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RESEARCH ARTICLE

Unraveling the Genetic and Morphological Diversity of Quinoa (*Chenopodium quinoa* Willd.)

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Abstract

Quinoa (*Chenopodium quinoa* Willd.) is a pseudo cereal used as a superfood because of its nutritional status. This study focuses on the morphological and molecular characterization of 36 quinoa genotypes, aiming to evaluate their genetic diversity and potential for breeding. Ten qualitative characters were selected for morphological analysis, revealing significant variations in traits such as spikelet color, leaf length, and plant height. Analysis of variance showed that most quantitative traits, including days to 50% flowering and seed yield, exhibited significant differences among genotypes, indicating substantial genetic variability. High heritability and genetic advance were observed for traits like leaf length and seed yield, suggesting strong potential for genetic improvement. The genotypic performance highlighted superior traits in genotypes ACQS1, EC 896115, IGKVC-12, ACQS8, EC 896208, and EC 896219 for leaf length, number of internodes, leaf width, petiole length, plant height, length of inflorescence, and number of inflorescences. Genotypes EC 896065, EC 896213, EC 896201, SHQ4, SHQ5, ACQS1, ACQS2, ACQS3, and EC 896218 exhibited higher seed weight, while EC 896109, ACQS3, ACQS1, and EC 896219 showed higher yield. High genotypic and phenotypic coefficient of variation (GCV and PCV) were recorded for leaf length (31.22, 34.71), leaf width (43.64, 44.91), number of internodes (40.47, 40.59), petiole length (35.46, 36.04), plant height (33.35, 54.47), length of inflorescence (36.41, 36.99), and seed yield (33.58, 34.53). Heritability was highest for the number of internodes (99.38%), with significant genetic advances observed in traits such as leaf length (57.86%) and seed yield (67.28%). Seed weight shows the highest positive direct effect (0.701), followed by the number of inflorescences per plant (0.700), whereas days to 50% flowering (-0.768) show the highest negative direct effect. Molecular diversity analysis using 16 ISSR markers revealed a polymorphism rate of 56.1%, with significant allelic variation among markers. The polymorphism information content (PIC) value ranged from 0.274 to 0.797, indicating varying levels of marker informativeness. Cluster analysis grouped the genotypes into two major clusters, demonstrating genetic diversity among the studied genotypes. Exploring the genetic basis of key traits and conducting further molecular characterization can provide deeper insights into the genetic architecture of quinoa. Additionally, incorporating more advanced genomic tools and expanding the genotypic pool could facilitate the development of high-yielding, resilient quinoa varieties.

Keywords: Cluster analysis, Genetic advance, Heritability, ISSR, Quinoa, Variation.

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Introduction

Quinoa (*Chenopodium quinoa* Willd.) is an annual and herbaceous plant that belongs to the Amaranthaceae family, having chromosome number 2n = 4x = 36, but formerly placed in the Chenopodiaceae family that originated in the Pacific slopes of the Andes in South America (Bhargava *et al.,* 2006). Quinoa is a pseudo cereal that is considered one of the most complete foods for humans. It is grown in South America, from Colombia to southern Chile. It is a dicotyledonous domesticated pseudo-cereal and one of the oldest nutritionally rich crops (Sentis D., 2018). Quinoa is a grain crop grown for its edible grains to support nutritional requirements. It provides a complete protein and is a source of all nine essential amino acids in the right proportions per 100 gm dry weight Nutrition content of quinoa is energy 399 Kcal, protein 16.5 g, fat 6.3 g, total carbohydrate 69 g, iron 13.2 mg, zinc 4.4 mg. Quinoa has also been termed as a superfood, mother of all grains, food for the future, power food, gold for people and food for global health security (Singh, 2019). It is cultivated in the world in more than 70

countries with a production of 175,180 metric ton (FAOSTAT, 2021). According to the Food and Agriculture Organization of the United Nations (FAO), in recent years (2000–2019). There has been a significant global increase in the area cultivated with quinoa crops, mainly in Peru and Bolivia, with increases between 36 and 72%, respectively. Quinoa was introduced in India during 1975-76 at Agra (Singh, 2019). Till 2017-18, the area under quinoa cultivation in India was 8630 hectares and the total production was 206257 quintals. The average productivity of quinoa in India was 23.2 q ha^{-1} (Singh, 2019).

Characterization of germplasm is important to distinguish one genotype from another and to provide information on the extent of variation and other genetic parameters in respect of yield and other quality characteristics. However, there is limited information on the characterization of quinoa germplasm for morphological and molecular traits (Santis D. 2018). Quinoa comprises a broad genetic variability. Therefore, a preliminary approach to understanding the genetics of quinoa materials entails a morphologic characterization, which can provide the basis for the selection of materials that satisfy the needs of farmers and consumers. Additionally, molecular marker analysis plays a vital role in the genetic characterization of quinoa. It allows for the identification of genetic variations at the DNA level, providing a more precise and comprehensive understanding of the genetic diversity within quinoa germplasm. Therefore, this study aimed to evaluate the morphological and molecular characterization of quinoa germplasm. It was hypothesized that there exists substantial variation for morphological and molecular traits in quinoa germplasm, which may be used in breeding programmes.

Material and Methods

The experimental materials consisted of 36 quinoa genotypes under the All India Coordinated Research Network project on Potential Crops (Table 1). The field experiment was conducted in the experimental field of the Raj Mohini Devi CARS, Ambikapur (Chhattisgarh), during the *rabi* season 2020-21 and 2021-22. The experiment was laid out in a randomized block design with three replications, and a plot size 3 × 1.8 m was used for the constitution of the experiment. All the recommended package of practices for the region was followed to raise a good crop.

Qualitative and Quantitative Data Collection

All the genotypes were evaluated for 10 qualitative characters *viz*. early plant vigor, plant growth habit, flower color, leaf color, leaf margin color, leaf blade shape, stem color, seed shattering, seed shape and seed color) based on morphological descriptors and to analyze genetic variability 12 quantitative characters (days to 50% flowering, leaf length (cm), leaf width (cm), number of primary branches, number of internodes, petiole length (cm), number of inflorescences per plant, length of inflorescence (cm), plant height (cm), days to 80% maturity, seed weight {g/10 mL tube (standardized by AICRN on PC)} and seed yield (q/ha) for all the genotypes were recorded.

PCR and Molecular Data Generation

About 1 g fresh leaflets of 36 quinoa genotypes were ground in a pestle and mortal for DNA extraction. Total DNA was extracted according to the CTAB extraction protocol. DNA concentration was determined with electrophoresis in agarose gel, ethidium bromide staining solution and visualization on UV transilluminator. DNA was PCR amplified using 16 ISSR markers in a 96-well thermal cycler. Reactions were carried out in a total volume of 22 µL consisting of 2 µL (20 ng) of template DNA, 18 µL of PCR mix (cocktail made by adding PCR buffer 2.5 µL, dNTPs 1.5 µL, sterile DD water 13.5 µL and Taq. polymerase 0.5 µL) and 2 µL of ISSR primer. Amplification was performed under the following conditions: PCR cycling consisted of initial denaturation at 94°C for 5 minutes, followed by 40 cycles of amplification at 94°C for 30 seconds (denaturation), 40 to 50°C, 40 seconds for (annealing) and 72°C for 45 seconds (extension). A final extension step at 72°C for 7 minutes was followed by the termination of the cycle and storing the PCA product at 4°C. The amplification products were electrophoresed on 2% agarose gel in 1X TAE buffer.

Data Analysis

Molecular data was recorded after PCR amplification and visualization using gel documentation and analyzed for polymorphism information content (PIC). Cluster analysis of recorded molecular data was done using the unweighted pair-group method with arithmetic average (UPGMA) (Kumar *et al.,* 2016). Jaccard's similarity coefficient, with the help of dendrogram and quantitative data, was analyzed using statistical software, namely OPSTAT and STAR 2.0.1 for Windows.

Results and Discussion

Morphological Characterization

Ten qualitative characters were selected for the morphological characterization of quinoa, with the observed morphological descriptors presented in Figure 1. Variations in spikelet color among various important quinoa genotypes are shown in Figure 4. The mean performance of the genotypes is displayed in Table 3. The analysis of variance for all 12 characters is summarized in Table 3, indicating that the mean sum of squares due to genotypes was highly significant for days to 50% flowering, leaf length, leaf width, number of internodes, petiole length, plant height, days to 80% maturity, length of inflorescence, number of inflorescences per plant, and seed yield. In contrast, the number of primary branches and seed weight were not significant. The significant mean squares for seed yield and related characteristics suggest substantial variability

Table 1: Mean performance for quantitative data in quinoa

Characters / Genotypes	Days to 50% flowering	Leaf length (cm)	Leaf width (cm)	Number of internodes	Petiole length (cm)	Number of primary branches	Plant height (cm)	Days to 80% maturity	Length of inflorescence (cm)	Number of inflorescences per plant	seed weight (g/10 mL)	Seed yield (q/ha)
EC896065	51.00	3.85	3.37	18.37	2.99	1.60	79.73	116.33	25.33	15.27	7.08	8.03
EC896069	50.33	4.80	2.76	8.67	2.97	1.67	56.37	113.67	21.00	14.17	6.48	7.99
EC896079	48.00	3.92	3.02	9.80	2.05	2.33	39.80	110.33	19.50	12.13	5.80	7.72
EC896237	50.33	2.64	2.23	13.10	1.90	1.67	43.33	113.00	16.27	11.33	5.57	9.00
EC896246	49.67	2.26	1.20	5.70	1.27	2.33	26.00	111.33	10.00	8.17	5.47	6.85
EC896213	49.67	3.56	1.66	8.77	2.38	2.33	51.00	109.00	16.00	13.13	7.10	6.75
EC896275	49.67	2.85	1.36	15.83	1.41	2.40	56.37	112.67	26.77	16.93	6.48	7.94
EC896276	50.33	3.74	2.89	21.00	2.26	1.80	52.70	109.67	28.07	17.10	6.39	12.68
EC896201	49.67	4.51	2.85	17.47	2.20	2.47	59.33	114.00	27.77	15.73	7.18	17.07
EC896208	51.00	5.15	3.63	22.07	4.74	1.73	83.43	112.33	32.87	17.43	6.75	8.26
EC896210	50.00	3.61	1.79	6.80	3.29	1.67	58.87	113.00	34.67	16.07	6.44	8.95
SHQ1	49.67	3.48	2.33	11.57	2.42	2.33	36.20	105.33	12.07	11.77	6.50	8.62
SHQ2	49.33	3.49	0.97	15.70	1.55	2.33	45.63	112.00	24.50	12.93	6.87	12.93
SHQ3	48.67	1.83	1.37	13.53	1.40	1.67	33.10	112.67	9.57	9.93	6.44	8.82
SHQ4	50.00	3.95	2.45	13.67	1.77	2.33	43.83	113.67	24.70	9.47	7.01	7.69
SHQ5	50.00	5.57	3.23	12.57	4.61	2.40	35.13	114.33	11.33	8.90	7.34	7.12
ACQS1	46.00	7.57	5.67	35.33	3.94	1.60	70.87	114.00	12.47	18.87	7.10	18.16
ACQS2	45.00	6.37	5.01	32.83	4.51	1.47	66.60	113.33	26.87	17.13	7.32	14.67
ACQS3	46.00	6.39	5.01	31.90	4.38	1.60	75.51	112.00	22.90	15.93	7.43	16.75
ACQS4	43.67	7.21	4.78	29.87	4.09	1.73	73.80	112.67	24.63	17.60	6.57	13.63
ACQS5	45.00	5.90	4.24	30.00	4.00	1.80	66.27	106.67	13.33	14.47	6.48	14.30
ACQS6	46.00	6.30	4.07	28.77	4.14	1.60	60.80	107.67	27.17	20.27	6.15	13.35
ACQS7	45.33	6.73	3.83	29.67	3.43	1.67	63.47	109.00	22.60	20.20	6.17	10.44
ACQS8	45.00	6.09	5.23	26.63	5.35	1.60	56.77	108.67	35.53	22.80	6.27	11.82
ACQS9	44.67	6.40	4.13	29.10	4.78	1.67	48.53	107.33	22.03	17.40	5.72	13.92
ACQS10	45.00	6.80	4.27	28.13	5.23	1.73	54.47	111.00	23.70	18.47	6.23	14.37
EC896062	43.33	5.85	4.63	33.47	5.07	1.73	79.07	112.33	31.63	13.43	6.65	10.41
EC896064	41.33	4.97	2.81	28.80	3.69	1.60	64.20	105.67	20.57	13.13	6.05	8.87
EC896097	42.67	6.45	5.23	33.93	4.95	1.80	78.60	111.67	21.63	19.60	6.50	8.77
EC896098	42.67	5.03	5.39	29.90	4.65	1.60	93.20	110.00	47.80	15.47	6.10	12.07
EC896109	41.67	7.44	5.45	31.33	4.71	1.80	79.07	110.00	42.73	20.47	6.38	15.70
EC896115	44.00	6.80	6.14	29.60	4.10	1.53	73.20	109.00	33.70	21.53	6.57	9.54
EC896218	43.00	7.15	5.47	28.17	4.58	1.53	63.40	105.33	36.57	22.13	6.60	14.21
EC896219	44.67	6.05	4.87	30.80	3.85	2.00	73.67	109.33	37.57	21.13	7.15	19.50
IGKVC-12	43.00	6.25	6.64	28.33	4.49	1.53	81.10	105.67	30.07	10.60	6.45	11.09
Him Shakti	43.00	9.09	8.04	30.50	4.13	1.80	91.33	97.67	29.90	23.07	7.63	22.75
Mean	**46.62**	**5.28**	**3.83**	**22.82**	**3.53**	**1.85**	**67.08**	**110.34**	**25.11**	**15.95**	**6.57**	**11.69**
CD	**2.32**	1.30	0.66	1.18	0.37	0.33	15.09	4.10	2.70	4.74	0.78	0.88
CV	3.05	15.17	10.62	3.18	6.46	9.89	15.02	2.22	6.60	18.25	7.24	6.35
SEm	0.821	0.462	0.235	0.419	0.132	0.294	0.342	0.451	0.957	0.678	0.275	0.311

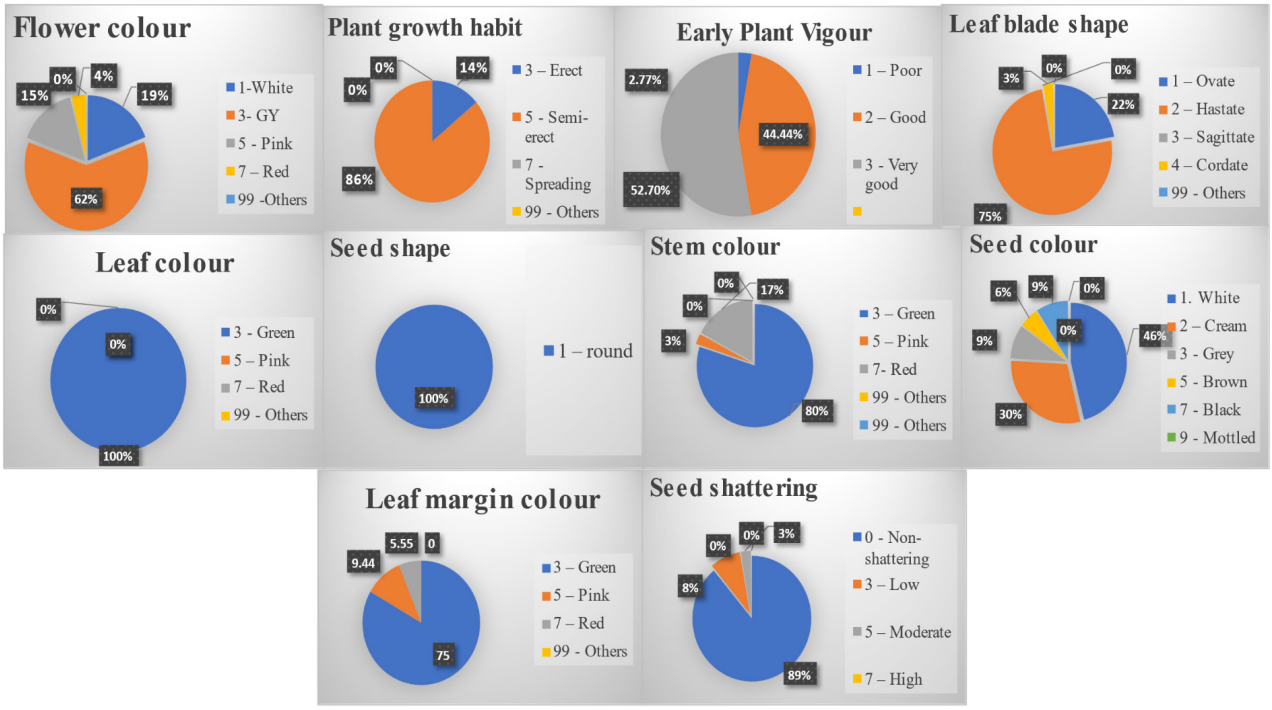

Figure1: Qualitative character variation among quinoa genotypes

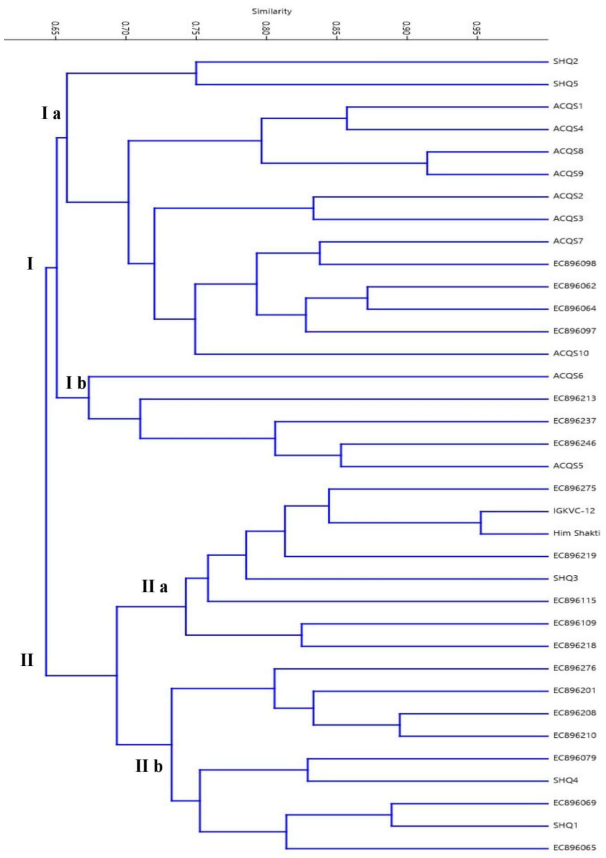

Figure 2: Dendrogram representing quinoa varieties based on ISSR markers.

Table 2: Analysis of variance for seed yield and its component in quinoa.

S. N.	Characters	Mean sum of squares		
		Replication	Genotypes	Error
	Degree of freedom (Df)	2	35	70
1	Days to 50% flowering	2.565	9.508**	2.022
2	Leaf length (cm)	0.385	8.794**	0.641
3	Leaf width (cm)	0.418	8.566**	0.166
4	Number of internodes	3.58	256.544**	0.528
5	Petiole length (cm)	0.213	4.766**	0.052
6	Number of primary branches	4.858	0.305	0.259
7	Plant height (cm)	2873.425	4610.789**	3109.369
8	Days to 80% maturity	12.954	8.636**	1.326
9	Length of inflorescence (cm)	2.189	253.358**	2.748
10	Number of inflorescences per plant	4.737	51.238**	8.449
11	Seed weight (g/10 mL)	1.522	0.823	0.226
12	Seed yield (q/ha)	8.906	47.103**	0.886

Note: ** at 1% significant

Table 3: Genetic parameters of variability for yield and attributes in quinoa

Characters	*General mean*	*Range*		*PCV (%)*	*GCV (%)*	*h2 (%)*	*Genetic advance*	*GA as % of mean*
		min.	*max.*					
Days to 50% flowering	46.62	41.33	51	7.17	6.49	81.92	5.64	12.11
Leaf length (cm)	5.28	1.82	9.08	34.72	31.23	80.9	3.05	57.86
Leaf width (cm)	3.83	1.2	8.04	44.92	43.64	94.41	3.35	87.35
Number of internodes	22.82	5.7	35.33	40.6	40.47	99.39	18.97	83.12
Petiole length (cm)	3.53	1.4	5.34	36.05	35.46	96.78	2.54	71.87
Number of primary branches	1.85	1.46	2.46	28.37	6.74	5.65	0.06	3.3
Plant height (cm)	67.08	26	93.2	51.47	33.35	13.86	17.16	25.58
Days to 80% maturity	110.34	97	116	3.75	2.98	63.04	5.37	4.87
Length of inflorescence (cm)	25.11	9.56	47.8	37	36.41	56.82	18.53	73.79
Number of inflorescences per plant	15.95	8.16	23.06	29.88	23.68	22.8	6.17	38.66
Seed weight (g/10 mL)	6.57	5.46	7.63	9.93	6.79	46.76	0.63	9.56
Seed yield (q/ha)	11.69	7.11	22.74	34.54	33.58	64.56	7.86	67.28

within the studied material, indicating a high potential for improvement through selective breeding.

The overall mean and range of the twelve quantitative traits are presented in Table 3, along with their genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability, genetic advance, and genetic advance as a percentage of the mean. All 36 quinoa genotypes exhibited a wide range of variations for all quantitative traits. Based on morphological descriptors, only leaf color and seed shape showed no variation. High mean performance was observed in genotypes ACQS1, EC 896115, IGKVC-12, ACQS8, EC 896208, and EC 896219 for leaf length, number of internodes, leaf width, petiole length, plant height, length of inflorescence, and number of inflorescences. Genotypes EC 896065, EC 896213, EC 896201, SHQ4, SHQ5, ACQS1, ACQS2, ACQS3, and EC 896218 exhibited higher seed weight. High yield was shown by genotypes EC 896109, ACQS3, ACQS1, and EC 896219, along with Him Shakti.

High GCV was recorded for leaf length (31.22), leaf width (43.64), number of internodes (40.47), petiole length (35.46), plant height (33.35), length of inflorescence (36.41), and seed yield (33.58). High PCV was observed for leaf length (34.71), leaf width (44.91), number of internodes (40.59), petiole length (36.04), plant height (54.47), length of inflorescence (36.99), and seed yield (34.53). Among the 12 quantitative characters, eight exhibited high heritability (60–99%), four showed moderate heritability, and two had low heritability, with the highest heritability found in the number of internodes (99.38%). Genetic advance as a percentage of the mean was high for leaf length (57.86), leaf width (87.35), number of internodes (83.12), petiole length (71.87), plant height (25.68), number of inflorescences per plant (38.66), length of inflorescence (73.79), and seed yield (67.28), and low for the number of primary branches (3.3), days to 80% maturity (4.87), and seed weight (9.56).

The degree of association between plant characters is crucial for selection, especially in yield, which is influenced by multiple factors. Positive correlation ensures simultaneous improvement in two or more variables, while negative correlation necessitates a compromise between desirable characters. The phenotypic correlation coefficients among different characters are presented in Table 4.

Days to 50% flowering showed a significantly positive correlation with the number of primary branches plant height, and days to 80% maturity, and a negative correlation with leaf length, leaf width, number of internodes, petiole length, length of inflorescence, number of inflorescences per plant, and seed yield. Leaf length was positively correlated with leaf width, number of internodes, petiole length, plant height, length of inflorescence, number of inflorescences, seed weight, and plant height, and negatively correlated with the number of primary branches and days to 80% maturity (Pandya *et al.*, 2015). Leaf width showed a significantly positive correlation with the number of internodes, petiole length, plant height, length of inflorescence, number of inflorescences per plant, seed weight, and seed yield, and a negative correlation with the number of primary branches and days to 80% maturity. The number of internodes showed a significantly positive correlation with petiole length, plant height, length of inflorescence, number of inflorescences per plant, seed weight, and seed yield, and a negative correlation with the number of primary branches and days to 80% maturity. Petiole length was positively correlated with plant height, length of inflorescence, number of inflorescences per plant, seed weight, and seed yield and negatively correlated with the number of primary branches and days to 80% maturity. The number of primary branches

Table 4: Estimation of genotypic (G) and phenotypic (P) correlation coefficient among various characters in quinoa

Characters		*Leaf length (cm)*	*Leaf width (cm)*	*Number of internodes*	*Petiole length (cm)*	*Number of primary branches*	*Plant height (cm)*	*Days to 80% maturity*	*Length of inflorescence (cm)*	*Number of inflorescences per plant*	*10ml tube seed weight (g)*	*Seed yield (q/ha)*
Days to 50% flowering	(G)	-0.800**	-0.796**	-0.862**	-0.715**	0.920**	0.565**	0.578**	-0.438**	-0.571**	0.079	-0.502**
	(P)	-0.654**	-0.694**	-0.778**	-0.649**	0.239*	0.250**	0.433**	-0.389**	-0.403**	0.040	-0.444**
Leaf length (cm)	(G)		0.942**	0.853**	0.845**	-0.800**	0.617**	-0.474**	0.396**	0.816**	0.388**	0.695**
	(P)		0.816**	0.764**	0.759**	-0.288**	0.222*	-0.335**	0.333**	0.552**	0.226*	0.617**
Leaf width (cm)	(G)			0.823**	0.791**	-0.900**	0.738**	-0.497**	0.486**	0.697**	0.330**	0.635**
	(P)			0.790**	0.759**	-0.324**	0.261**	-0.401**	0.459**	0.534**	0.212*	0.599**
Number of internodes	(G)				0.762**	-0.600**	0.774**	-0.333**	0.410**	0.710**	0.209*	0.640**
	(P)				0.745**	-0.373**	0.282**	-0.267**	0.401**	0.553**	0.138	0.621**
Petiole length (cm)	(G)					-1.000**	0.543**	-0.261**	0.455**	0.599**	0.164	0.383**
	(P)					-0.330**	0.201*	-0.216*	0.441**	0.485**	0.117	0.361**
Number of primary branches	(G)						-0.515**	0.551**	-0.871**	1.000**	0.081	-0.618**
	(P)						-0.109	0.031	-0.199*	-0.186	0.140	-0.168
Plant height (cm)	(G)							-0.147	0.858**	0.920**	0.784**	0.899**
	(P)							-0.134	0.303**	0.191*	0.092	0.290**
Days to 80% maturity	(G)								-0.178	-0.439**	0.113	-0.426**
	(P)								-0.119	-0.209*	0.037	-0.298**
Length of inflorescence (cm)	(G)									0.620**	0.094	0.329**
	(P)									0.517**	0.068	0.318**
NO of inflorescences per plant	(G)										0.157	0.668**
	(P)										0.185	0.495**
Seed weight (g/10ml)	(G)											0.511**
	(P)											0.285**

Note: ** at 1% significant, * at 5% significant

Table 5: Genotypic path coefficient analysis for yield and its component characters in quinoa

characters	*Days to 50% flowering*	*Leaf length (cm)*	*Leaf width (cm)*	*Number of internodes*	*Petiole length (cm)*	*Number of primary branches*	*Plant height (cm)*	*Days to 80% maturity*	*Length of inflorescence (cm)*	*Number of inflorescences per plant*	*seed weight (g/10ml)*	*Seed yield (q/ha)*
Days to 50% flowering	-0.768	0.358	0.045	0.240	0.171	-0.210	-0.077	-0.065	0.148	-0.400	0.056	-0.768
Leaf length (cm)	0.615	-0.448	-0.053	-0.238	-0.202	0.174	0.084	0.053	-0.134	0.572	0.272	-0.448
Leaf width (cm)	0.611	-0.422	-0.056	-0.229	-0.189	0.209	0.100	0.056	-0.164	0.488	0.232	-0.056
Number of internodes	0.662	-0.382	-0.046	-0.279	-0.182	0.219	0.105	0.037	-0.138	0.497	0.147	-0.279
Petiole length (cm)	0.549	-0.378	-0.044	-0.213	-0.239	0.224	0.074	0.029	-0.154	0.420	0.115	-0.239
Number of primary branches	-1.158	0.560	0.084	0.438	0.385	-0.139	-0.070	-0.062	0.294	-1.006	0.057	-0.139
Plant height (cm)	0.434	-0.276	-0.041	-0.216	-0.130	0.072	0.136	0.016	-0.290	0.644	0.549	0.139
Days to 80% maturity	-0.443	0.212	0.028	0.093	0.062	-0.077	-0.020	-0.112	0.060	-0.308	0.079	-0.112
Length of inflorescence (cm)	0.336	-0.177	-0.027	-0.114	-0.109	0.121	0.117	0.020	0.337	0.434	0.066	0.337
Number of inflorescences per plant	0.439	-0.366	-0.039	-0.198	-0.143	0.200	0.125	0.049	-0.209	0.700	0.110	0.700
Seed weight (g/10 mL)	-0.061	-0.174	-0.019	-0.058	-0.039	-0.011	0.107	-0.013	-0.032	0.110	0.701	0.701

showed a significantly positive correlation with days to 80% maturity, number of inflorescences per plant, and seed weight, and a negative correlation with plant height, length of inflorescence, and seed yield. Plant height showed a positive correlation with length of inflorescence, number of inflorescences per plant, seed weight, and seed yield, and a negative correlation with days to 80% maturity. Length of inflorescence showed a significant positive correlation with the number of inflorescences per plant, seed weight, and seed yield. The number of inflorescences per plant showed a significantly positive correlation with seed yield. Seed weight (g/10 mL) showed a significantly positive correlation with seed yield.

Path Coefficient Analysis

Path coefficient analysis is crucial for selection criteria as it is challenging to exploit various yield-contributing characters based solely on correlation knowledge. The coefficients generated by path analysis measure the direct and indirect influence of one variable upon another (Table 5). The current analysis indicated that plant height (0.136), length of inflorescence (0.337), number of inflorescences per plant (0.700), and seed weight (g/10 mL) (0.701) had a direct positive effect on seed yield. In contrast, days to 50% flowering (-0.769), leaf length (-0.448), leaf width (-0.056), number of internodes (-0.279), petiole length (-0.139), and days to 80% maturity (-0.112) had a direct negative effect on seed yield. Characters with the highest significant positive direct effect on seed yield were the number of inflorescences and seed weight, while plant height and length of inflorescence had comparatively less significant direct positive effects. The trait days to 50% flowering showed the highest significant direct negative effect, while leaf length, number of internodes, petiole length, and number of primary branches had moderate direct effects. Leaf width exhibited a negative direct and negligible influence on seed yield. Similar results have been reported by Singh and Yadav (1985), and Singh *et al.* (1998). Our study also revealed significant variation in key traits such as days to 50% flowering, leaf length, leaf width, number of internodes, petiole length, plant height, days to 80% maturity, length of inflorescence, number of inflorescences per plant, and seed yield. These findings align with those of Wu *et al*. (2020), who also observed substantial phenotypic diversity in quinoa germplasm, suggesting that these traits are crucial for selection in breeding programs. High genotypic and phenotypic coefficients of variation were noted for several traits, indicating their high heritability and the potential for effective selection, as corroborated by Nowak *et al.* (2022).

Path coefficient analysis demonstrated that traits like plant height, length of inflorescence, number of inflorescences per plant, and seed weight had direct positive effects on seed yield, while days to 50% flowering and other traits had negative effects. These results are consistent

with the findings of Bazile *et al.* (2016), who emphasized the importance of these traits in enhancing yield through breeding strategies. The significant positive correlations observed among yield-contributing traits suggest that simultaneous selection for these traits can lead to yield improvements.

The extensive genetic variability observed in both morphological and molecular traits underscores quinoa's potential for breeding improvement. The high heritability of key traits indicates that selective breeding can effectively enhance these characteristics, contributing to improved yield and agronomic performance. Recent studies by Murphy *et al.* (2018) and Danielsen *et al.* (2019) support the notion that exploiting genetic diversity through molecular markers and morphological selection can lead to significant advancements in quinoa breeding.

Molecular Diversity

To analyze molecular diversity, PCR amplification of all 36 quinoa genotypes was performed using 16 inter-simple sequence repeat (ISSR) markers (Ana-cuz *et al*., 2017; Christensen *et al*., 2007). The Polymorphism Information Content (PIC) value for 12 ISSR markers ranged from 0.274 (UBC841) to 0.797 (UBC840), with an average of 0.56 (Table 6). About 16 ISSR markers were amplified, of which twelve were polymorphic. A total of 32 alleles were obtained from these 12 markers: seven markers produced two alleles, three markers produced three alleles, one marker produced four alleles, and one marker produced five alleles (Figure 3). The 12 markers produced 921 bands across the 36 genotypes, 517 of which were polymorphic (56.1%). Based on the level of polymorphism, two ISSR markers (UBC840 and UBC835) were identified as effective primers for high amplification. Marker alleles were converted into binary scores based on their presence (1) or absence (0). UPGMA cluster analysis, using Jaccard's similarity coefficient matrices calculated from ISSR markers, generated a dendrogram for the 36 varieties.

The varieties were grouped into two major clusters, Cluster I and Cluster II, with a similarity coefficient of 64%, as revealed by the dendrogram depicted in Figure 2. Cluster

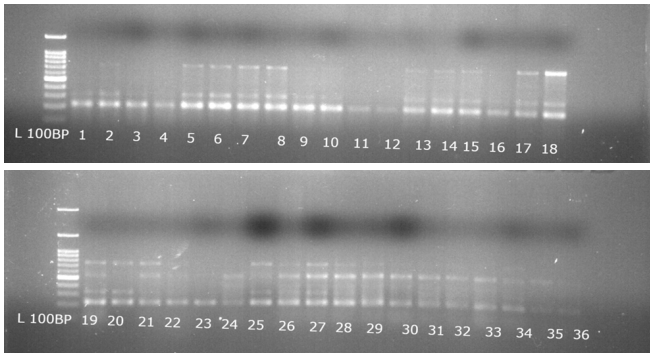

Figure 3: PCR amplification of 36 accessions of quinoa with ISSR primer UGC835.

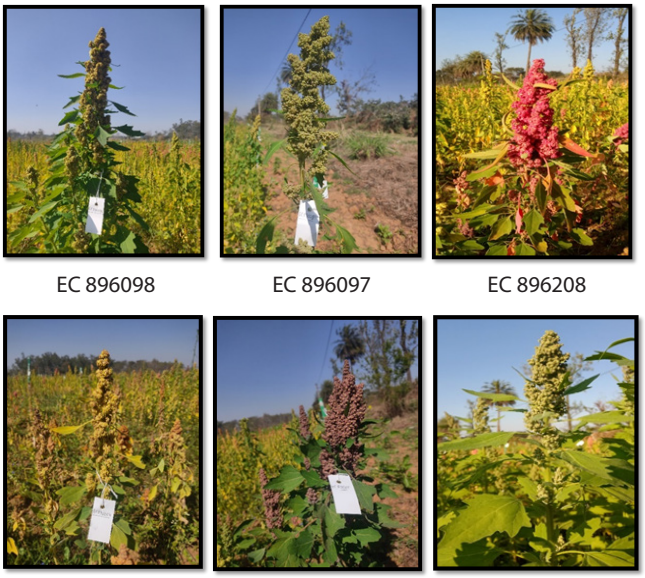

Figure 4: Variation in spikelet color in various important quinoa genotypes.

I is further divided into two sub-clusters, 'Ia' and 'Ib,' with a similarity coefficient of 68% and a maximum similarity rate of 91% between genotypes ACQS8 and ACQS9. Sub-cluster 'Ia' consists of genotypes SHQ2, SHQ5, ACQS1, ACQS4, ACQS8, ACQS9, ACQS2, ACQS3, ACQS7, EC 896098, EC 896064, EC 896062, and ACQS10, while Cluster 'Ib' comprises ACQS6, EC 896213, EC 896237, EC 896246, and ACQS5. Cluster II is divided into two sub-clusters, 'IIa' and 'IIb,' with a similarity coefficient range of 68% and a maximum similarity rate of 94% between IGKVC-12 and Him Shakti, indicating that the accessions in these clusters were genetically less diverse and had almost the same genetic makeup. This narrow range of genetic variability within the clusters has also been reported by other authors (Chandirakala and Manivannan,

Table 6: Frequency of alleles by 12 ISSR markers in 36 germplasm lines (included checks) of quinoa

S. N.	*Issr markers*	*Number of allele*	*Allele freqency*	*Pic value*
1	UBC810	2	0.701	0.299
2	UBC815	2	0.499	0.501
3	UBC824	3	0.568	0.431
4	UBC840	5	0.203	0.797
5	UBC841	2	0.726	0.274
6	UBC842	2	0.135	0.865
7	UBC884	3	0.336	0.664
8	UBC885	3	0.369	0.631
9	UBC808	2	0.318	0.682
10	UBC809	2	0.538	0.462
11	UBC835	4	0.257	0.743
12	UBC836	2	0.513	0.487

2014; Srinivas *et al*., 2006). Cluster 'IIa' consists of EC 896275, IGKVC-12, Him Shakti, EC 896219, SHQ3, EC 896115, EC 896109, and EC 896218, while Cluster 'IIb' comprises EC 896276, EC 896201, EC 896208, EC 896210, EC 896079, SHQ4, EC 896069, EC 896065, and SHQ4.

The use of ISSR markers in our study revealed considerable molecular diversity among the quinoa genotypes, with 12 polymorphic markers showing high levels of polymorphism. This genetic variability is essential for breeding programs aiming to improve quinoa's agronomic performance and stress tolerance. The polymorphism information content (PIC) values obtained in our study are comparable to those reported by Zhang *et al.* (2017), who highlighted the effectiveness of ISSR markers in assessing genetic diversity in quinoa.

Cluster analysis grouped the genotypes into two major clusters, indicating varying degrees of genetic similarity. This clustering is similar to the genetic diversity patterns reported by Jarvis *et al.* (2021), who found that quinoa accessions could be categorized into distinct genetic groups based on their molecular profiles. The identification of genetically diverse clusters in our study provides a basis for selecting parents with desirable traits for hybridization, potentially maximizing heterosis and genetic gain.

Conclusion

Quinoa showed wide variation for selected traits along with high heritability. Therefore, it is concluded that the characters that showed high genotypic value coupled with high heritability and genetic advance should be considered for direct selection, so there is ample scope for improvement of yield and other associated characters especially plant height and seed weight. These traits should be used while selecting elite genotypes of quinoa. Morphological descriptors can be utilized effectively for identifying and categorizing germplasm lines, but that may or may not be sufficient for characterization requirements. So, several other markers/descriptors should be examined in addition to the morphological descriptor. ISSR markers used in this study were evaluated for their capacity to provide distinct DNA profiles on quinoa genotypes. If molecular markers are used as additional descriptors, they will improve the informativeness of morphological characters. ISSR markers can be used to efficiently generate locus-specific allele information, which can then be used to generate molecular IDs for 36 quinoa germplasms. In comparison to traditional breeding methods or morphological characterization, molecular characterization can be used effectively in assessing the increase of any particular character while saving time, resources, and energy.

Authors Contribution

Conceptualization of research (JK); Contribution of experimental materials (JK, HL); Execution of field/lab experiments and data collection (AB, JK, TP); Preparation of manuscript (JK, AB); Analysis of data and interpretation (JK, AB)

Conflict of Interest

The authors declare no conflict of interest.

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