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Morphometric Analysis for Intraspecific Diversity of *Oryza sativa* cv. 'Shamim' Populations (Morphotypes) from Southern Iran

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ABSTRACT

Understanding morphometric diversity in rice (*Oryza sativa* L.) is essential for improving genetic resources, enhancing breeding efficiency, and supporting conservation strategies. This study examines intraspecific and inter-population morphological variation in *Oryza sativa* cv. "Shamim" from 15 populations across southern Iran, in Fars (Rostam) and Kohgiluyeh and Boyer-Ahmad (Basht) regions. A total of 150 specimens (10 per population) were sampled in mid-November, with whole plants collected as herbarium vouchers. Fourteen quantitative traits, spikelet number, seed weight, awn length, panicle traits, culm length, blade dimensions, plant height, internode metrics, grain length, and width, plus eight qualitative traits, were measured using standard procedures. Habitat factors like altitude, soil pH, and electrical conductivity varied significantly, driving phenotypic differences. Correlation analyses showed strong links among traits: spikelet number positively correlated with ligule length, culm length, blade length, plant height, panicle length, and weight; awn length negatively correlated with internode number. The strongest correlation was between culm and plant height, indicating integrated stature. PCA and UPGMA clustering grouped the 15 populations into four clusters: first (P02, P10, P07, P03, P04, P12, P01, P13) with low genetic distance; second (P05, P09, P06, P15, P08); and independent (P14, P11) showing greater divergence. Key differentiating traits included spikelet number, seed and panicle weight, awn and panicle length, glume length, culm and blade dimensions, plant height, internode number, and grain attributes. Results highlight substantial diversity in Iranian "Shamim" populations, likely due to environmental variation and adaptation. These findings aid in selecting divergent parents for breeding to boost yield, adaptability, and conserve local rice resources.

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Introduction

Rice is one of the primary foods for much of the world and the main source of protein and calories in Asia (Aesomnuk *et al.*, 2021), with over 90% of global production (Farahzadi *et al.*, 2020). As the world population increases, the need for food and rice production will increase; on the other hand, the presence of biotic and abiotic stresses, especially drought stress, has limited the possibility of increasing the cultivated area of rice, hence, efforts to improve genetic improvement are inevitable for cereal and rice, especially (Zare *et al.*, 2019). Knowledge of population structure and genetic diversity is crucial for improving crop economic value (Badu-Apraku *et al.*, 2021), especially since rice has experienced a loss of genetic diversity in recent centuries. Rice genetic resources serve as primary breeding material and contribute directly to food security and economic development (Konak *et al.*, 2021). The population structure of domesticated species is influenced by the history of predomesticated populations (morphotypes), breeding systems, and human breeding practices (Garris *et al.*, 2005). Knowledge of genetic variation and relationships among genotypes is essential for designing effective breeding and conservation strategies (Begna, 2021). Population genetic analysis of local rice landraces helps understand the interaction between rice diversity and human cultivation practices, as cultivar structure results from adaptation to local environments and farmer selection (Roy *et al.*, 2015; 2021; Liu *et al.*, 2021). Global studies, though informative, often include few cultivars from a region and cannot fully capture local diversity. Wild rice populations (morphotypes), in contrast to cultivated ones, evolve primarily under natural selection and often show more stable geographic distributions, with studies in China and Guinea revealing differentiation within and between local populations (morphotypes) (Xu *et al.*, 2006; Barry *et al.*, 2007). A study demonstrated that morphometric diversity in Iranian rice genotypes can be identified using physicochemical traits and molecular markers. This research emphasized the importance of conserving and utilizing local genetic resources in breeding programs (Safiedin Ardebili *et al.*, 2024). In 2025, a study investigated the genetic diversity of Iranian rice cultivars using microsatellite (SSR) markers. The results can be valuable for selecting suitable parents in breeding programs aimed at blast disease resistance (Farahzadi *et al.*, 2020). Another study in 2025 evaluated genetic diversity in rice genotypes using molecular markers, showing high within-population diversity (Khazaie *et al.*, 2025). A 2024 study applied 3D models for in-field phenotyping of rice panicles to assess morphometric traits under natural conditions, which can improve the accuracy of trait evaluation (Yang *et al.*, 2024). Over generations, rice has become central to the cultures and customs of Asian rice-growing communities, yet the effects of farmer practices on the preservation, exchange, and evolution of landraces remain underexplored (Thomson *et al.*, 2009). This knowledge is essential for conservation and breeding programs, and therefore, this study aimed to quantify morphometric diversity among *Oryza sativa* populations in Iran.

Materials and Methods

Sampling

In this study, sampling was conducted in mid-November on farms located in Fars province (Rostam city) and Kohgiluyeh and Boyer-Ahmad (Basht city). The populations (morphotypes) were from 15 regions, and 15 samples were collected from each region (Table 1). For the morphometric studies, entire plants were collected, dried, and mounted as herbarium specimens. Voucher specimens are deposited in the Islamic Azad University herbarium (IAUH).

Studied traits

Morphological traits were measured on all 150 herbarium specimens using loops and calipers. Quantitative traits were evaluated using a caliper and qualitative traits were evaluated using a loop and coded into different states. The studied traits in this research included quantitative and qualitative properties. The quantities properties were spikelet number (SN), seed weight (SW), panicle weight (g) (PW), awn length (mm) (AL), panicle length(mm) (LL), length of glumes (LG), culm length(mm) (UL), blade length(mm) (BL), blade width(mm) (BW), plant height(mm) (PH), number of internodes (NI), last inthernode length (LI), grain length(mm) (GL), grain width(mm) (GW) and the qualitative traits were grain color (GC): creamy white (0) and pearl white (1); awn presence (AP): fully awned (0), partly owned (1), terminally awned (2) and awnless (3); color of glumes (CG): colorless (0) and straw (1); marginal of ligule (ML): glabrous (0) and ciliate (1); panicle type (PT): open (0), compact (1) and intermediate (2); panicle form (PF): equilateral (paired branching) (0) and unilateral (one-sided branching) (1); awn color (AC): straw (0), gold (1), pink (2) and brown (3); hulls color (HC): straw (0), gold (1) and tawny (2).

For more accurate estimation of inter-population and intraspecific variations, all analyses of the morphological data were conducted in SPSSSPSS version. 23 (IBM). The correlation of morphological characters and habitat traits was measured. Chi-square test and Pearson's coefficient of contingency were used (Antonius 2003).

Data analysis

The Analyses were performed using SAS 9.2 software (SAS Institute, Cary, NC). Also, PCA and UPGMA clustering were performed using SPSS 19 software, and Duncan's multiple-range test was performed at 5% statistically level for mean comparison.

Result and Discussion

Habitat characteristics

Altitude, pH, EC, DO, and the salinity of habitats were measured (Table 2). The Soil pH ranged from 6.7 at population P01 to 7.98 at P06. Based on the pH, populations (morphotypes) P05, P10, P13, and P15 occur on slightly alkaline soils (pH 7.5 to 7.9). The P02, P03, P07, P08, P11, P12, and P14 populations (morphotypes) occur on the neutral range (pH= 7 to 7.4). Also, the lowest and highest values of EC ranged from 0.17 μS cm^{-1} at P10 to 1.93 μS cm^{-1} at P03. All sampling populations (morphotypes) were located below 1000 m, except for the P01 location. The altitude for P01 was 1062 m above sea level.

Table 1. Habitat characteristics of *Oryza sativa* cv. ‘Shamim’ populations (morphotypes) in Southern Iran.

Locality	Field No.	pH of soil	EC of soil (μS.cm^{-1})	Altitude (m)	Leaf Length(cm)	Panicle Length (cm)	Habitat
P01	FRS01	6.7	0.28	1062	28	9	GC
P02	FRS02	7.37	0.35	841	34	14	CL
P03	FRS03	7.31	1.93	815	21	45	CG- GM
P04	FRS04	6.95	0.47	849	30	10	GC
P05	KB05	7.52	0.41	803	33	12	CL
P06	FRS06	7.98	0.36	873	25	8	SC
P07	FRS07	7.04	0.76	871	21	5	CG- GM
P08	FRS08	7.36	0.26	786	26	8	GP- GM
P09	FRS09	7.46	0.24	802	23	7	GC- GM
P10	FRS10	7.73	0.17	780	25	7	GM
P11	FRS11	7.27	0.42	794	29	9	GC
P12	FRS12	7.33	0.31	787	27	8	GC
P13	FRS13	7.71	0.56	915	23	7	GP-GC
P14	FRS14	7.16	0.90	783	31	11	CL
P15	FRS15	7.65	0.21	787	27	8	SC

Table 2. Origin of *Oryza sativa* cv: “shamim” Slocality and populations (morphotypes) with their herbarium code. For each population, a voucher specimen is deposited at the IAUH herbarium.

Field No.	Province	City	Locality	Altitude(m)	Latitude	Longitude	Coll Date*
FRS01	Fars	Rostam	Faryab village,	1062	30° 28′ 17.634″	51° 19′ 42.924″	2017/Oct/7
FRS02	Fars	Rostam	Khong Taheri village	841	30° 18′ 4.397″	51° 25′ 55.228″	2017/Oct/5
FRS03	Fars	Rostam	Kul Koochak, Dozak village	815	30° 18′ 7.914″	51° 23′ 50.134″	2017/Oct/5
FRS04	Fars	Rostam	Hossein Abad village	849	30° 23′ 54.258″	51° 20′ 1.239″	2017/Oct/8
KB05	K&B-A*	Basht	next to Bonyadi poultry house	803	30° 21′ 30.433″	51° 9′ 7.888″	2017/Oct/1
FRS06	Fars	Rostam	Tol Pir village	873	30° 16′ 13.259″	51° 29’ 56.874″	2017/Sep/28
FRS07	Fars	Rostam	between Babameidan and Masiri	871	30° 16’ 9.262″	51° 29′ 58.423″	2017/Sep/28
FRS08	Fars	Rostam	Arab Khakak village	786	30° 20′ 26.540″	51° 17′ 41.33″	2017/Oct/8
FRS09	Fars	Rostam	Dehnou Mogomi's village	802	30° 19′ 6.165″	51° 23′ 19.265″	2017/Oct/7
FRS10	Fars	Rostam	Kupen village	780	30° 19′ 58.096″	51° 16′ 13.151″	2017/Oct/7
FRS11	Fars	Rostam	Dehnou Markazi village	794	30° 19′ 57.665″	51° 22′ 42.927″	2017/Oct/5
FRS12	Fars	Rostam	Karamabad village	787	30° 20′ 58.549″	51° 21′ 39.461″	2017/Oct/4
FRS13	Fars	Rostam	next to Emamzadeh, Konareh village	915	30° 25′ 8.307″	51° 21′ 8.307″	2017/Oct/6
FRS14	Fars	Rostam	next to the village, Konareh village	783	30° 20′56.245″	51° 17′ 12.854″	2017/Oct/6
FRS15	Fars	Rostam	Dehnou Torkal village	787	30° 19′12.273″	51° 21′ 12.273″	2017/Oct/13

* K&B-A= Kohgiluyeh and Boyer-Ahmad; ** Coll Date = Collection Date

Morphometric analyses

Table 3 shows the Pearson correlation between the studied traits of *Oryza sativa* cv. “Shamim.” There were significant correlations between spikelet number and ligule length (0.811), culm length (0.55), blade length (0.75), plant height (0.58), number of internodes (0.52), panicle length (0.54), and panicle weight (0.88). The awn length had a significant negative correlation with the number of internodes (-0.52). In addition, ligule length showed significant correlations with culm length (0.67), blade length (0.53), plant height (0.64), and panicle weight (0.71). The highest positive correlation was found between culm length and plant height (0.95), and the highest negative correlation was observed between grain length and grain width (-0.68) (Table 3). At this order, Kumar *et al.* (2022) evaluated correlation analysis for yield- and quality-contributing characters in rice (*Oryza sativa* L.) genotypes and reported that the highest positive significant genotypic and phenotypic correlation coefficient was observed between grain yield per plot and grain yield per plant (0.98), followed by days to flowering and days to maturity (0.96), grain length and decorticated grain length (0.93), grain breadth and decorticated grain breadth (0.93), The grain length was positively correlated with decorticated grain length (0.93) and decorticated grain length-breadth ratio (0.93), suggesting that these traits could be used as selection criteria for improving both grain yield and quality. In another report, Liu *et al*. (2021) investigated geographical adaptation to soil nitrogen in rice and noted that high tillering response in modern rice cultivars boosts grain yield and nitrogen-use efficiency under low or moderate nitrogen levels, demonstrating substantial potential for rice breeding and the amelioration of negative environmental effects by reducing nitrogen application.

Recent studies have further highlighted the importance of trait correlations in rice breeding; in this regard, the present research also demonstrated that there was variation of morphometric traits in different locals. It was reported that significant positive associations between grain yield per plant and traits such as biological yield per plant, panicle-bearing tillers per plant, spikelets per panicle, grains per panicle, and chlorophyll content under sodic soil conditions, emphasizing their role as reliable selection criteria (Shrivastav and Verma, 2023). Moreover, Yang *et al.* (2024) developed PanicleNeRF, a smartphone-based phenotyping method, and found strong correlations between 3D-measured panicle traits and grain number and mass, offering a practical tool for large-scale evaluation of morphometric traits in breeding programs.

Character associations have been reported to vary with environmental conditions; hence, studying these associations under target environments helps breeders establish proper selection criteria for grain yield in germplasm lines, ensuring that selected lines possess the desired combination of traits.

PCA analysis and clustering analysis

The PCA analysis was performed based on the quantitative morphological traits. The Scree test criterion determines the number of suitable significant factors for analyzing and interpreting the PCA. UPGMA clustering analysis of morphotypes data separated the 15 populations (morphotypes) into four main groups (Fig. 1). According to clustering, it was found that the populations (morphotypes) of P02, P10, P07, P03, P04, P12, P01, and P13 were in one Cluster; the second Cluster included P05, P09, P06, P15, and P08. Two locations were in separate clusters: P14 and P11 (Fig. 1). This suggests that the morphometric analysis used to distinguish the subspecies is indeed important morphological characters to delimit the intraspecific morphological variation. Apart from habitat factors, our analysis of morphological characters also categorized individuals into separate *Oryza sativa* cv. “Shamim” subspecies populations (morphotypes) rather than according to subspecies. However, subspecies in *Oryza sativa* cv. “Shamim” is considered a group of populations (morphotypes) of homogeneous morphology, separated from other groups of populations (morphotypes) by consistent morphological characters and by geography. Therefore, we consider them in the following as morphotypes rather than subspecies. Differences in spikelet number (SN), seed weight (SW), Panicle Weight (PW), Awn Length (AL), Panicle Length (PL), Length of Glumes (LG), Culm Length (CL), Blade Length (BL), Blade Width (BW), Plant Height (PH), Number of Internodes (NI), Last Internode Length (LI), Grain Length (GL), and Grain Width (GW) were most important to predict the overall variation and diversity of *Oryza sativa* cv. “Shamim” populations (morphotypes) (Table 4). Previous PCA and clustering studies have similarly identified that characters like days to reach maturity, panicle length, and spikelets per panicle directly influenced grain yield and are important contributors in selecting traits for breeding programs (Jeke *et al.*, 2021).

Table 3. Pearson correlation between studied traits of *Oryza sativa* cv: “shamim”

	NS	**AL**	**LL**	**UL**	**BL**	**BW**	**PH**	**NI**	**LI**	**GL**	**GW**	**LG**	**SW**	**PL**	**PW**
NS	1														
AL	-0.259	1													
LL	0.811**	0.036	1												
UL	0.553*	0.287	0.670**	1											
BL	0.751**	-0.344	0.528*	0.597*	1										
BW	0.364	0.025	0.414	0.250	0.242	1									
PH	0.578*	0.357	0.636*	0.950**	0.511	0.202	1								
NI	0.524*	-0.550*	0.453	0.363	0.634*	0.467	0.237	1							
LI	-0.112	0.385	0.067	0.450	0.096	0.565*	0.330	0.360	1						
GL	-0.191	-0.217	-0.269	-0.240	-0.133	-0.586*	-0.223	-0.139	-0.503	1					
GW	-.0043	0.317	0.129	0.143	0.109	0.310	0.055	-0.027	0.503	-0.683**	1				
LG	-0.328	0.114	-0.431	-0.072	-0.124	-0.487	0.000	-0.384	-0.223	0.236	-0.097	1			
WS	0.400	-0.196	0.344	0.163	0.389	0.007	0.124	0.324	-0.012	-0.026	0.402	0.161	1		
PL	0.543*	0.063	0.247	0.405	0.330	-0.142	0.598*	-0.105	-0.364	0.229	-0.508	0.214	-0.060	1	
PW	0.886**	-0.250	0.709**	0.491	0.649**	0.270	0.505	0.516*	0.012	-0.242	0.202	-0.354	0.633*	0.360	1

*and ** show significant value at 5 and 1% statistically levels; spikelet number (SN), seed weight (SW), panicle weight (g) (PW), awn length (mm) (AL), panicle length(mm) (LL), length of glumes (LG), culm length(mm) (UL), blade length(mm) (BL), blade width(mm) (BW), plant height(mm) (PH), number of internodes (NI), last inthernode length (LI), grain length(mm) (GL), grain width(mm) (GW).

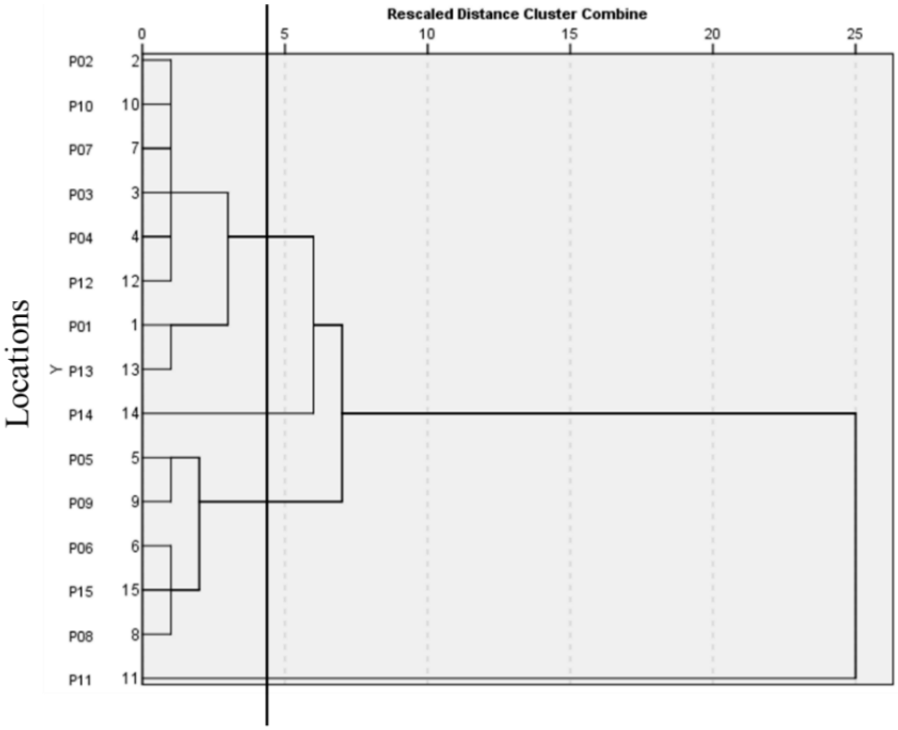

Fig. 1. Comparison of the relative contribution of morphotypes of *Oryza sativa* cv: Shamim" subspecies in the populations (morphotypes) with UPGMA clustering analysis.

Table 4. Distribution of quantitative variables in six main factors in PCA analysis. Measures ± 0.5 are shown only.

Variables	1	2	3	4	5	6
spikelet number	0.865	.348		-.134	-.204	
Awn Length			.746			
Ligule Length	0.837					
Culm Length	0.764					
Blade Length	0.759					
Blade Width		-.509		-.509		
Plant Height	0.732					
Number of Internodes	0.583					
Last inthernod length		-.702				
Grain length		.627				
Grain width		-.790				
Length of glumes				.625		
Weight seed	0.536			.606		
Panicle Length		.779				
Panicle Weight	0.885					
PL			.584		-.592	
LL						-.740
Altitude			-.576			
EC			.657		-.552	
pH	-0.593			-.582		

Similarly, recent studies by Ahmed *et al*. (2023) have confirmed that morphometric traits such as panicle length, culm length, and spikelet number show strong correlation with grain yield in diverse rice populations (morphotypes), emphasizing their importance for PCA-based diversity analysis. Lasalita-Zapico *et al.* (2010) reported that the relative discriminating power of the two principal components, as inferred from PCA analysis, was high for PCA 1 (6.7) and quite low for PCA 2 (1.7). The first principal component was correlated with plant height, culm diameter, panicle length, leaf length, leaf width, leaf area, and culm length. Only the flag leaf angle made a substantial contribution to the second component. Garris *et al.* (2005), in the evaluation of diversity in Oryza sativa, mentioned that five distinct groups were detected, corresponding to indica, aus, aromatic, temperate japonica, and tropical japonica rices. Nuclear and chloroplast data support a closer evolutionary relationship between the indica and the aus, and among the tropical japonica, temperate japonica, and aromatic groups. Zhang *et al.* (2011) mentioned that the differentiation within the indica or japonica populations (morphotypes) was different: japonica presented clearer differentiation between soil-watery ecotypes than indica, and indica presented clearer differentiation between seasonal ecotypes than japonica.

A similar finding has been noted in rice accessions collected in Guinea, where two generic names designating small- and long-grain varieties also showed low genetic consistency across accessions (Barry *et al.*, 2007). More recent PCA and clustering studies in 2023-2025 by Li *et al.* (2023) demonstrated that combining morphometric and molecular markers improves the resolution of intraspecific population structure in rice, allowing more precise grouping and identification of high-yielding morphotypes.

Conclusion

Rice populations (morphotypes) may provide the genetic diversity needed to diversify the depauperate gene pool of improved rice varieties. So, the selection of parents with genetic distance led to a successful breeding program. In this research, it was concluded that the populations (morphotypes) of P02, P10, P07, P03, P04, P12, and P1 and P13 were in one group, so these populations (morphotypes) have low genetic distance, and the second group included P05, P09, P06, P15, and P08. Also, Differences in the spikelet number, seed weight, Panicle Weight, Awn Length, and Panicle Length had the highest proportion in diversity, so these traits were most important to predict the overall variation and diversity of *Oryza sativa* cv: Shamim” subspecies populations (morphotypes).

Conflict of interests

The authors declare that they have no conflict of interest.

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