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Unveiling Genetic Variation in Garlic Genotypes in Response to Rust Disease Using RAPD Markers

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Abstract

Garlic (*Allium sativum*), cultivated worldwide for its medicinal and nutritional value, faces challenges due to diseases caused by various pathogens. In this study, eleven garlic genotypes from Iran and one from China were selected and sown under natural infection

rendered by the rust fungus (*Puccinia alli*) over two consecutive years. Subsequently, disease distribution and severity, percentage of infection and susceptibility of different garlic genotypes to rust disease were investigated. The results showed that genotype Solan was the most susceptible, with disease severity of 30.81%. In comparison, genotypes Aliabad and Bahar were resistant against rust disease with the lowest infection percentages of 13% and 16.37%, respectively. Further, genetic diversity was assessed using random-amplified polymorphic DNA (RAPD) markers. Out of 10 primers used, 95 scorable bands were generated, of which 66 (69.48%) were found to be polymorphic. A dendrogram was constructed based on RAPD polymorphism using the UPGMA method, and the genotypes were separated into six distinct clusters based on Jaccard's coefficient of similarity. Additionally, it was observed that there is no genetic differentiation among the genotypes based on their geographical origin. This study highlights the significant diversity in resistance and susceptibility among garlic genotypes, which can be harnessed in plant breeding programs.

Keywords
Allium sativum; polymorphism; RAPD; resistance; susceptibility; *Puccinia alli*

1. Introduction

Garlic (*Allium sativum*) is the second most significant crop in Allium. This bulb vegetable crop is next to onion as an essential nutritional crop grown in many countries around the globe [1]. Garlic production worldwide is more than 28 million tons per year, which has grown significantly in the last decade, with China alone accounting for 74% of the total world production [2]. Despite the agricultural potential in Iran, a scarcity of garlic persists in the market, primarily attributed to the widespread occurrence of plant diseases. The incidence of garlic diseases has reached 100% in some regions of cultivation in Iran [3].

Plant diseases are one of the significant constraints on crop production. Of various plant pathogens, fungi are of particular importance [4-7]. Despite using protective fungicides, garlic, like many other crops, is infected by some pathogens, particularly fungi [8, 9]. One of the most devastating diseases of garlic is rust disease, caused by the fungus *Puccinia Alli*. It significantly threatens garlic plants during the growing season [10]. Early rust symptoms appear as tiny, white specks on the leaves and stem that develop into a circular or elongated posture. In severe cases when conditions are conducive, leaves turn yellow, wither, and die, leading to substantial yield losses. The disease spreads in the field by wind-blown spores [11].

Resistant genotypes are valuable genetic resources that can improve crop yield [12-14]. Identifying and using resistant genotypes is one of the essential ways to manage and control disease epidemics and reduce crop damage. In this case, pesticide application is reduced, and the environment and human health are preserved [15].

The growing significance of garlic in commercial, medicinal, and industrial contexts has led to a surging interest in enhancing production, performance, and the development of novel genotypes. Molecular markers can screen and identify resistant and susceptible genotypes even at early

growth stages [16]. Selection for resistant genotypes based on phenotype is a time-consuming process in plant breeding, whereas molecular markers allow for the direct selection of favorable genotypes [17]. The use of molecular markers and the concept of polymorphism in systematic studies to determine the relationships between organisms, geographic distribution, speciation studies, genetic drift, genetic diversity, and the interpretation of population structure have received much attention in recent decades [18]. This is because the accuracy of phylogenetic information can play a significant role in the successful advancement of ecological research, preparation, protection, and maintenance of gene banks and breeding programs [19].

It is estimated that a 10% error in phylogenetics classification can reduce the genetic progress of a breeding program by three to four percent [20]. A group of diagnostic markers that can distinguish homogenous species from each other can be found among the repetitive sequences of genomes. Among conventional markers, random amplified polymorphic DNA (RAPD) is one of the most widely used markers due to its uniform distribution in the whole genome and its generality among different organisms [21]. In addition, the RAPD procedure is simple, requires a meager amount of DNA for analysis, costs less, and is faster and more accessible technology than amplified fragment length polymorphism (AFLP) or simple sequence repeat (SSR) analysis. RAPD markers are recommended for selecting resistant cultivars [16, 22].

The comprehensive evaluation of garlic genotypes in this study suggests that the selected genotypes have high genetic variability in rust disease resistance. RAPD markers are expected to reveal unique genetic profiles in genotypes, especially resistant ones. Additionally, using RAPD markers to characterize these garlic genotypes may help manage and control rust disease in garlic fields by selecting resistant cultivars more efficiently and precisely. These molecular markers should also speed up breeding and help create rust-resistant garlic varieties.

This study aimed to elucidate the genetic basis of rust resistance in garlic breeding programs to improve garlic production and reduce dependence on protective fungicides in disease management.

2. Materials and Methods

2.1 Plant Materials

Eleven local Iranian garlic genotypes (Gandagh, Barfgin, Toein, Aliabad, Maryang, Moein, Bahar, Solan, Toyserkan, Hydare, Shorin) and one Chinese garlic genotype were obtained from the Plant Pathology Collection of the Agriculture and Natural Resources Research Center, Esfahan, Iran. Garlic is cultured as a winter crop between September and October and harvested from April to May the following year. A greenhouse experiment was laid out at the Agriculture and Natural Resources Research Center, Esfahan, Iran (51°34' E; 32°36' N) at 1500 meters above sea level during the 2020 growing season. Garlic genotypes were planted in pots containing pasteurized soil and maintained under greenhouse conditions. Bulbs were harvested at physiological maturity when the leaves fell and 70% dried. Each treatment was replicated five times in a randomized complete block design.

3. Disease Assessment

3.1 Frequency of Infection

The number of healthy and infected plants was meticulously counted independently to evaluate the frequency of rust fungus infection among the genotypes Negash *et al*. described [23]. The ratio of the total number of infected plants to the total number of samples was calculated as the percentage of plants infected with rust disease. The frequency of infection was calculated using the following formula:

$$\mathrm{DF}\ (\%) = \frac{\sum_{i=2}^{N} R_i}{N} \times 100$$

Where R: Total number of infected plants; N: Total number of plants.

3.2 Disease Severity

Disease severity was recorded on 10 bulbs from each phenotype after the appearance of the disease symptom. Symptoms (rust postulates) in each bulb sample were scored based on disease categories (0, 5, 10, 25, 50, 75, and 100), which corresponded to the estimated percentage of lesions in the entire disease area (0, 1, 2, 4, 8, 16 and above 32), respectively [24]. A score of zero represents the most resistant phenotype, whereas a score of 100 represents the most susceptible one. The scores were converted to a susceptibility index:

SI = [(Sum of grade value × Number of bulbs in that grade)/(Total bulb number × Highest grade value)] ×100.

$$\mathrm{DS} = \frac{\sum_{i=2}^{N} R_i . S_i}{N} \times 100$$

The average SI across the two-year study was used as the final SI for each genotype. Based on this, the resistance level of each genotype was classified into five classes based on its SI: Immune (IS), SI = 0, class 1; Highly resistant (HR), SI = 0.1-0.5, class 2; Resistant (R), SI = 5.1-25, class 3; Susceptible (S), SI = 25-50, class 4; Highly susceptible (HS), SI = 50-100, class 5 [25, 26].

3.3 DNA Extraction

Total genomic DNA was extracted from dried leaf tissue following the standard CTAB (cetyltrimethylammonium bromide) method described by Doyle and Doyle [27]. Leaves (100 mg) were pulverized in liquid nitrogen, then homogenized in 25 ml of extraction buffer (2% CTAB, 20 mM EDTA, 2% PVP, 1.4 M NaCl, 100 mM Tris-HCl, pH 8.0, and 1% β-mercaptoethanol) containing 4 µl RNAse and incubated at 65°C for 1 h. The supernatant was extracted twice with chloroform: isoamyl alcohol (24:1 v/v). RNase was again added, and the mixture was incubated at room temperature for 30 minutes. The DNA was pelleted with chilled isopropanol, washed twice with 70% ethanol, air-dried, dissolved in 500 mL TE buffer, and stored at -20°C. DNA quality and quantity were evaluated using a NanoDrop spectrometer and 2% (w/v) agarose gel electrophoresis [28].

3.4 RAPD Amplification

The genomic DNA was subjected to polymerase chain reaction (PCR) amplification using RAPD markers. The PCR was run with 20 RAPD primers in a 25 µL total volume, containing 500 mM KCl, 100 mM Tris-HCl, pH 9.0, 1% Triton X-100, 1.5 mM $MgCl_2$, 0.2 mM each dNTP, 0.15 µM primer, 45 ng genomic DNA, and 1 U Taq DNA polymerase (Bioneer Company, Seoul, South Korea). The amplification was performed in a DNA thermal cycler with pre-denaturation set at 95°C for 5 min followed by 45 cycles of 1 min at 95°C, 1 min at 36°C, and 2 min at 72°C, with a final extension step at 72°C for 7 min [29]. The amplification products were resolved by electrophoresis on 2% (w/v) agarose gel containing 0.5 µg/mL ethidium bromide in 1× TAE buffer (40 mM Tris-acetate, pH 8.0, 1 mM EDTA) at 120 V for 1 h. In all cases, 1 kb DNA ladder was utilized as the size marker. The gel was examined and photographed on a UV transilluminator. All reactions were repeated at least three times [30, 31].

3.5 Statistical Analysis

Each treatment was prepared in three replicates, and the experiment was repeated twice. All statistical analysis was performed using SAS 9.1 software. Data were analyzed using the SIMQUAL route to generate Jaccard's similarity coefficient using NTSYS-pc version 2.02 (Numerical Taxonomy System), and significant differences were identified by one-way analysis of variance (ANOVA) followed by Turkey's HSD (Honestly Significant Difference) test. Mean comparisons of every treatment were carried out using Duncan's multiple-range test. Differences were considered to be significant at $p \leq 0.01$. Clustering of genotypes was done based on UPGMA using NTSYS version 2.02 software [32, 33].

4. Results

Rust postulates initially appeared on the lower leaf surface of garlic plants, as shown in Figure 1. Yellow spots turned orange and gradually spread to the other leaves. Microscopic examination revealed the presence of uredospores characterized as spherical or oval structures measuring 20-24 ×23-29 µm (Figure 1). Based on symptom development, morphology, and microscopic analysis, the causal agent was identified as *Puccinia allii*.

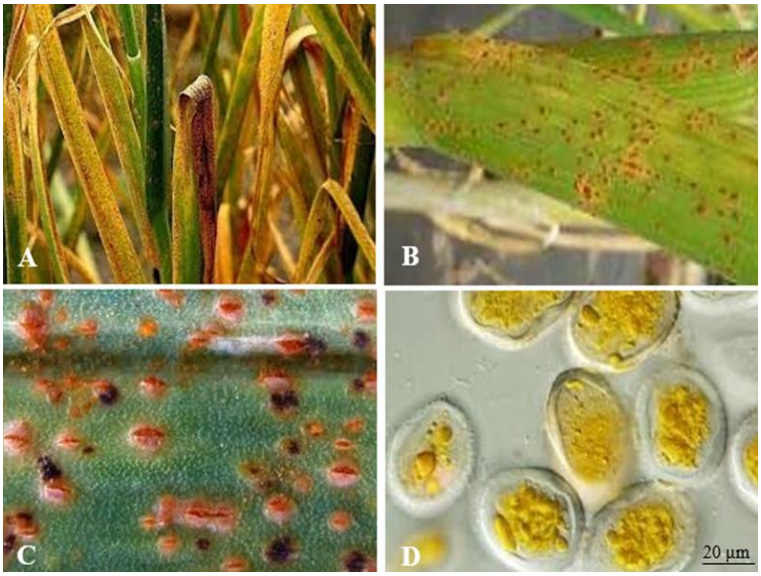

Figure 1 Garlic rust disease: A) *Infected garlic plant* with *rust disease; B)* Yellow spots on garlic leaves; and C, D) Uredospores. (Scales bars = 20 µm).

4.1 Evaluation of Rust Disease Resistance Performance in Garlic Genotypes

Results from the two-year combined analysis of variance showed significant differences in resistance to the rust fungus among garlic genotypes at 1% level (Table 1 and Table 2). During the initial growth stage, the genotypes Shorin, Toein, and Toyserkan exhibited the highest percentages of infection at 55%, 51.52%, and 46.25%, respectively (Table 3), while the Solan genotype recorded the lowest at 75.28% (Table 3). Moving to the second stage, the genotypes Moein, Toein, Hydare, and Aliabad displayed the highest infection percentages at 83.75%, 76.25%, 75%, and 73.75%, respectively (Table 4). Further, the data indicated that during the first stage, the genotype Toein exhibited the highest disease severity at 8.85%, followed by Shorin at 8.5%, and Toyserkan at 8.43%, placing them in a statistically significant group compared to others (Table 3).

Table 1 Results of combined analysis of variance for resistance to rust in garlic genotypes at the first growth stage.

SOV	df	SS	MS	F	CV
Infection Frequency	9	5011.25	556.806	3.58**	29.446
Disease severity	9	231.591	25.732	11.78**	22.759
disease index	9	1.519	0.169	9.43**	23.207

****** significant at p ≤ 0.01; S.O.V: source of variance; df: degree of freedom; SS: sum of squares; MS: mean of squares; F: F value; CV: coefficient of variation.

Table 2 Results of combined analysis of variance for resistance to rust in garlic genotypes at the second growth stage.

SOV	df	SS	MS	F	CV
Infection Frequency	9	3045.00	338.33	4.30**	12.495
Disease severity	9	1668.113	185.346	30.51**	13.11

disease index	9	17.508	1.945	28.93**	15.751

** significant at p ≤ 0.01; S.O.V: source of variance; df: degree of freedom; SS: sum of squares; MS: mean of squares; F: F value; CV: coefficient of variation.

Table 3 Mean comparison of resistance to rust in garlic genotypes at the first growth stage.

Genotypes	Infection Frequency (%)		Disease severity		Disease index	
	0.05	0.01	0.05	0.01	0.05	0.01
Shorin	55.00 ^{a}	55.00 ^{a}	8.50 ^{a}	8.50 ^{ab}	0.75 ^{a}	0.75 ^{a}
Solan	28.75 ^{c}	28.75 ^{c}	3.063 ^{d}	3.063 ^{d}	0.3 ^{d}	0.30 ^{c}
Toein	51.52 ^{a}	51.52 ^{ab}	8.875 ^{a}	8.875 ^{a}	0.763 ^{a}	0.763 ^{a}
Toyserkan	46.25 ^{ab}	46.25 ^{abc}	8.438 ^{a}	8.438 ^{ab}	0.725 ^{ab}	0.725 ^{a}
Aliabad	32.50 ^{bc}	32.50 ^{bc}	5.063 ^{c}	5.063 ^{c}	0.45 ^{c}	0.45 ^{bc}
Hydare	45.00 ^{ab}	45.00 ^{abc}	5.438 ^{bc}	5.438 ^{c}	0.513 ^{c}	0.513 ^{b}
Bahar	33.75 ^{bc}	33.75 ^{bc}	5.813 ^{bc}	5.813 ^{c}	0.50 ^{c}	0.50 ^{b}
Mariyang	42.50 ^{ab}	42.50 ^{abc}	6.563 ^{bc}	6.563 ^{bc}	0.588 ^{bc}	0.588 ^{ab}
Moein	43.75 ^{ab}	43.75 ^{abc}	6.438 ^{bc}	6.438 ^{bc}	0.575 ^{c}	0.575 ^{ab}
Barfgin	45.00 ^{ab}	45.00 ^{abc}	615.75 ^{b}	615.75 ^{bc}	0.60 ^{bc}	0.60 ^{ab}

Values in a column followed by the same letter(s) are not statistically significantly different (P ≤ 0.01 and P ≤ 0.05) according to Duncan's multiple range test.

Table 4 Mean comparison of resistance to rust in garlic genotypes at the second growth stage.

Genotypes	Infection Frequency (%)		Disease severity		Disease index	
	0.05	0.01	0.05	0.01	0.05	0.01
Shorin	67.50 ^{bc}	67.50 ^{bc}	14.688 ^{ef}	14.688 ^{ef}	1.238 ^{de}	1.238 ^{d}
Solan	66.25 ^{bc}	66.25 ^{bc}	17.223 ^{de}	17.223 ^{cde}	1.488 ^{cd}	1.488 ^{cd}
Toein	76.25 ^{b}	76.25 ^{ab}	18.00 ^{d}	18.00 ^{cde}	1.538 ^{c}	1.538 ^{c}
Toyserkan	67.50 ^{bc}	67.50 ^{bc}	12.938 ^{f}	12.938 ^{f}	1.113 ^{e}	1.113 ^{d}
Aliabad	73.75 ^{b}	73.75 ^{abc}	30.25 ^{a}	30.25 ^{a}	2.838 ^{a}	2.838 ^{a}
Hydare	75.00 ^{ab}	75.00 ^{ab}	21.813 ^{b}	21.813 ^{b}	1.95 ^{b}	1.95 ^{b}
Bahar	61.25 ^{c}	61.25 ^{c}	16.375 ^{de}	16.375 ^{de}	1.413 ^{cd}	1.413 ^{cd}
Mariyang	72.50 ^{b}	72.50 ^{abc}	20.813 ^{bc}	20.813 ^{bc}	1.90 ^{b}	1.90 ^{b}
Moein	83.75 ^{a}	83.75 ^{a}	19.063 ^{cd}	19.063 ^{bcd}	1.60 ^{c}	1.60 ^{bc}
Barfgin	66.25 ^{bc}	66.25 ^{bc}	16.75 ^{e}	16.75 ^{de}	1.386 ^{cd}	1.386 ^{cd}

Values in a column followed by the same letter(s) are not statistically significantly different (P ≤ 0.01 and P ≤ 0.05) according to Duncan's multiple range test.

The combined analysis of the disease index demonstrated a significant variation among the genotypes examined (Table 1). The highest indices were associated with the Toein, Shorin, and Toyserkan genotypes at 0.76, 0.75, and 0.72, respectively, thus placing them in a distinct statistical group different from other genotypes. On the other hand, the lowest disease indices were observed in the Solan, Aliabad, and Bahar genotypes at 0.3, 0.45, and 0.5, respectively. These

genotypes occupied a distinctive statistical position, showing a significant impact compared to others, while the remaining genotypes fell within the intermediate range between these two groups (Table 3).

Analysis of variance revealed a significant variation among garlic genotypes about disease severity over the two years during the second growth stage (Tables 2-4). It significantly increased in the second growth stage compared to the first. Notably, the Aliabad genotype displayed the highest disease severity at 25.3% in the second growth stage compared to the first. Thus, this genotype seems the most susceptible among other genotypes tested (Table 4).

Disease index based on the two-year dataset at the first growth stage correlated with disease severity. More specifically, the Aliabad genotype showed the highest disease index at 2.83, thus placing it in a distinct statistical group with a significant impact (Table 2). On the other hand, both Shorin and Toyserkan genotypes exhibited the lowest disease index of 1.11, forming a separate statistical group that differed significantly from other genotypes (Table 4). This divergence in disease indices suggests that garlic genotypes exhibited distinct behaviors across growth stages, indicative of varied genetic factors influencing their resistance to garlic rust fungus. The outcome of this study highlights the diverse responses of garlic genotypes, classifying them into resistant, partially resistant, partially susceptible, and susceptible categories.

4.2 Identification of RAPD Markers Associated with the Rust Disease

Genomic DNA extracted from various garlic genotypes was subjected to RAPD-PCR analysis utilizing a panel of 20 RAPD primers. In the initial experiments, 10 of the 20 tested primers yielded distinct and reproducible polymorphic banding profiles. These selected primers, namely OPA-07, OPA-09, OPA-10, OPA-16, OPA-19, OPAB-04, OPC-06, OPC-09, OPD-06, and OPG-13, generated polymorphic bands across all garlic genotypes as shown in Figure 2.

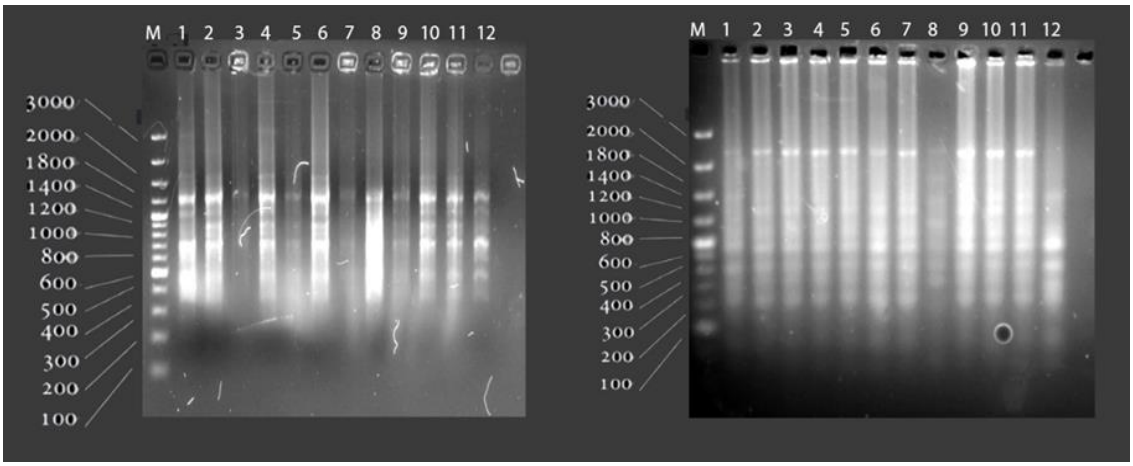

Figure 2 RAPD patterns on 2% agarose gel of amplified fragments generated from garlic genotypes with primers OPA-16 and OPC-06.

The percentage of polymorphism varied from 42.86% (OPA-10) to 84.62% (OPAB-04), with an average polymorphism rate of 67.66% (Table 5). A total of 95 fragments were detected, spanning the range of 300 to 1500 bp. Of the total, 66 fragments exhibited polymorphism ranging from 5 to 15 pieces per primer and averaging 9.5 fragments per primer. The OPC-06 primer generated the

highest number of bands, whereas the OPC-03 and OPC-19 primers exhibited the lowest. Notably, the OPAB-04 primer demonstrated the highest polymorphism, featuring 11 bands, whereas the OPA-19, OPA-10, and OPD-03 primers displayed the lowest polymorphism with 3 bands each (Table 5). Our data show that RAPD markers can be a convenient and reliable tool for distinguishing garlic genotypes. It further demonstrates a significant genetic variation among these genotypes.

Table 5 RAPD primers amplified specific bands (bp) for resistant and susceptible garlic genotypes.

Row	Primer	Sequences 5'-3'	Total number of bands	Polymorphism bands	Polymorphism %
1	OPA-07	GAAACGGGTG	6	5	83.34
2	OPA-09	GGGTAACGCC	7	4	57.15
3	OPA-10	GTGATCGCAG	7	3	42.86
4	OPA-16	AGCCAGCGAA	12	10	83.34
5	OPA-19	CAAACGTCGG	5	3	60.00
6	OPAB-04	GGCACGCGTT	13	11	84.62
7	OPC-06	GAACGGACTC	15	8	53.34
8	OPC-09	CTCACCGTCC	12	9	75.00
9	OPD-03	GTCGCCGTCA	5	3	60.00
10	OPG-13	CTCTCCGCCA	13	10	76.93

The UPGMA (Unweighted Pair Group Method with Arithmetic Average) dendrogram derived from the cluster analysis of RAPD data is shown in Figure 3. It categorizes the garlic genotypes into six distinct clusters, making up 76% of the observed variation, with Jaccard's similarity coefficient falling within the specified range. Specifically, the first cluster only included the genotype Gandagh; the second cluster comprised genotypes Barfgin, Toein, and Aliabad; the third cluster involved genotypes Mariyang and Moein; the fourth cluster constituted genotypes Bahar, Solan, Toyserkan, and Hydare; the fifth cluster featured genotype Shorin whereas the sixth cluster included the Chinese genotype.

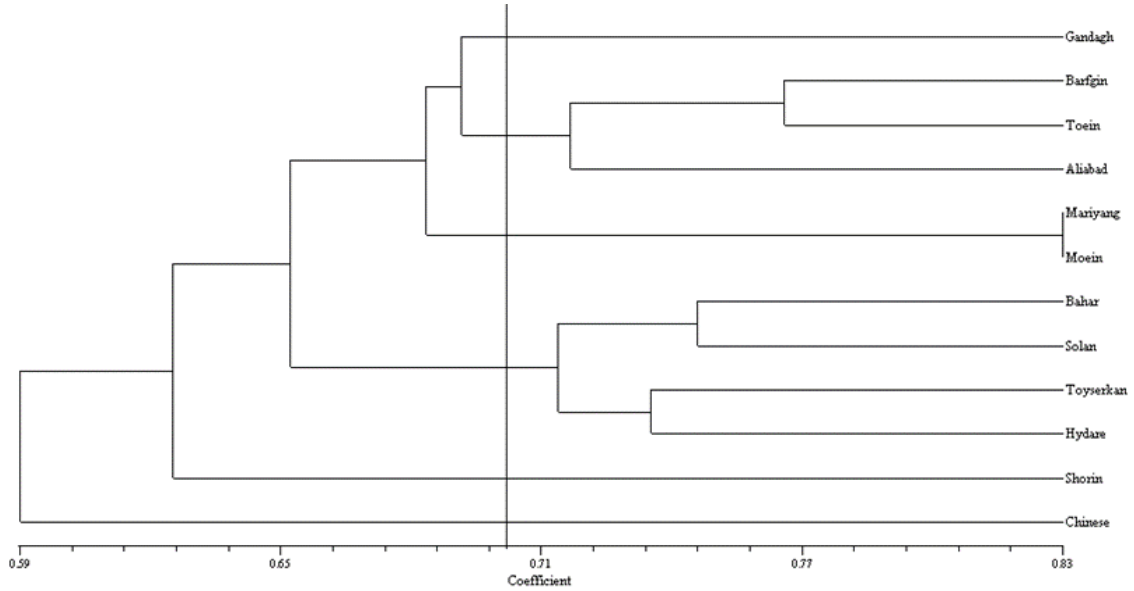

Figure 3 UPGMA dendrogram of the accessions studied, based on the RAPD data using Jaccard's similarity coefficients.

The highest genetic similarity was observed between the Mariyang and Moein genotypes, with a similarity coefficient 0.826 (Table 6). This suggests a common ancestral origin, or they were initially the same genotype, albeit assigned different names over time. On the other hand, the lowest similarity coefficient of 0.520 was obtained between the Bahar and Chinese genotypes and between the Solan and Chinese genotypes at 0.529. Notably, the Chinese genotype exhibited the slightest similarity with the other genotypes under investigation. Within the native genotypes, the Shurin genotype displayed the lowest similarity with others, forming a distinct cluster. The genetic similarity coefficients among genotypes ranged from 0.520 to 0.826, with an average estimated at 0.70 (Table 6).

Table 6 Jaccard similarity coefficient of garlic genotypes accessions based on RAPD data analysis.

Rows\Cols	Gandagh	Barfgin	Bahar	Toein	Toyserkan	Hydare	Solan	Shorin	Aliabad	Mariyang	Moein	Chinese
Gandagh	1.000											
Barfgin	0.700	1.000										
Bahar	0.690	0.635	1.000									
Toein	0.722	0.762	0.671	1.000								
Toyserkan	0.654	0.691	0.718	0.685	1.000							
Hydare	0.620	0.635	0.704	0.697	0.731	1.000						
Solan	0.583	0.637	0.742	0.604	0.696	0.721	1.000					
Shorin	0.552	0.666	0.589	0.630	0.621	0.646	0.607	1.000				
Aliabad	0.700	0.728	0.676	0.697	0.675	0.653	0.640	0.616	1.000			
Mariyang	0.693	0.698	0.568	0.699	0.579	0.623	0.590	0.615	0.689	1.000		
Moein	0.662	0.594	0.630	0.675	0.652	0.685	0.647	0.661	0.656	0.826	1.000	
Chinese	0.642	0.594	0.520	0.589	0.585	0.617	0.529	0.590	0.555	0.603	0.611	1.000

5. Discussion

Molecular markers are crucial in assessing the genetic diversity among different genotypes. The RAPD markers, in particular, are favored over alternative molecular markers for genetic diversity analysis due to their cost-effectiveness, rapid assessment of DNA-level variability, simplicity, ease of use, dominant nature, and the fact that it does not necessitate specific knowledge of the DNA sequence. These attributes make the RAPD markers a practical and efficient tool for exploring genetic diversity in various genotypes [12]. Phylogenetic analysis of RAPD profiles further reveals intergenic and intraspecies variation. Despite its many advantages, there are certain limitations to RAPD [34]. RAPD markers predominate, and amplification may or may not occur at a locus, leading to scores based on the presence or absence of bands. This means that homozygotes cannot be distinguished from heterozygotes [35]. Furthermore, the absence of a band due to the lack of a target sequence cannot be distinguished from DNA non-amplification for technical reasons (poor quality DNA), thus contributing to the ambiguity in interpreting the results [12].

Patel *et al*. [36] reported that morphological propensity, environmental factors, variation, and inter-hybridization may cause misidentification of genotypes. Polymorphism occurs when two or more different phenotypes exist in a population of the same species or when more than one morph or form exists [37]. Polymorphism is common in nature and is related to biodiversity, genetic diversity, and adaptation. It usually acts to maintain different forms in a population living in a diverse environment [38]. Polymorphism results from evolutionary processes, as does any aspect of a species. It is inherited and modified by natural selection. The ability of a species to respond adaptively to environmental changes depends on its level of genetic diversity [39]. Extensive studies on evaluating the genetic diversity of a diverse plant species using molecular markers have proven the relationship between geographic distance and genetic similarity between individuals [12]. Likewise, many studies have evaluated the establishment of genetic relationships in various cultivars and plant species [36].

The present study, 20 RAPD primers were used to achieve diversity within and between 11 Iranian garlic genotypes and one Chinese garlic genotype. 10 RAPD primers scored 66 bands in eleven Iranian and one Chinese garlic genotype. In a study on the genetic diversity of Iranian garlic genotypes using AFLP as a molecular marker, Vafaei *et al.* [40] classified Iranian garlic genotypes into 6 groups. The results showed that garlic genotypes react differently to rust, indicating the existence of genetic diversity among garlic genotypes. Garlic rust disease spreads in all genotypes with differences in infection percentage, severity, and disease index in the first and second growth stages. These results are supported by those reported by Periyannan *et al*. [41] regarding the genetics of resistance against rusts. In another study, Anjomshoaa *et al*. [12] showed that Iranian garlic genotypes reacted differently to the rust fungus (*Puccinia allii*) and were separated into resistant, partially resistant, partially susceptible, and fully susceptible genotypes. A study in Pakistan on garlic rust resistance showed 0.8% rust severity recorded on the Hazero variety [42]. Another study in California reported that the early and late maturing garlic genotypes, including Spanish and Chinese cultivars, were relatively tolerant to rust disease [43]. A California breeding program evaluating garlic rust resistance revealed that most genotypes were infected, and only about 1% had less than 26% infection [44]. Merga *et al.* [45] reported that using resistant or tolerant garlic cultivars is economically, sustainably effective, and environmentally friendly for managing rust disease.

Various researchers have utilized diverse markers to identify the genetic diversity of resistance genes in garlic and other crops. For example, the utilization of RAPD markers to uncover genetic diversity and resistance genes is further exemplified in the research by Poulsen *et al*. [46], where bulk segregant analysis led to the identification of an RAPD marker linked to leaf rust resistance in barley. This approach demonstrates the potential of molecular markers in breeding programs to enhance resistance against rust pathogens [47].

A study by Fernández-Aparicio *et al*. [48] delved into identifying and characterizing resistance against *Puccinia allii* in garlic germplasm, shedding light on the defense mechanisms impeding fungal development at various stages.

Additionally, Junghans *et al*. [49] identified a major gene associated with resistance to *Puccinia psidii* Winter in Eucalyptus, highlighting the involvement of a significant gene in a non-coevolved pathosystem. The work by Becerra *et al*. [50] explored the high genetic diversity in Chilean wheat yellow rust (Puccinia striiformis) populations, emphasizing the need to consider multiple significant genes in cultivars. These studies underscore the significance of genetic markers and diversity in understanding rust diseases caused by *Puccinia* species. These findings emphasize the importance of leveraging genetic diversity to develop cultivars with durable resistance to rust pathogens [51].

Applying RAPD markers in this study showed a significant genetic variation among garlic genotypes. As demonstrated in our research, this technique is proper in breeding programs, integrating with marker-assisted selection and comparative genomics studies. Further investigation may shed some light on garlic genes involved in resistance against rust pathogen and the resistance mechanism involved.

6. Conclusion

The OPAB-04 primer exhibited the highest level of polymorphism, revealing 11 bands in the study. Notably, genotype Solan displayed the highest susceptibility to rust disease, with a severity rate of 30.81%, while genotypes Aliabad and Bahar demonstrated resistance, with infection rates as low as 13% and 16.37%, respectively. Additionally, genetic diversity was evaluated using random-amplified polymorphic DNA (RAPD) markers, generating 95 scorable bands, of which 66 (69.48%) were polymorphic. A dendrogram was constructed using the UPGMA method based on Jaccard's coefficient of similarity, revealing six distinct clusters among the genotypes. Garlic rust, attributed to Puccinia alli, poses a significant threat to garlic crops globally, leading to economic constraints. To combat this challenge, various disease management strategies are employed. Utilizing resistant or tolerant garlic cultivars has emerged as a cost-effective, sustainable, and environmentally friendly approach. RAPD markers are a robust tool for identifying resistant and susceptible garlic genotypes against rust disease, offering valuable insights for breeding programs and disease management strategies.

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Author Contributions

SAA: Running the project, Resources, Writing - original draft. **MNE**: Supervision, Conceptualization, Methodology, Data curtain, review and editing. **KS**, **ANE**, **HZT** and **MM**: Data curtain and analysis, review and editing.

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Competing Interests

The authors stated that, this research was conducted in the absence of any commercial and or financial relationships that could be construed as a potential conflict of interest.

References

1. Aswani N, Azmi C, Cartika I, Basuki RS. Agronomic responses of four garlic genotypes in two different locations. Indones J Agron. 2023; 51: 134-145.
2. FAO. FAOSTAT [Internet]. Rome, Italy: FAO; 2024. Available from: https://www.fao.org/faostat/en/#data.
3. Entezari A, Mehrvar M, Zakiaghl M. Identification and molecular characterization of onion yellow dwarf virus isolates in some garlic growing areas of Iran. J Iran Plant Prot Res. 2020; 34: 401-411.
4. Forghani D, Bazgir E, Nasr-Esfahani M, Darvishnia M. Genomic structure of novel Iranian rhizoctonia solani AG-3PT isolates on potato, solanum tuberosum. Sydowia. 2021; 73: 217-238.
5. Gholamaliyan A, Esfahani MN, Dababat AA. Novel Iranian wheat cultivars resistant to bipolaris sorokiniana. Sydowia. 2021; 73: 257-269.
6. Chen J, Yan R, Hu Y, Zhang N, Hu H. Compositional shifts in the fungal diversity of garlic scapes during postharvest transportation and cold storage. LWT. 2019; 115: 108453.
7. Hejazi R, Esfahani MN, Maleki M, Sedaghatfar E. Susceptibility assessment and genetic population structure associated with rhizoctonia solani AG3-PT-potato stem canker disease. Physiol Mol Plant Pathol. 2022; 119: 101835.
8. Mishra R, Jaiswal R, Kumar D, Saabale P, Singh A. Management of major diseases and insect pests of onion and garlic: A comprehensive review. J Plant Breed Crop Sci. 2014; 6: 160-170.
9. Palevsky E, Konopická J, Rueda-Ramírez D, Zemek R. A review of prospective biocontrol agents and sustainable soil practices for bulb mite (Acari: Acaridae) management. Agronomy. 2022; 12: 1491.
10. Mallmann G, Fernandes JMC, Monteiro FP, Valmorbida J, Wamser AF, Feltrim AL, et al. Validation of a generic model for predicting garlic rust. Plant Pathol Quar. 2023; 13: 75-83.
11. Avasthi S, Gautam AK, Niranjan M, Verma RK, Karunarathna SC, Kumar A, et al. Insights into diversity, distribution, and systematics of rust genus puccinia. J Fungi. 2023; 9: 639.
12. Anjomshoaa A, Jafary H, Hassandokht MR, Taheri M, Abdossi V. Study on genetic diversity of resistance to the rust in Iranian garlic clones (Allium sativum L.) using NBS profiling technique. J Plant Mol Breed. 2019; 7: 93-100.

13. Monazzah M, Esfahani MN, Enferadi ST. Genetic structure and proteomic analysis associated in potato to rhizoctonia solani AG-3PT-stem canker and black scurf. Physiol Mol Plant Pathol. 2022; 122: 101905.
14. Alizadeh-Moghaddam G, Nasr-Esfahani M, Rezayatmand Z, Khozaei M. Genomic markers analysis associated with resistance to alternaria alternata (fr.) keissler-tomato pathotype, solanum lycopersicum L. Breed Sci. 2022; 72: 285-296.
15. Soheili-Moghaddam B, Mousanejad S, Nasr-Esfahani M, Hassanzade-Khankahdani H, Karbalaie-Khiyavie H. Identification of novel associations of candidate genes with resistance to rhizoctonia solani AG-3PT in solanum tuberosum stem canker. Int J Biol Macromol. 2022; 215: 321-333.
16. Hromadová Z, Gálová Z, Mikolášová L, Balážová Ž, Vivodík M, Chňapek M. Efficiency of RAPD and SCoT markers in the genetic diversity assessment of the common bean. Plants. 2023; 12: 2763.
17. Soheili-Moghaddam B, Nasr-Esfahani M, Mousanejad S, Hassanzadeh-Khankahdani H, Karbalaie-Khiyavie H. Biochemical defense mechanism associated with host-specific disease resistance pathways against Rhizoctonia solani AG3-PT potatoes canker disease. Planta. 2023; 257: 13.
18. Malysheva A, Kokhmetova A, Urazaliev R, Kumarbayeva M, Keishilov Z, Nurzhuma M, et al. Phenotyping and identification of molecular markers associated with leaf rust resistance in the wheat germplasm from kazakhstan, CIMMYT and ICARDA. Plants. 2023; 12: 2786.
19. Qalavand F, Nasr-Esfahani M, Vatandoost J. Transcriptome-based analysis of resistance mechanism to bipolaris sorokiniana-wheat common root rot disease. Plant Biol. 2022; 25: 119-130.
20. Gilbert GS, Parker IM. The evolutionary ecology of plant disease: A phylogenetic perspective. Annu Rev Phytopathol. 2016; 54: 549-578.
21. Sharma R, Joshi A, Maloo S, Rajaman G. Assessment of genetic finger printing using molecular marker in plants: A Review. Sci Res Impact. 2012; 1: 29-36.
22. Qalavand F, Esfahani MN, Vatandoost J, Azarm DA. Enzyme activity and population genetic structure analysis in wheat associated with resistance to bipolaris sorokiniana-common root rot diseases. Phytochemistry. 2022; 200: 113208.
23. Negash T, Shifa H, Regassa T. Fungicidal management of garlic rust (Puccinia allii) and assessment of yield losses due to the disease. J Plant Pathol Microbiol. 2019; 10: 471.
24. Koike ST, Smith RF, Davis RM, Nunez JJ, Voss RE. Characterization and control of garlic rust in California. Plant Dis. 2001; 85: 585-591.
25. Nasehi A, Kadir J, Esfahani MN, Mahmodi F, Ghadirian H, Ashtiani FA, et al. An outbreak of leaf spot caused by stemphylium solani on eggplant in Malaysia. Plant Dis. 2013; 97: 689.
26. Sadeghpoor N, Gharneh HA, Nasr-Esfahani M, Khankahdani HH, Golabadi M. Assessing genetic diversity and population structure of Iranian melons (Cucumis melo) collection using primer pair markers in association with resistance to Fusarium wilt. Funct Plant Biol. 2023; 50: 347-362.
27. Doyle JJ. Isolation of plant DNA from fresh tissue. Focus. 1990; 12: 13-15.
28. Nasehi A, Kadir JB, Esfahani MN, Mahmodi F, Golkhandan E, Akter S, et al. Cultural and physiological characteristics of Stemphylium lycopersici causing leaf blight disease on vegetable crops. Arch Phytopathol Plant Prot. 2014; 47: 1658-1665.

29. Poursakhi S, Asadi-Gharneh HA, Nasr-Esfahani M, Abbasi Z, Khankahdani HH. Identification of novel associations of candidate marker genes with resistance to onion-fusarium basal rot interaction pathosystem. Plant Gene. 2024; 37: 100440.
30. Nasehi A, Al-Sadi AM, Nasr Esfahani M, Ostovar T, Rezaie M, Atghia O, et al. Molecular re-identification of stemphylium lycopersici and stemphylium solani isolates deposited in NCBI genbank and morphological characteristics of malaysian isolates. Eur J Plant Pathol. 2019; 153: 965-974.
31. Esfahani MN. Identification of ulocladium atrum causing potato leaf blight in Iran. Phytopathol Mediterr. 2018; 57: 112-114.
32. Nagy S, Poczai P, Cernák I, Gorji AM, Hegedűs G, Taller J. PICcalc: An online program to calculate polymorphic information content for molecular genetic studies. Biochem Genet. 2012; 50: 670-672.
33. Sadeghpour N, Asadi-Gharneh HA, Nasr-Esfahani M, Khankahdani HH, Golabadi M. Antioxidant enzymes associated with resistance to Fusarium oxysporum f. sp. melonis race 1.2 in melon. Physiol Mol Plant Pathol. 2022; 121: 101880.
34. Singh S, Doshi A, Yadav M, Kamal S. Molecular characterization of specialty mushrooms of western Rajasthan, India. Curr Sci. 2006; 91: 1225-1230.
35. Amiteye S. Basic concepts and methodologies of DNA marker systems in plant molecular breeding. Heliyon. 2021; 7: e08093.
36. Patel DM, Fougat RS, Sakure AA, Kumar S, Kumar M, Mistry JG. Detection of genetic variation in sandalwood using various DNA markers. 3 Biotech. 2016; 6: 55.
37. Sohn SI, Thamilarasan SK, Pandian S, Oh YJ, Ryu TH, Lee GS, et al. Interspecific hybridization of transgenic brassica napus and brassica rapa-an overview. Genes. 2022; 13: 1442.
38. Terletskaya NV, Turzhanova AS, Khapilina ON, Zhumagul MZ, Meduntseva ND, Kudrina NO, et al. Genetic diversity in natural populations of rhodiola species of different adaptation strategies. Genes. 2023; 14: 794.
39. Valadez-Moctezuma E, Arroyo-Álvarez E, Samah S. Activation of transposable elements and insertional polymorphism in opuntia offspring as assessed by inter-retrotransposon amplified polymorphism markers. Plant Biosyst. 2019; 153: 450-460.
40. Vafaii Y, Dashti F, Mardi M, Ershadi A. A detection of genetic diversity among Iranian garlic clones (Allium sativum L.) clones via morphological characters and AFLP markers. Iran J Agric Sci. 2009; 40: 142623.
41. Periyannan S, Moore J, Ayliffe M, Bansal U, Wang X, Huang L, et al. The gene Sr33, an ortholog of barley Mla genes, encodes resistance to wheat stem rust race Ug99. Science. 2013; 341: 786-788.
42. Alam SS, Ahmad M, Alam S, Usman A, Ahmad M, Naveedullah M. Variation in garlic varieties for reaction to natural infection of puccinia porri wint and alternaria porri clif at swabi, NWFP. Sarhad J Agric. 2007; 23: 149-152.
43. Coviello RL. US IPM pest management guidelines: Onion and garlic-insects section. US ANR publication 3453, statewide IPM program, agriculture and natural resources. Oakland, CA: University of California; 2007.
44. Davis RM. US IPM pest management guidelines: Onion and garlic-diseases section. US ANR publication 3453, statewide IPM program, agriculture and natural resources. Oakland, CA: University of California; 2007.

45. Merga D, Bekele M, Obsa C. Garlic rust (Puccinia alli) disease and management practices: A review. Int J Agric Agribus. 2019; 5: 98-107.
46. Poulsen D, Henry R, Johnston R, Irwin J, Rees R. The use of bulk segregant analysis to identify a RAPD marker linked to leaf rust resistance in barley. Theor Appl Genet. 1995; 91: 270-273.
47. Zolfaghary Z, Esfahan MN, Nourollahi K, Akbarzadeh H. Genetic diversity and virulence of Iranian bipolaris sorokiniana isolates causing common root rot disease of wheat. Sydowia. 2021; 74: 107-119.
48. Fernández-Aparicio M, Barilli E, Mansilla F, Rubiales D. Identification and characterisation of resistance against rust (Puccinia allii) in garlic (Allium sp.) germplasm. Ann Appl Biol. 2011; 159: 93-98.
49. Junghans D, Alfenas A, Brommonschenkel S, Oda S, Mello E, Grattapaglia D. Resistance to rust (Puccinia psidii Winter) in Eucalyptus: Mode of inheritance and mapping of a major gene with RAPD markers. Theor Appl Genet. 2003; 108: 175-180.
50. Becerra V, Paredes M, Madariaga R, Bariana H, Mellado M, Rojo C. High genetic diversity in Chilean populations of wheat yellow rust (Puccinia striiformis f. sp. tritici West.) assessed by RAPD and AFLP. Aust J Agric Res. 2007; 58: 525-531.
51. Correia GS, Araujo Filho JV, Silva WR, Moccellin R, Resende FV, Pinheiro JB, et al. Reaction of garlic genotypes to Ditylenchus dipsaci and aspects related to productivity in a naturally infested area. Hortic Bras. 2022; 40: 451-456.