. It was found that various taxa differed in PHS resistance and agronomic traits. Several comparisons of the aggregated data led to the selection of five varieties of quinoa, of which CDU-2 presented excellent agronomic qualities and strong PHS resistance. This study has provided a reference for breeding excellent quinoa varieties with PHS resistance.

KEYWORDS

Quinoa; pre-harvest sprouting (PHS); agronomic traits; cluster analysis

1 Introduction

Quinoa (*Chenopodium quinoa* Willd.), an annual dicotyledonous plant of the *Chenopodium* genus in the *Amaranthaceae* family, is native to the Andes region of South America and an agricultural crop similar to grains [1]. About 7000 years ago, quinoa was domesticated and cultivated by the local population as a main food crop [2]. Quinoa can withstand extreme conditions that common crops (rice, wheat, corn, sorghum) cannot survive [3], including drought salinity and frost tolerance, and can be grown at high altitude areas. Quinoa is rich in starch (52.0%–69.0%), lipids (2.0%–9.5%), protein (13.8%–16.5%), dietary fiber (7.0%–9.7%) [4] and nutrients that meet the body's daily intake [5]. Despite the high cultivation, nutritional and functional value of quinoa, it was neglected for thousands of years [6,7]. Until the last 50 years, as the global population grew dramatically and the natural environment and agricultural production conditions became increasingly severe, there was an urgent need to find a high-quality grain to satisfy the daily consumption of people [8]. In recent years, quinoa has been rediscovered due to its excellent agronomic property and nutritional value, so its area of cultivation and yield are rapidly increasing. In recent years, quinoa has been grown in China, France, India, Sweden, Denmark, Netherlands, Italy, and other countries [9,10]. Due to the Andean people's contribution to the development of quinoa, the International Food and Agriculture Organization (FAO) has declared 2013 the "International Year of Quinoa" [1].

However, in Southwest China [11], especially Chengdu Plain, the harvest season of quinoa is often accompanied by high temperatures and high humidity, and Quinoa is extremely vulnerable to PHS if it is not harvested in time or not handled properly after harvested [8,12,13]. PHS will promote the hydrolysis of starch in grain endosperm [8,14,15], the change of protein spatial structure, the damage of seed internal structure which reduce the nutritional value of grain, create a favorable environment for saprophytic fungi, and lead to seed spoilage and deterioration [16] and loss of vitality. The annual global economic loss due to PHS is up to $1 billion [15]. Consequently, it is crucial to find a solution to the quinoa PHS method as soon as possible, both in terms of boosting agricultural production and promoting economic development.

Through natural variation and artificial selection, selective breeding is an effective method of evaluating PHS resistance of quinoa resources with excellent resistance in years of cultivation (the conventional methods to identify pre-harvest sprouting resistance mainly include whole-spike germination test and grain germination test) [17–19]. Varieties with excellent agronomic characters are selected from the screened resources for planting, so as far as possible to reduce the grain PHS caused by agricultural production loss. Quinoa yield is generally determined by effective spike length, number of spike branches and thousand grain weight [20]. Thousand grain weight is closely related to the grain phenotypic traits, which can be divided into grain size (grain area, grain width, grain length) and grain morphology (aspect ration, roundness value) [21,22]. The improvement of quinoa yield and quality can be achieved by improving the phenotypic traits of the grain of quinoa. In this study, varieties with PHS resistant were selected through grain germination and whole spike germination experiments, varieties with high quality agronomic traits were selected through thousand grain weight, grain length, grain width and grain area. The data were integrated and 37 quinoa resources were analyzed, and the excellent quinoa varieties suitable for planting in the Chengdu Plain were screened, which provided a reference for developing quinoa resources and improving quinoa yield.

2 Materials and Methods

2.1 Preparation and Treatment of Test Materials

In this experiment, 37 quinoa resources were provided by the Key Laboratory of Coarse Cereal Processing, Ministry of Agriculture and Rural Affairs at Chengdu University. Approximately 100–110 days were the most fertile period for the thirty-seven quinoa resources. The experimental site was located in Yuanba Village, Xinshi Street, Jianyang City, East New District, Chengdu City, Sichuan Province (104°56′59″N, 30°32′90″E; 387 m). The average temperature of the test site from March to July is 25°C in two years. All the materials were sowed in a plot of measured 3 m × 3.3 m, with a 30 cm × 30 cm plant spacing, cultivated 1 quinoa plant every 30 cm and cultivated 11 quinoa plants within 3.3 m. The experiments were conducted in 2021 and 2022, respectively, and three biological replicates were performed in each year. All the quinoa materials were sowed on March 15 and were harvested on July 11 in the first year and sowed on March 8 and were harvested before July 10 in the second year. Conventional field management was adopted during the growth period. During harvest, each plot was divided into five parts, and a top spike approximately 15 cm long was randomly selected from each part. Hence, each quinoa material was numbered and five spikes were selected in a plot.

All the quinoa spikes were dried in an oven at 37°C for 72 h until the moisture content of the grain less than 12%. An evaluation of spike germination was conducted on two spikes of each quinoa. Ninety grains were randomly selected from the remaining dried quinoa spikes for the grain germination test after manual threshing. Furthermore, 37 quinoa resources were assessed for their grain agronomic traits (including thousand grain weight, grain length, grain width, and grain area).

All the test materials were disinfected by immersion with 0.5% NaClO (grains immersion for 3 min and spikes immersion for 10 min), rinsed slowly with distilled water to remove residual reagents. A dark climate chamber at 25°C and 85% relative humidity was used to test germination of grains and spikes [23].

2.2 Germination Test

2.2.1 Whole Spike Germination Test

A whole spike germination test was conducted using wet tissue wrapping [23,24]. For each spike, wet paper towels were wrapped around it and it was placed upright in a 250 mL beaker for one hour until completely moist. During incubation, the samples were placed in a 25°C artificial climate chamber. In order to prevent the paper from drying out, 10 mL of distilled water was added to the beaker. The germination status of the spikes in the paper towels was observed every four hours. The 100% humidity of the paper towels was maintained at all times to provide a moist environment for the germination of quinoa spikes. The number of germinated seeds was observed and counted at 8, 16, 24, 32, 40, 48, 72 and 96 h respectively (the grain coat was considered to be broken or buds of 1–2 mm appeared named germinate) [25] and the whole spike germination rate (SR represents the percentage of PHS grains in total grains) and index (SI represents the average germination degree of quinoa PHS) were calculated. Among them, the whole spike germination resistance test was conducted within 8–48 h and the whole spike vitality test was carried out at 72, 96 and 120 h.

2.2.2 Grain Germination Test

Each treated grain of quinoa was placed in a Petri dish covered with double filter paper (diameter 9 cm), 30 seeds were placed in each dish and 5 mL of distilled water was added, and the process was repeated three times. The Petri dishes were observed every two hours in a constant light incubator. Each grain germination rate (GR) and grain germination index (GI) was recorded every 4 h for 32 h on the filter paper with a moisture level of 100%. Subsequent, seed vitality test was carried out at 44, 56 and 68 h.

2.2.3 Agronomic Trait Determination

The length, width and area of each quinoa resource was performed using a WinRHIZO Reg STD4800 root system analyzer, while the thousand grain weight was determined with an electronic analytical balance FA2004.

2.2.4 Statistics and Analysis

Germination rate (%): $GR = n_{(1\sim k)}/N \times 100\%$.

Germination index: $GI = (K \times n_1 + \ldots\ldots + 3 \times n_{(k-2)} + 2 \times n_{(k-1)} + 1 \times n_k)/K \times N$ [26].

In the formula: $n_{(1\sim k)}$ denotes the number of grains sprouting each time from the 1st to the next k times. The k represents the total number of germination tests. The N represents the total number of grains used for germination.

A modified version of the standard method for wheat spike germination resistance (NY/T1739-2009) was used for the whole spike germination resistance test [27,28]. References to relevant literature were used to develop the grading method for quinoa grains germination resistance in Table 1. The results of dormancy grade and grain germination resistance grade are consistent.

Table 1: Resistance grading criteria

Resistance level	Classification criteria	
	Germination resistance of grains	Germination resistance in spikes
High resistance (HR)	GR ≤ 10%	GI ≤ 5%
Resistance (R)	10% < GR ≤ 30%	5% < GI ≤ 20%
Moderately resistance (MR)	30% < GR ≤ 50%	20% < GI ≤ 40%
Moderately susceptible (MS)	50% < GR ≤ 70%	40% < GI ≤ 60%
Highly susceptible (HR)	70% < GR	60% < GI

Coefficient of variation (CV) is defined as mean standard deviation (SD)/mean value (Mean) × 100%.

PHS resistance indicators were used for cluster analysis. Agronomic traits were used for grey correlation analysis and cluster analysis.

All the results were the average of two years. All statistical results were analyzed and plotted using Microsoft Excel 2019 software, SPSS Statistics 25 and Origin 2021. All data were averaged, and in this study, single factor ANOVA test method were used for significance analysis.

3 Results and Analysis

3.1 Evaluation and Analysis of Whole Spike Germination Trials

The results of the whole spikes germination trials were shown in Table 2. There were significant differences in whole spike germination resistance within 48 h among 37 quinoa resources treated under the same conditions. According to the standard, nine highly resistant (HR) varieties, seven resistant (R) varieties, nine moderately resistant (MR) varieties, seven moderately susceptible (MS) varieties and five highly susceptible (HS) varieties were selected. Among high resistance (HR) varieties, the spike germination index was in the order of CDU-23 < CDU-15 < CDU-1 < CDU-36 < CDU-5 < CDU-2 < CDU-18 < CDU-6 < CDU-14. CDU-23 spike had the lowest germination rate of 1.05% and germination index of 0.44%. It was followed by CDU-15 with 1.35% and 0.70% spike germination rate and index. The spike germination rate and index of CDU-1 were 2.33% and 1.56%, respectively. The germination rate of CDU-23 spikes was 0% during the first 32 h, and slowly increased to 1.05% within next 16 h. The germination process of CDU-15 was similar to that of CDU-23, with 0% of spikes germinating in the first 8 h and rose to 1.35% within next 40 h and remained unchanged. For CDU-1, the spike germination rate was 0% during the first 16 h and rose to 2.33% in the subsequent period. In a comprehensive analysis, CDU-31 had the lowest spike germination resistance among the 37 quinoa resources, followed by CDU-16 and CDU-35, which the spikes germination rates were 76.43%, 75.63% and 56.99%, respectively. CDU-31 and CDU-16 had 0 germination in the first 8 h, while CDU-35 had the same germination in the first 8 h. However, the spike germination rate of the three varieties aforementioned gradually increased in the subsequent time, and by 24 h, the spike germination rate had far exceeded that of the other varieties.

3.2 Evaluation and Analysis of Grain Germination Trials

Table 2 showed that there were significant differences in grain germination rates within 32 h among 37 quinoa resources treated under the same conditions. According to the standard, CDU-23 was screened as a highly PHS resistance (HR) variety with the grain germination rate and index of 2.67%. For CDU-23, there were zero grain germinated in the first 16 h and the germination rate increased to 2.67% in the second 16 h with no change in the subsequent time. CDU-2 was selected as a resistant (R) variety with a grain germination rate of 16.00% and grain germination index of 7.83%. Moreover, the germination process of CDU-1 was similar to that of CDU-23, with no germination during the first 16 h and no change after the germination rate reached 16.00% in the subsequent time. Twenty out of thirty-seven quinoa materials were screened as high-sensitive (HS) varieties. The top three grain germination rates were CDU-34, CDU-26 and CDU-31. According to the above sequence, with 100.00%, 97.33% and 96.00% of germination rates and 260.33%, 227.17% and 200.83% of germination indices, respectively. The CDU-34 grains had all sprouted within 20 h, while CDU-26 and CDU-31 reached the peak within 24 and 28 h, respectively. CDU-15 was selected as one of the medium PHS resistant (MR) varieties, which germination rates and germination indices were 45.33% and 77.33%. Moreover, when CDU-15 was subjected to a 32 h of germination treatment, its germination rate was the highest and tended to increase continuously.

3.3 Resistance Analyses of Grain Germination Rate and Whole Spike Germination Index

As a statistical indicator of seed germination resistance, grain germination rate indirectly indicated the dormancy resistance of each resource in Table 3. The variation in grain germination resistance among the 37 quinoa resources ranged from 2.67% to 100.00%, with a coefficient of variation of 34.78% and germination was discrete and highly variable among the species. There were only one highly resistant (HR) and one resistant (R) variety in seed germination, which were CDU-23 and CDU-2, respectively. There were twenty seed germination high susceptibility (HS) varieties, accounting for 54.05% of the total resources, with a variation range of 72.00%–100.00%, which was the most representative part. However, the coefficient of variation of the high-sensitive (HS) varieties was only 9.64% with a good degree of dispersion, indicating that the differences in germination rate between each HS variety were not significant. The second was followed by ten seed germination moderately susceptible (MS) varieties with the least variation range from 53.33% to 65.33% and the least dispersion of coefficient of variation of 7.60%, indicating that the germination rates of each moderately susceptible variety were very close.

The whole-spike germination index was used as a grading index. In the whole spike germination resistance results, the variation of germination index of 37 quinoa resources ranged from 0.44% to 81.50% with a coefficient of variation as high as 82.13%, indicating that there were significant differences in quinoa spike resistance among the resources. The coefficient of variation of the medium-sense (MS) variety of spike germination was the smallest at 11.30%, and the range of variation was also small at 41.73% to 56.30% for a total of seven resources. The second was the highly sensitive (HS) varieties with a slightly larger coefficient of variation. There were five varieties with a coefficient of variation of 13.18%. Spike germination high resistance (HR) varieties were the most numerous, with a total of nine varieties and a minimum variation range of 0.44% to 4.66%, but the coefficient of variation was as high as 61.86%. Among highly resistant (HR) varieties, spike seed germination resistance differed significantly, and spike seed vigor differed significantly among highly resistant (HR) varieties, while highly resistant (HR) varieties differed significantly in spike seed vigor, they were all capable of inhibiting PHS to varying degrees.

Table 2: Evaluation of 37 quinoa resources for germination trial

Resources	Whole spike germination rate (%)									Resistance grade	Index ranking	Grain germination rate (%)											Dormancy grade	Germination rate ranking	Spike/grain germination index (%)
	8 h	16 h	24 h	32 h	40 h	48 h	72 h	96 h	120 h			4 h	8 h	12 h	16 h	20 h	24 h	28 h	32 h	44 h	56 h	68 h			
CDU-1	0.00a	0.00a	0.10a	0.49a	1.25a	2.33a	10.97	17.67	62.69	HR	35	0.00d	1.33d	10.67d	34.67c	48.00b	53.33ab	62.67a	64.00a	72.67	84.00	100.00	MS	24	1.56/98.33
CDU-2	0.00c	0.00c	0.00c	1.27c	4.67b	9.21a	18.94	30.45	74.39	HR	32	0.00d	0.00d	0.00d	0.00d	1.33cd	6.67bc	10.67ab	16.00a	36.33	42.67	100.00	R	36	3.87/7.83
CDU-3	0.00b	1.48b	4.75b	10.29ab	14.74ab	25.91a	39.47	50.33	100.00	R	22	1.33e	2.67e	4.00e	13.33de	28.00cd	34.67bc	52.00ab	57.33a	71.33	80.00	100.00	MS	25	19.81/62.17
CDU-4	0.18d	0.78cd	1.79cd	5.49bc	9.19b	13.40a	28.69	42.27	85.73	R	26	0.00d	1.33d	6.67cd	22.67c	65.33b	68.00b	85.33a	89.33a	100.00	100.00	100.00	HS	14	10.11/111.00
CDU-5	0.00b	0.29b	0.51b	0.98b	1.87bc	4.00a	13.33	26.04	68.67	HR	33	0.00c	2.67c	8.00bc	14.67abc	16.00abc	22.67ab	22.67ab	32.00a	47.33	64.67	100.00	MR	35	1.89/43.67
CDU-6	0.50c	0.63bc	0.79bc	1.36bc	2.25ab	3.42a	11.46	24.98	67.79	HR	30	1.33d	2.67d	5.33d	9.33d	21.33c	32.00bc	37.33b	54.67a	66.67	78.67	100.00	MS	28	3.96/52.33
CDU-7	0.00c	0.35c	5.65c	13.66bc	22.41ab	35.23a	50.23	64.29	100.00	MR	21	0.00e	0.00e	4.00e	10.67de	17.33cd	29.33bc	36.00ab	48.00a	61.33	70.67	100.00	MR	31	23.32/44.33
CDU-8	0.00c	0.40c	2.73c	13.75b	16.82ab	24.15a	38.29	48.67	100.00	R	23	4.00d	12.00cd	25.33c	45.33b	60.00ab	68.00a	73.33a	73.33a	100.00	100.00	100.00	HS	19	18.34/144.83
CDU-9	0.00c	3.45bc	14.61abc	22.03ab	28.01a	35.57a	54.28	68.78	100.00	MS	8	1.33f	22.67e	60.00d	69.33c	76.00b	89.33a	92.00a	93.33a	100.00	100.00	100.00	HS	10	53.64/215.67
CDU-10	0.00c	0.00c	22.46bc	37.25ab	46.68ab	61.19a	76.49	89.63	100.00	HS	4	0.00d	2.67d	13.33d	36.00c	48.00bc	57.33b	61.33b	85.33a	100.00	100.00	100.00	HS	15	63.08/158.33
CDU-11	0.00d	0.18d	1.50cd	8.97c	18.87b	36.85a	52.37	66.18	100.00	R	24	0.00d	0.00d	0.00d	4.00cd	8.00cd	12.00c	25.33b	41.33a	54.67	68.67	100.00	MR	34	17.93/22.50
CDU-12	0.00c	0.18c	7.38bc	10.54abc	21.55ab	28.28a	43.78	59.45	100.00	MR	18	0.00c	0.00c	5.33c	21.33bc	37.33abc	52.00ab	56.00ab	64.00a	80.00	87.33	100.00	MS	22	23.92/77.50
CDU-13	0.00b	0.60b	12.32ab	22.43ab	36.37ab	50.94a	66.13	80.94	100.00	MR	13	0.00e	1.33e	6.67e	17.33d	30.67c	42.67b	46.67b	65.33a	80.67	90.00	100.00	MS	21	39.93/68.17
CDU-14	0.14b	0.18b	0.40b	2.15b	4.78b	10.71a	19.13	27.68	70.38	HR	29	0.00d	0.00d	8.00d	29.33c	44.00b	49.33b	54.67ab	64.00a	78.00	85.33	100.00	MS	22	4.66/56.50
CDU-15	0.00c	0.02c	0.02c	0.36bc	0.70b	1.35a	11.17	23.42	67.58	HR	36	1.33d	5.33d	12.00cd	25.33bc	33.33ab	38.67ab	38.67ab	45.33a	59.33	70.00	100.00	MR	32	0.70/77.33
CDU-16	0.00d	3.25d	36.43c	47.48bc	63.03ab	75.63a	90.89	100.00	100.00	HS	2	0.00c	1.33c	21.33c	46.67b	62.67ab	69.33ab	80.00a	81.33a	100.00	100.00	100.00	HS	16	80.80/133.83
CDU-17	0.00d	0.71d	11.72cd	21.19bc	35.17ab	45.48a	60.72	71.56	100.00	MS	12	0.00d	0.00d	34.67c	56.00bc	62.67ab	70.67ab	77.33ab	78.67a	100.00	100.00	100.00	HS	17	41.73/148.00
CDU-18	0.00c	0.44c	0.88bc	2.06bc	2.85b	5.23a	14.78	29.23	72.49	HR	31	0.00d	1.33cd	10.67c	52.00b	89.33a	96.00a	96.00a	96.00a	100.00	100.00	100.00	HS	4	3.91/58.33
CDU-19	0.07d	0.77d	5.74cd	9.86bc	14.10ab	19.25a	30.47	48.06	100.00	R	25	6.67d	10.67cd	20.00cd	26.67bc	42.67ab	48.00a	48.00a	53.33a	65.33	73.33	100.00	MS	30	16.76/105.67
CDU-20	0.00c	0.77c	9.34bc	15.71abc	21.98ab	29.23a	48.53	62.53	100.00	MR	15	2.67f	5.33f	25.33e	49.33d	66.67c	78.67b	93.33a	94.67a	100.00	100.00	100.00	HS	5	33.03/155.17
CDU-21	0.00c	0.18c	4.40bc	9.10abc	15.19ab	21.51a	39.24	56.44	100.00	MR	19	2.67f	2.67f	13.33ef	28.00de	44.00cd	52.00bc	66.67ab	76.00a	100.00	100.00	100.00	HS	20	23.55/100.17
CDU-22	0.09d	0.73d	1.74cd	3.94bc	6.80ab	8.41a	20.39	34.17	80.27	R	27	0.00c	1.33c	42.67b	62.67b	88.00a	90.67a	93.33a	94.67a	100.00	100.00	100.00	HS	5	8.12/185.50
CDU-23	0.00b	0.00b	0.00b	0.23b	0.50ab	1.05a	10.32	19.32	53.67	HR	37	0.00a	0.00a	0.00a	0.00a	1.33a	2.67a	2.67a	2.67a	10.67	30.00	100.00	HR	37	0.44/2.67
CDU-24	0.00c	2.79c	10.41bc	22.06abc	27.99ab	32.44a	68.47	79.47	100.00	MS	11	0.00d	9.33d	46.67c	57.33bc	65.33bc	74.67ab	89.33a	90.67a	100.00	100.00	100.00	HS	13	44.76/173.33

(Continued)

Table 2 (continued)

Resources	Whole spike germination rate (%)									Resistance grade	Index ranking	Grain germination rate (%)											Dormancy grade	Germination rate ranking	Spike/grain germination index (%)
	8 h	16 h	24 h	32 h	40 h	48 h	72 h	96 h	120 h			4 h	8 h	12 h	16 h	20 h	24 h	28 h	32 h	44 h	56 h	68 h			
CDU-25	0.00b	0.37b	6.88ab	13.21ab	19.99ab	28.47a	46.59	62.19	100.00	MR	14	0.00d	1.33d	2.67cd	16.00bc	26.67b	41.33a	44.00a	54.67a	77.33	87.33	100.00	MS	28	37.86/59.83
CDU-26	2.04c	6.25c	8.16bc	14.28ab	15.24ab	17.04a	38.54	58.54	100.00	MR	16	0.00e	20.00d	64.00c	73.33b	89.33a	93.33a	96.00a	97.33a	100.00	100.00	100.00	HS	2	29.27/227.17
CDU-27	0.00c	5.30bc	17.83abc	27.52ab	34.03a	39.53a	59.33	73.47	100.00	MS	9	2.67e	21.33d	40.00c	69.33b	69.33b	90.67a	93.33a	94.67a	100.00	100.00	100.00	HS	5	48.52/198.50
CDU-28	0.44d	3.36cd	5.13bcd	11.58abc	13.66ab	20.18a	38.16	59.81	100.00	MR	20	5.33c	13.33c	44.00b	80.00a	89.33a	89.33a	94.67a	94.67a	100.00	100.00	100.00	HS	5	23.54/213.67
CDU-29	0.00c	4.38c	24.15bc	36.08abc	44.84ab	55.85a	74.57	84.57	100.00	MS	7	10.67f	16.00f	45.33e	58.67cd	72.00bc	86.67ab	94.67a	94.67a	100.00	100.00	100.00	HS	5	55.16/199.33
CDU-30	0.00d	4.72cd	17.58c	33.31b	47.02a	56.91a	77.43	87.56	100.00	MS	6	0.00d	1.33d	9.33cd	18.67bcd	25.33bcd	33.33abc	54.67ab	45.33a	61.33	72.00	100.00	MR	32	56.30/64.33
CDU-31	0.00d	5.66d	31.07c	40.51c	60.65b	76.43a	96.79	100.00	100.00	HS	1	1.33e	12.00e	38.67d	76.00c	84.00bc	92.00ab	96.00a	96.00a	100.00	100.00	100.00	HS	3	81.50/200.83
CDU-32	0.00d	2.08d	9.38cd	12.81bc	16.80ab	23.73a	44.33	58.33	100.00	MR	17	1.33e	5.33e	17.33d	61.33c	76.00b	86.67a	90.67a	92.00a	100.00	100.00	100.00	HS	11	24.64/162.00
CDU-33	0.00d	0.03d	1.31cd	3.61bc	6.32b	13.78a	23.79	32.65	75.68	R	28	0.00d	1.33d	10.67cd	20.00bc	30.67b	49.33a	56.00a	57.33a	70.67	81.33	100.00	MS	25	7.32/76.67
CDU-34	2.22e	11.28d	16.40d	30.52c	43.62b	56.78a	83.32	100.00	100.00	HS	5	2.67d	22.67c	69.33b	97.33a	100.00a	100.00a	100.00a	100.00a	100.00	100.00	100.00	HS	1	62.14/260.33
CDU-35	0.00c	3.68c	21.56bc	36.50ab	47.18ab	56.99a	89.19	100.00	100.00	HS	3	0.00e	6.67e	14.67de	36.00cd	52.00bc	60.00ab	74.67a	74.67a	100.00	100.00	100.00	HS	18	68.64/115.83
CDU-36	0.00c	0.25c	0.36c	0.94bc	1.83b	3.44a	14.74	28.47	70.56	HR	34	1.33e	1.33e	8.00e	21.33d	32.00cd	40.00bc	46.67ab	57.33a	70.00	79.33	100.00	MS	25	1.88/71.67
CDU-37	0.00c	1.73c	11.69bc	28.54ab	33.65a	42.11a	55.37	65.87	100.00	MS	10	0.00e	0.00e	13.33d	53.33c	68.00b	76.00b	89.33a	92.00a	100.00	100.00	100.00	HS	11	46.56/139.67

Note: Different lowercase letters indicate significant differences in germination rates of the same variety (grain and whole spike) at different germination stages ($p < 0.05$). Among them, the whole spike germination resistance test was conducted within 8–48 h, the whole spike germination index, germination index ranking and resistance grade classification were calculated in addition. The whole spike continuous germination test was carried out at 72, 96 and 120 h. The grain germination resistance test was conducted within 4–32 h, the grain germination index, germination index ranking and dormancy grade classification were calculated in addition. The grain germination test was carried out at 44, 56 and 68 h. Only the germination resistance experimental data were significantly labeled.

Table 3: Analysis of the diversity of PHS resistance in 37 quinoa resources

Indicators	Projects					
	Grain germination rate			Whole spike germination index		
	Number	Range of variation (%)	Coefficient of variation (%)	Number	Range of variation (%)	Coefficient of variation (%)
HR	1	2.67	100.00	9	0.44~4.66	61.86
R	1	16.00	100.00	7	7.32–19.81	37.86
MR	5	32.00–48.00	14.82	9	23.32–39.93	22.99
MS	10	53.33–65.33	7.60	7	41.73–56.30	11.30
HS	20	72.00–100.00	9.64	5	62.14–81.50	13.18
Mean ± standard deviation (%)	70.45 ± 24.50			29.27 ± 24.04		
Coefficient of variation (%)	34.78			82.13		

3.4 Analysis of High-Quality Agronomic Traits of Quinoa Resources

Fig. 1 showed that the phenotypic trait characteristics of quinoa seeds showed a roughly normal distribution trend. There was an R^2 greater than 0.9 in grain length (Fig. 1A), grain width (Fig. 1B), grain area (Fig. 1C), and thousand grain weight (Fig. 1D), suggesting that the phenotypic traits were well modeled and the overall variation was small.

Agronomic statistics and analysis of quinoa grains were presented in Tables 4 and 5. According to the 37 quinoa varieties tested, variety CDU-18 had the maximum weight of 3.70 grams, while variety CDU-5 had the smallest weight of 1.60 grams, indicating there was a difference of 2.10 g between the two varieties. The CDU-2 variety had a maximum grain length of 0.25 cm and a maximum grain area of 0.05 cm^2. There was a coefficient of variation of 19.79% for thousand grain weight, followed by a coefficient of variation of 18.35% for grain area. The results showed a high degree of dispersion in phenotypic data, indicating a large difference in yield between varieties. There was relatively little variation in grain length and width, both less than 10%, indicating that phenotypic traits were relatively stable in quinoa.

Based on the results of Table 5, it was apparent that agronomic traits were more closely related to quinoa yields. For each variety, thousand grain weight, grain length, grain area, and grain width correlated with yield, in descending order. According to the classification principle, the higher the correlation degree, the closer the relationship between the traits and the yield. Among phenotypic traits, thousand grain weight was a decisive factor for quinoa yield, while seed length and seed area had a weaker effect.

3.5 Correlation Analyses of Each Index of Quinoa Spike Germination with Agronomic Traits

The correlation analysis results between the PHS indicators and agronomic traits of quinoa are shown in the Table 6. The indicators had degrees of correlation. Especially, each of the evaluation indices of spike germination was highly significant and positive correlated, only the whole spike germination rate and seed germination rate (r = 0.399) were significantly and positively correlated. All indicators of agronomic traits were highly significantly positively correlated. The correlation between different types of indicators was also evident. Grain germination rate was highly significantly positively correlated with thousand grain weight (r = 0.436), and grain germination index was significantly positively correlated with thousand grain weight (r = 0.391), grain area (r = 0.340), and grain width (r = 0.349). Seed area, seed length, and seed width negatively correlated with whole spike germination rate, correlations between the remaining indicators were not significant.

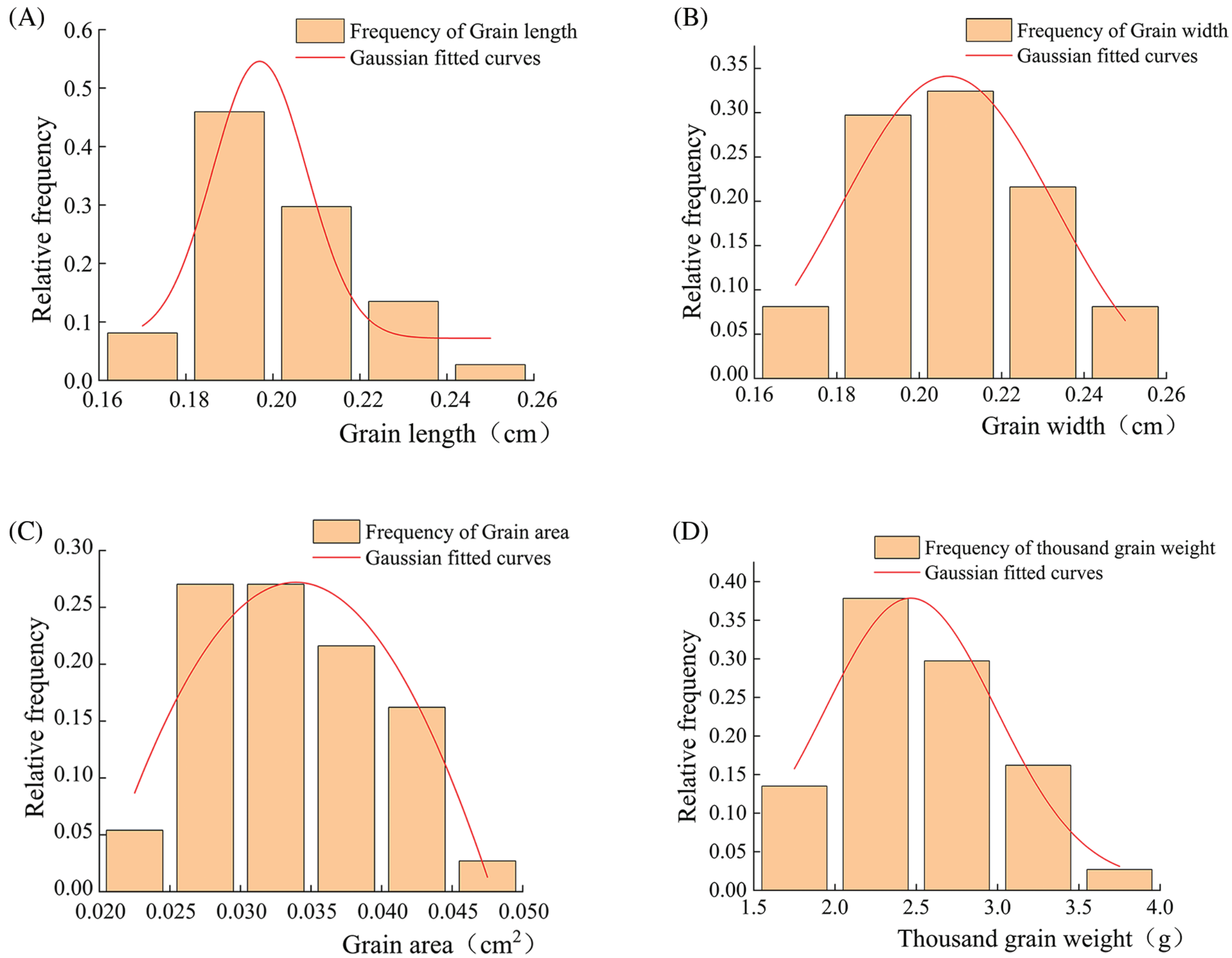

Figure 1: Frequency distribution of agronomic traits in the grains of 37 quinoa resources. (A) Grain length; (B) Grain width; (C) Grain area; (D) Thousand grain weight

Table 4: Mean values of basic agronomic traits for 37 quinoa resources

	Trait			
Species	Grain length (cm)	Grain width (cm)	Grain area (cm^2)	Thousand grain weight (g)
CDU-1	0.2042 ± 0.0119defghijkl	0.2262 ± 0.0119bcdef	0.0370 ± 0.0015defghi	2.3510 ± 0.0273lm
CDU-2	0.2526 ± 0.0110a	0.2450 ± 0.0198ab	0.0478 ± 0.0064a	3.0804 ± 0.0474def
CDU-3	0.1802 ± 0.0118lmno	0.1856 ± 0.0096mnop	0.0264 ± 0.0027pqrs	2.0718 ± 0.0535p
CDU-4	0.1966 ± 0.0089efghijklm	0.2092 ± 0.0139fghijk	0.0322 ± 0.0031ghijklmnop	2.1905 ± 0.0495o
CDU-5	0.1856 ± 0.0103klmno	0.1836 ± 0.0084nopq	0.0264 ± 0.0019pqrs	1.6030 ± 0.0314t

(Continued)

Table 4 (continued)

Species	Trait			
	Grain length (cm)	Grain width (cm)	Grain area (cm^2)	Thousand grain weight (g)
CDU-6	0.1980 ± 0.0110efghijklm	0.1996 ± 0.0104ijklmn	0.0306 ± 0.0031ijklmnopq	2.0669 ± 0.0576p
CDU-7	0.1980 ± 0.0129efghijklm	0.1984 ± 0.0062ijklmno	0.0312 ± 0.0028hijklmnopq	2.2209 ± 0.0718no
CDU-8	0.1914 ± 0.0524hijklmn	0.2144 ± 0.0114efghi	0.0372 ± 0.0052defgh	2.8483 ± 0.0609g
CDU-9	0.2180 ± 0.0086bcdef	0.2376 ± 0.0152abcd	0.0406 ± 0.0036bcde	2.8046 ± 0.0474g
CDU-10	0.1916 ± 0.0101hijklmn	0.1918 ± 0.0117jklmnop	0.0288 ± 0.0035mnopqrs	2.3518 ± 0.0717lmn
CDU-11	0.2054 ± 0.0087defghijk	0.2032 ± 0.0108ghijklmn	0.0330 ± 0.0033ghijklmno	2.8199 ± 0.0783g
CDU-12	0.2156 ± 0.0166bcdefgh	0.2070 ± 0.0053fghijkl	0.0354 ± 0.0020efghijkl	2.6395 ± 0.0462hi
CDU-13	0.1650 ± 0.0038o	0.1650 ± 0.0054q	0.0226 ± 0.0005s	1.6921 ± 0.0448st
CDU-14	0.1788 ± 0.0124mno	0.1882 ± 0.0089klmnop	0.0276 ± 0.0031nopqrs	1.8869 ± 0.0617r
CDU-15	0.2056 ± 0.0064defghijk	0.2070 ± 0.0053fghijkl	0.0342 ± 0.0012efghijklm	2.4525 ± 0.0773jk
CDU-16	0.1714 ± 0.0184no	0.1738 ± 0.0066pq	0.0240 ± 0.0028rs	2.5260 ± 0.0930jk
CDU-17	0.2196 ± 0.0087bcdef	0.2072 ± 0.0157fghijkl	0.0356 ± 0.0030efghijk	3.1470 ± 0.0465de
CDU-18	0.2156 ± 0.0139bcdefgh	0.2362 ± 0.0178abcd	0.0404 ± 0.0036bcde	3.7010 ± 0.0744a
CDU-19	0.2270 ± 0.0061bcd	0.2488 ± 0.0152a	0.0436 ± 0.0017abc	2.5008 ± 0.0773j
CDU-20	0.2206 ± 0.0073bcde	0.2174 ± 0.0205defghi	0.0364 ± 0.0021defghij	3.4470 ± 0.0730b
CDU-21	0.1992 ± 0.0204efghijklm	0.1866 ± 0.0147lmnop	0.0290 ± 0.0036lmnopqrs	2.7010 ± 0.0797h
CDU-22	0.2104 ± 0.0062defghij	0.2236 ± 0.0107cdefg	0.0380 ± 0.0024bcdefg	2.7110 ± 0.0902h
CDU-23	0.1828 ± 0.0128klmno	0.1984 ± 0.0123ijklmno	0.0272 ± 0.0040nopqrs	2.0701 ± 0.0659pq
CDU-24	0.1928 ± 0.0119ghijklmn	0.2148 ± 0.0160efghi	0.0318 ± 0.0015ghijklmnop	2.3820 ± 0.0647hl
CDU-25	0.1864 ± 0.0048jklmno	0.2020 ± 0.0083hijklmn	0.0296 ± 0.0014klmnopqr	2.3039 ± 0.0814lm
CDU-26	0.2346 ± 0.0058abc	0.2414 ± 0.0115abc	0.0442 ± 0.0023ab	3.3968 ± 0.1103c
CDU-27	0.2386 ± 0.0250ab	0.2300 ± 0.0210abcde	0.0422 ± 0.0055abcd	2.9530 ± 0.0405f
CDU-28	0.1978 ± 0.0074efghijklm	0.1970 ± 0.0091ijklmno	0.0300 ± 0.0015jklmnopqr	3.0668 ± 0.0868ef
CDU-29	0.2168 ± 0.0159bcdefg	0.2224 ± 0.0058cdefgh	0.0378 ± 0.0035cdefg	2.8016 ± 0.0403g
CDU-30	0.2028 ± 0.0069defghijklm	0.2110 ± 0.0107efghij	0.0344 ± 0.0015efghijklm	2.3140 ± 0.0858mn
CDU-31	0.2208 ± 0.0128bcde	0.2312 ± 0.0115abcde	0.0398 ± 0.0040bcdef	3.1839 ± 0.0679d
CDU-32	0.1916 ± 0.0124hijklmne	0.1894 ± 0.0189klmnop	0.0274 ± 0.0027nopqrs	2.5683 ± 0.0755ij
CDU-33	0.1966 ± 0.0081efghijklm	0.2062 ± 0.0142fghijklm	0.0306 ± 0.0030ijklmnopq	1.9680 ± 0.0474qr
CDU-34	0.2142 ± 0.0074cdefghi	0.2364 ± 0.0123abcd	0.0400 ± 0.0021bcde	2.0534 ± 0.0476pq
CDU-35	0.1954 ± 0.0101fghijklmn	0.2158 ± 0.0108efghi	0.0334 ± 0.0021fghijklmn	2.3580 ± 0.0499lmn
CDU-36	0.1902 ± 0.0097ijklmn	0.1932 ± 0.0052jklmnop	0.0268 ± 0.0007opqrs	1.7618 ± 0.0533s
CDU-37	0.1802 ± 0.0115lmno	0.1784 ± 0.0099opq	0.0250 ± 0.0017qrs	2.3094 ± 0.0603mn

Note: Different lowercase letters indicate significance of differences at the 0.05 level.

3.6 Cluster Analyses of PHS Indicators and Agronomic Traits of Quinoa Material

Results of the spike germination index by cluster analysis were shown in Fig. 2A. It was possible to divide the 37 quinoa resources into three major categories when the squared Euclidean distance was 9.66. First-class quinoa materials included CDU-2 and CDU-23, accounting for 5.41% of the test materials. The average spike and seed germination rate were 5.13% and 9.34%, and the average spike and seed germination index were 0.22 and 0.05. Based on the spike germination index, it was evident that a class of materials are highly resistant to spike germination. A total of 19 materials were included in the second category, representing 51.35 percent of the materials tested. According to the results, pre-harvest germination rate, grain germination rate, pre-harvest germination index, and grain germination index of these materials were 39.02%, 89.75%, 0.43% and 1.70%, respectively. All the relevant pre-harvest germination indices were high, belonging to the materials with low pre-harvest germination resistance, indicating that they were easy to sprout. The third category contained 16 materials that accounted for 43.24% of all test materials. It was 19.72%, 55.17%, 0.16% and 0.74% for the spike germination index, seed germination index, spike germination index, and seed germination index, respectively, and there was some resistance to PHS at the middle level.

Table 5: Agronomic traits and grey correlation analysis of quinoa resources

Agronomic traits	Maximum value	Minimum value	Range	Mean ± standard deviation (%)	Coefficient of variation (%)	Relevance	Ranking
Thousand grain weight	3.7010	1.6030	2.0980	2.5216 ± 0.4491	19.7912	0.911	1
Grain length	0.2526	0.1651	0.0880	0.2025 ± 0.0188	9.2818	0.685	2
Grain area	0.0478	0.0226	0.0250	0.0335 ± 0.0061	18.3451	0.674	3
Grain width	0.2488	0.1654	0.0830	0.2087 ± 0.0206	9.8904	0.638	4

Table 6: Correlation analysis of each indicator of quinoa PHS with agronomic traits

Indicators	Whole spike germination rate	Whole spike germination index	Grains germination rate	Grains germination index	Grain length	Grain width	Grain area	Thousand grain weight
Whole spike germination rate	1							
Whole spike germination index	0.955**	1						
Grains germination rate	0.399*	0507**	1					
Grains germination index	0.431**	0.573**	0.857**	1				
Grain length	−0.054	0.009	0.150	0.298	1			
Grain width	−0.079	0.027	0.194	0.349*	0.859**	1		
Grain area	−0.035	0.037	0.185	0.340*	0.963**	0.963**	1	
Thousand grain weight	0.110	0.170	0.436**	0.391*	0.564**	0.564**	0.645**	1

Note: "*" indicates a significant level of correlation ($p < 0.05$), "**" indicates a highly significant correlation ($p < 0.01$).

Results of the agronomic traits by cluster analysis were shown in Fig. 2B. The 37 quinoa resources could be divided into three categories based on the squared Euclidean distance of 9.66. Three materials were in the first category, representing 8.11% of the test material, namely CDU-18, CDU-26, and CDU-19. There was an average grain length of 0.22, a grain width of 0.23, a grain area of 0.04 and a thousand grain weight of 3.52 g. It was a quinoa resource with yield and quality potential, as its agronomic traits were higher than the other two categories. A total of 12 materials were tested in the second category, representing 32.43 percent of all materials tested. In terms of grain length, grain width, grain area, and thousand grain weight, the average grain was 0.22, 0.228, 0.04, and 2.85, which was an excellent quinoa resource for transformation, all agronomic indexes place it slightly below the first category, but significantly higher than the third category. The third category contains another 22 materials, accounting for 59.46%. Approximately 0.18, 0.19, 0.03 and 2.11 grains were averaged for length, width, grain area and thousand grains.

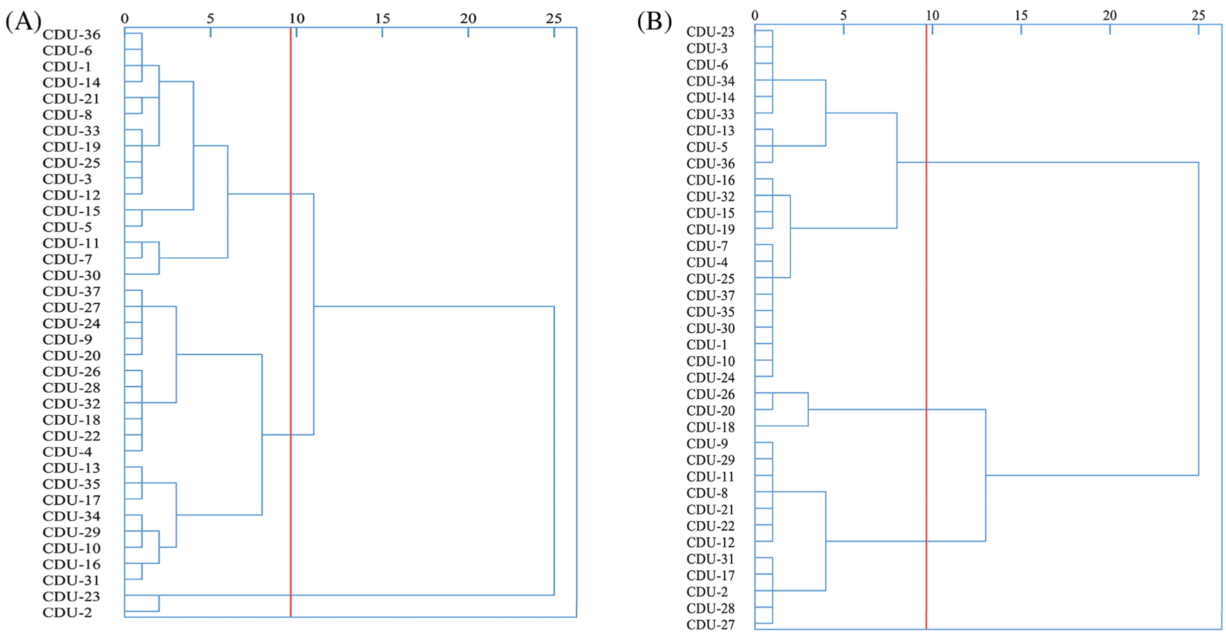

Figure 2: Cluster analysis of 37 quinoa resources. (A) Cluster analysis of PHS resistance indicators; (B) Cluster analysis of agronomic traits

4 Discussion

PHS in grains is an irreversible and extremely damaging natural disaster, the imbalance between grain dormancy and germination is the main reason for cause of PHS [13,29,30], which can lead to a decrease in grain yields and quality. Agronomic traits of germplasm resources that can be used for estimating grain yield and quality. Grain yield is determined by thousand grain weight, while grain quality is determined by seed length, seed width, and seed area [28,31]. One of the most effective ways to reduce PHS and improve nutrition of grain is by selecting and breeding varieties that have excellent agronomic attributes and are resistant to PHS.

This study selected quinoa varieties with PHS resistance based on seed germination and whole spike germination test referring to the experimental methods for identifying PHS resistance in wheat, rice and barley [23,32–34] adjusted for quinoa germination characteristics. By analyzing thousand grain weight, seed length, seed width, and seed area, excellent agronomic traits were selected. Dormancy could explain seed PHS resistance, which is closely related to seed dormancy characteristics. The strength of seed dormancy characteristics could be determined by grain germination rate, grain germination index, whole spike germination rate and whole spike germination index. According to the seed germination rate and the whole spike germination index, PHS resistance of quinoa varieties were divided into five categories:

high resistance (HR), medium resistance (MR), resistance (R), medium sensitivity (MS), and high sensitivity (HS). After analysis of variation coefficient, seed germination rate, whole spike germination rate, seed germination rate and whole spike germination index, it was found that the seed germination rate of 37 quinoa varieties was much higher than the germination rate of the whole spike, and the grain germination index was higher than the germination index of the whole spike, indicating that significant differences in PHS resistance among different varieties. Correlation analysis revealed that PHS resistance indexes of all varieties were significantly and positively correlated.

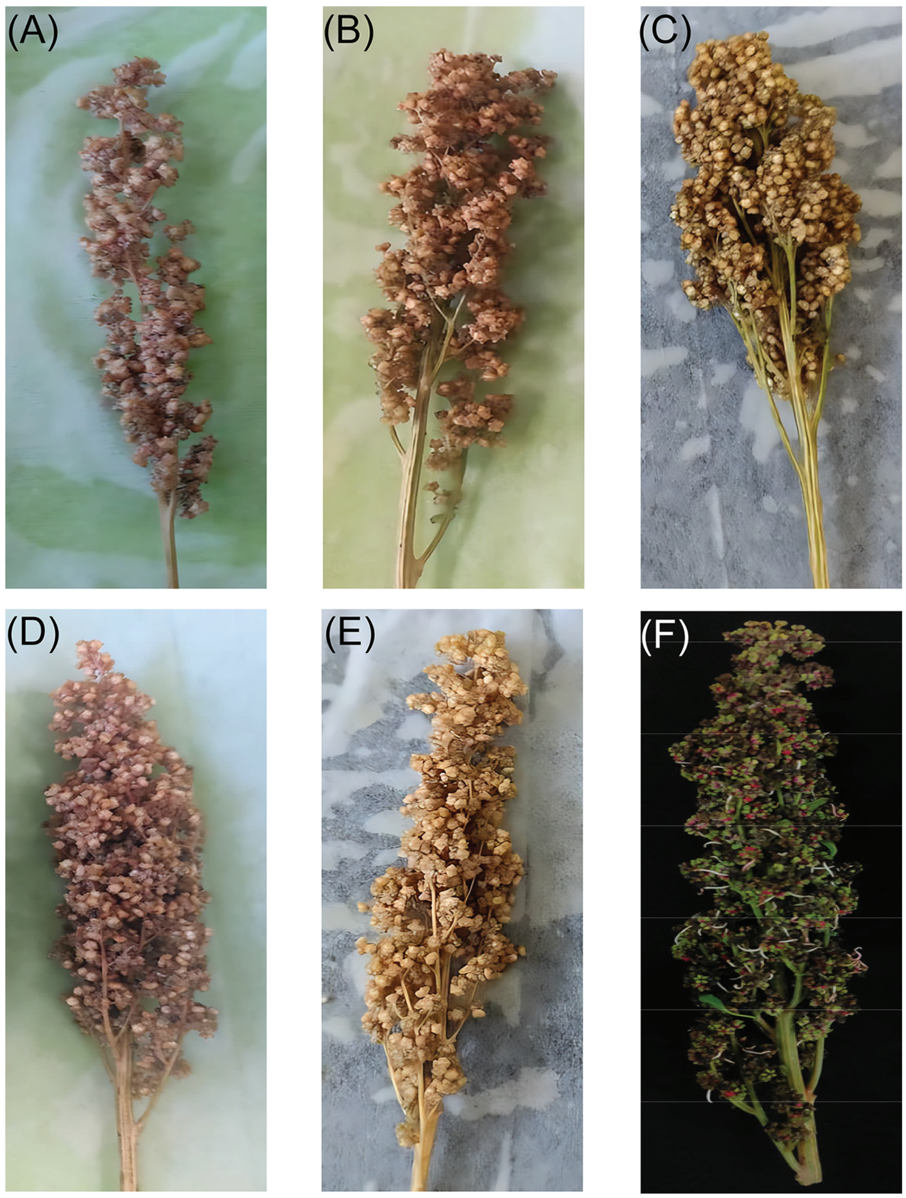

Figure 3: The picture of whole spike germination in 48 h. The excellent agronomic traits and PHS resistance of quinoa varieties were as follows: (A) represents CDU-2 with germination rate of 9.21%, (B) represents CDU-11 with germination rate of 36.85%, (C) represents CDU-12 with germination rate of 28.28%, (D) represents CDU-21 with germination rate of 21.51%, (E) represents CDU-8 with germination rate of 24.15%. (F) represents CDU-31 as the most sensitive variety to PHS, with germination rate of 76.43%

Furthermore, during the experimental process, it was found that the middle and bottom of the spike were the main germination sites. A first possibility is that there are substances in the peripheral perianth of grains that inhibit or promote grain germination, thus, the variety acquired PHS resistance, which was consistent with previous studies [35]. Secondly, water in the tissue at the top of the spike may flow downward due to gravity, the spike bottom stayed wet for a long time and provided the conditions for grain germination. The third reason might be that the seeds at the top of the spike were exposed to more sunlight and had lower water content, which dormancy of seed was enhanced and obtained stronger PHS resistance. The last reason might be that α-amylase activity and endogenous hormone content in the embryo of the seed dormancy [12,36–38]. This study of agronomic traits has been shown to follow a normal distribution. According to the coefficient of variation, there was a large difference between thousand grain weight and seed area. It was found that the traits were highly significantly correlated in the correlation analysis. Gray correlation analysis revealed that thousand grain weight, grain length, and grain area were the three most important traits affecting yield. Variations in genetic variation may lead to differences in yield and quality under the same treatment. Cluster analysis was used to classify 37 quinoa resources based on agronomic traits and PHS resistance at a squared Euclidean distance of 9.66. The germplasm resources with similar PHS resistance and genetic proximity were clustered into one category, for cultivation, PHS resistant varieties with excellent agronomic qualities (CDU-2) were selected to alleviate damage caused by quinoa PHS and to increase yield and quality, and promote the healthy development of quinoa industry.

5 Conclusion

In this study, 37 quinoa resources were tested for PHS resistance in 48 h, excellent agronomic traits and were compared. Five quinoa materials, CDU-2, CDU-11, CDU-12, CDU-21 and CDU-8, were selected from the comprehensive multiple comparison analysis, and all had better PHS resistance and excellent agronomic traits. The CDU-31 was selected as the most sensitive material to PHS. The photos of the 6 quinoa varieties were shown in Fig. 3. There was one material, CDU-2, which had the best PHS resistance and thousand grain weight, length, width, and area of seed. Besides screening high quality and PHS resistant quinoa varieties suitable for low altitude cultivation in Chengdu plain, this study also provides some theoretical support for the creation of subsequent quinoa germplasm resistant to PHS, which has important implications in regards to cultivation extension and production guidance.

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Availability of Data and Materials: The datasets used and/or analyzed during the current study are available from the author and/or corresponding author on reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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