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Study on relationship between morphological and physiological traits with resistance to rust fungus (*Puccinia allii)* in Iranian garlic clones

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Key words: *Allium sativum* L., garlic rust, combined variance analysis, infection frequency.

Abstract: In the present study we collected 12 clones of garlic from different geographical origin in Iran. The clones were sown in a field trial under natural infection of the rust fungus during two consecutive years. After 210 days, the reactions of the clones to the disease as well as the morphological features of the clones were evaluated. The results of analysis of variance on morphological traits showed a significant difference among the clones in terms of bulb weight, mean clove weight, number of bulb skin, number of cloves in the bulb, leaf temperature and the percentage of clove dry weight, and nutrient uptake for N,P,K, Mn and Zn. The results showed a positive and significant correlation between the leaf temperature, photosynthesis, nitrogen and manganese uptake and percentage of leaf infection at 1% probability level. The results of the infection frequency showed that the clones 'Gilvan1' and 'Lalejin' had the lowest percentage of infection and were identified as resistant clones to the rust disease. The results also showed that garlic clones reacted differently to the rust fungus and are separated into resistant, semi resistant, semi-susceptible and susceptible clones.

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1. Introduction

Garlic *(Allium sativum* L.) is one of the most important vegetable crops in the world. Asian countries such as China, India, Afghanistan and Iran are known as the main growing area of this vegetable. Currently, garlic cultivation and production has been developed with a wide variety from East Asia to South America. Due to the commercial, economic and pharmaceutical-industrial importance of garlic, the interest in increasing pro-

duction, yield and development of new varieties is rapidly growing (Cunha *et al*., 2012). The world average yield of garlic in the last 20 years has been around 10 tons per hectare, while in recent years it increased to 18 tons per hectare (FAOSTATS, 2018). The Iranian plateau is one of the most important centers of vegetation diversity in the world (Mousavi, 1994) and among scientists, it is known as the arc of garlic distribution. Morphological and molecular studies have indicated a wide variety of Iranian garlic clones distributed all over the country (Baghalian *et al*., 2005; Vafaee, 2007). Despite the long history of forced apomixis in the garlic, its cultivars show a great diversity of morphological, physiological and biochemical differences (Etoh and Simon, 2002; Ammarellou *et al*., 2014). Garlic colonies have a wide variety in vegetative traits, taste and flavor, bolting and fertility capacity. The cultivar characteristics differ significantly according to the location of cultivation and climate, and the weather conditions has a significant effect on cloves, flowering and taste of garlic. All garlic colonies are sterile, and therefore genetic variation in this plant may only be due to random mutations or non-sexual variation and to the introduction of new genetic variation using modern molecular techniques. Biochemical and molecular studies indicate that the highest level of heterogenesity occurs within the genetic reserves of central Asia, which may include favorite genes for use in future genetic studies, as well as the improvement of plant programs. Kallo and Bergh stated that cloves' morphological traits, in particular number and size of leaves, height of the flowering stem, number of bulb shells, reaction to temperature, seed dormancy, quality of maintenance, vegetative and maturity period duration, are genetically controlled and these are polygenic traits (Kallo and Bergh, 1993).

One of the most important problems faced by farmers in relation to garlic cultivation is the outbreak of fungal rust disease due to high humidity of cultivated areas. Most herbaceous fungi are destroyed by fungicides, but for many of them there is still no proper and effective fungicide. In addition, the use of fungicides generally causes pollution of the environment, groundwater resources, and products themselves. The cause of garlic rust is the fungus *Puccinia allii* that is an airborne fungus, and its release of spores is very fast (Michael and Sarah, 1995); spores are commonly transported by the wind and causes the disease characterized by a sore or pustules at the leaf level. The teliospores live in non-crop seasons in the soil and plant infected residuals. The disease develops at temperatures below 10 °C and is destroyed by frozen dew. If the garlic is subjected to drought stress or excess water or condensed due to excessive consumption of nitrogen fertilizer, it will become susceptible to the disease (Houshiarfard and Pourabdollah, 2015).

In the world, an overgrowth of rust on garlic has been reported in many cases (Schwartz and Mohan, 1999). For example, garlic rust is one of the major problems in garlic cultivation in the United States. Studies show that the clones do not have complete resistance to garlic rust, they may be tolerant showing (on average) 51% reduction in the product (Coviello, 2007). Selection of resistant clones from the genetic complex of Iranian local clones is one of the prebreeding research priorities of the country in Iran. Identification of resistant crop cultivars has many advantages over the use of chemicals and other control methods. Since the economic importance of garlic rust has been grown up in recent years, few studies have been focused on the identification of morphological traits of garlic related to the yield, but none of the studies addressed on the relationship between morphological, physiological component and the resistance to the rust fungus. This study aimed to investigate the physiological and morphological features in the resistant and the susceptible Iranian garlic clones, with the ultimate scope to achieve resistant colonies to the garlic rust in selected clones.

2. Materials and Methods

Plant materials

Based on geographical distribution of garlic in Iran, we indicated the main cultivation regions with long crop history throughout the country. In each region, we collected one sample which represented the main geographical characteristic of that region to avoid any doubling in sampling and to collect as much as distinct clones. In total we collected 12 superior clones from all over the country (Table 1). The cloves were planted after disinfection with fungicide carbendazim and confidor insecticide in a field trial in Chavarzagh Tarom Zanjan (48 46' 43.34" E - 36 59' 51.33" N) and altitude of 484 meter from sea level and temperate-semi-arid climate in a completely randomized block design with three replications during two consecutive years. Soil texture of cultivation site was clay loam with pH= 7.6 and electrical conductivity of 0.8 dS/m, percentage of neutralizing matter was 6.2%, and available phosphorus and

Table 1 - Origin specifications of Iranian garlic clones

Row	Clone	Country	Province	City/Region	Longitude	Latitude	Altitude
1	Dezfol	Iran	Khozestan	Dezfol	48° 25' 52.57" E	32° 22' 59. 08" N	143
3	Sahneh	Iran	Kermanshah	Sahneh	47° 41' 41.08" E	34° 28' 26. 53" N	1354
4	Lalejin	Iran	Hamedan	Lalejin	48° 28' 34.42" E	34° 58' 25. 22" N	1700
5	Azar shahr	Iran	Azarbayejan sharghy	Azar shahr	45° 58' 59.78" E	37° 44' 1. 64" N	1415
6	Lahijan	Iran	Gilan	Lahijan	50° 0' 12.07" E	37° 12' 25. 46" N	4
7	Khaf	Iran	Khorasan razavi	Khaf	60° 8' 50.48" E	34° 34' 18. 53" N	975
8	Sojas	Iran	Zanjan	Sojas	48° 33' 04.45" E	36° 14' 24. 14" N	1774
9	Hesar	Iran	Zanjan	Hesar	47° 43' 7.24" E	36° 57' 26. 62" N	1158
10	Gilvan 1	Iran	Zanjan	Gilvan 1	49° 4' 48.88" E	36° 48' 10. 53" N	340
11	Gilvan 2	Iran	Zanjan	Gilvan 2	49° 7' 51.54" E	36° 47' 10. 94" N	340
12	Chavarzagh	Iran	Zanjan	Chavarzagh	48° 46' 43.34" E	36° 59' 51. 31" N	484

potassium in the soil were 3.8 and 261 mg/kg, respectively. In the annual cultivation process, the cloves of each clone were grown on 35 cm stacks with cultivation depth of 3 cm and 10 cm spacing. Due to possible genetic variation on resistance to the rust fungus within each clone, the most resistant seedlings of clones were selected for diseases resistance evaluation in the second year. At garlic harvesting stage, the bulbs were harvested at the time of physiological maturity, when the leaves fell and 70% dried (Rubatzky and Yamaguchi, 1997).

Evaluation of morphological traits

The morphological traits were evaluated according to the descriptor recommended by the International Plant Genetic Resources Institute (IPGRI, 2000). The evaluated morphological traits included bulb weight, bulb diameter, number of leaves, mean clove weight, number of bulb sheet, number of cloves in a bulb, color of clove, bulb and leaf, side view of bulb, shape of mature bulb and type of bulb structure. Leaf color and number were evaluated when the plants were fully developed, while the traits of bulb were studied after complete maturation of the plant and bulb harvesting.

Evaluation of physiological traits

At the end of March each year, the leaf chlorophyll content was estimated by measuring 10 leaf samples per replication and estimation of the mean data was done with the help of SPAD device. Other physiological measurements on leaf such as pure photosynthesis, stomatal conductance of H_2O, leaf surface temperature and transpiration sub stomatal CO_2 at the full leaf growth stage were performed by UK manufacturing ADC device (ADC BioScientific LCi Analyser Serial No. 32648). Sampling of green leaves was done at full leaf growth stage to estimate the rate of nutrients absorption and dry matter percentage of the leaf and immediately transferred to laboratory of Zanjan Agricultural Research Center. The percentage of dry weight of cloves and leaves was calculated and recorded by placing a sample of cloves or leaves of each treatment for 24 hours in oven at 70°C and measuring the weight of samples before and after drying. The nutrient uptake was evaluated in Water and Soil laboratory of Zanjan Agriculture Research Center. After sampling the plant leaves, they were immediately transferred to the laboratory and after washing they were dried immediately in a 70° C oven for 48 hours. Then, the samples were digested with acid and the plant extract was prepared. Finally, macro elements such as nitrogen, potassium, phosphorus and micro elements such as zinc, copper and manganese were measured by atomic absorption and calorimetric (Emami, 1996).

Evaluation of resistance to the rust fungus

In order to evaluate the frequency of leaf infection by rust fungus among the clones, the numbers of pustules were recorded at 4 stages (20 April, 5 May, 20 May and 5 June). The evaluation method of infection frequency of the disease was as follows: 10 plants per plot were randomly selected. The average of number of pustules on leaves of 10 plants was determined as the percentage of rust infection in each clone (Clifford and Jones, 1983; Dhingra and Sinclair, 1995).

Methods and tools for analyzing the data

In this study, statistical software SAS and SPSS was used to analyze the statistical data. Analysis of variance of traits and their mean comparison were done using Duncan test at 1% level. The correlation between traits was measured by Pearson's two-

domain test with the SPSS software. At the end of the second year, combined variance analysis was performed.

3. Results

Analysis of variance

The results of two-year combined analysis of variance of morphological traits showed a significant difference between the clones in terms of bulb weight, mean clove weight, number of bulb skin, number of cloves in the bulb at 1% level, while there was no significant difference between the bulb diameter and number of leaves (Table 2).

The results of combined analysis of variance for two years from physiological traits showed a significant difference between the studied clones in leaf surface temperature and clove dry weight percentage at 1% probability level. In terms of stomatal conductance and photosynthesis, the difference was significant at 5% probability level, while there was no significant difference between substomatal CO_2, transpiration, chlorophyll and leaf dry weight percentage among the clones (Table 3).

Combined analysis of variance in terms of nutrient uptake of N, P, K, Mn, and Zn showed that a significant difference was found between the clones at 1% level and in terms of Cu uptake at 5% probability level (Table 4).

The results of combined analysis of variance in terms of the resistance of the clones to the rust fun-

Table 2 - Results of combined analysis of variance for morphological characteristics in some Iranian garlic clones

S.O.V	df	MS					
		Bulb weight	Bulb diameter	Mean clove weight	Number of bulb skin	Number of cloves bulb	Leaf number
Year	1	11295.04 **	5.24 *	2.41 NS	0.12 NS	0.05 NS	0.00 NS
Rep/Year	4	120.94	0.46	1.68	0.06	3.81	0.44
Treat	11	351.53 **	0.59 NS	5.77 **	22.31**	32.77 **	0.46 NS
Treat × Year	11	363.21 **	0.57 NS	0.53 NS	0.42 NS	0.38 NS	0.00 NS
Error	44	103.00	0.57	0.95	0.87	1.84	0.56
CV%		17.48	15.30	25.46	18.73	11.41	10.83

*, **, NS= significant at 5%, 1% and not significant probability levels, respectively.

Table 3 - Results of combined analysis of variance for physiological characteristics in some Iranian garlic clones

S.O.V	df	MS							
		Clove dry weight	Leaf dry weight	Chlorophyl	Sub stomatal CO_2	Stomatal conductance of H_2O	Photosynthesis	Transpiration	Leaf surface temperature
Year	1	590.64 **	0.61 NS	123.76 NS	9531.20 NS	0.0013 NS	233.24 **	160.68 **	894.64 **
Rep/Year	4	0.21	1.84	39.75	2517.86	0.0036	0.37	1.70	7.26
Treat	11	8.55 **	1.89 NS	39.51 NS	455.23 NS	0.0032 *	10.53 *	0.38 NS	3.14 **
Treat × Year	11	13.66 **	1.44 NS	62.30 NS	1061.43 *	0.0032 *	3.74 NS	0.58 NS	1.74 **
Error	44	0.84	2.48	39.16	581.20	0.001	5.21	0.51	0.52
CV%		3.11	12.29	11.18	10.05	17.50	19.38	11.23	1.96

*, **, NS= significant at 5%, 1% and not significant probability levels, respectively.

Table 4 - Results of combined analysis of variance for physiological characteristics in some Iranian garlic clones

S.O.V	df	MS					
		Nitrogen	Phosphorus	Potassium	Manganese	Zinc	Copper
Year	1	26/21 **	0/026 NS	0/0004 NS	445/80 NS	3786/84 **	1/85 NS
Rep/Year	4	0/064	0/009	0/199	25/19	32/23	2.45
Treat	11	0/24 **	0/0071 **	1/326 **	67/36 **	56/87 **	1/28 *
Treat × Year	11	0/098 **	0/0075 NS	0/429 **	22/86 NS	15/60 NS	2/53 **
Error	44	0/017	0/0006	0/09	15/32	9.81	0/61
CV%		2.9	7.31	7.32	10.27	11.08	9.39

*, **, NS= significant at 5%, 1% and not significant probability levels, respectively.

gus at four stages of measurement showed that the difference at the first stage was significant at 1% level. At the next three stages, the difference in resistance between the clones was significant at 5% level (Table 5).

The effect of year was also significant in terms of the leaf surface temperature, photosynthesis, transpiration, percentage of clove dry weight, absorption of nutrients Zn and N and bulb weights at 1% level. In term of resistance to garlic rust, the effect of the year was significant at 4 stages at 1% level.

The effect of year on the treatment showed that the leaf surface temperature, N, P, K and copper nutrient uptake percentage, percentage of dry weight cloves, bulb weight, and percentage of resistance garlic rust were significant at the first and second stages of growth at 1% level and substomatal CO_2, stomatal conductance, and percentage of infection of garlic rust were significant at the 3rd and 4th stages of growth. Other physiological traits including photosynthesis, transpiration, chlorophyll content, dry matter percentage, manganese and zinc intake, and morphological traits including the bulb diameter, mean bulb weight, number of the bulb skin, number of cloves in the bulb and number of leaves did not showed significant differences. The highest coefficient of variation among the physiological traits was related to photosynthesis (19.38). Among the morphological traits, the highest coefficient of variation was related to the mean weight of the clove (25.46). In the study of the percentage of infection of garlic to the rust fungus, the highest coefficient of variation appeared at the second stage of evaluation in the early May (33.60).

Regarding to significance of analysis of variance among the clones, for some traits the mean comparison was done using Duncan's multi-domain test. The results of the two-year mean comparison of morphological traits showed that among the studied clones, the lowest weight was related to the clones ʿChavarzagh' (49.84) and 'Lahijan' (51.47) and the highest was related to the clones 'Sahne' (79.40) and 'Sojas' (61.11). In terms of the mean weight of the clove, the lowest weight belonged to the clone 'Khaf'(1.97) and the highest weight belonged to the clones 'Sahne' (5.14) and 'Gilvan1' (4.98). The mean comparison of the studied clones showed that the lowest number of bulb skin was for the clones 'Chavarzagh' and 'Lahijan' (2.33) and the highest was for the clone 'Azar Shahr' (7.16). In terms of number of cloves in a bulb, the lowest number was related to the clone 'Hesar' (8.66) and the highest was related to the clone 'Khaf (17.33) (Table 6).

Table 5 - Results of combined analysis of variance for resistance to rust in some Iranian garlic clones

S.O.V	df	MS			
		20/04/2019	05/05/2019	20/05/2019	05/06/2019
Year	1	491.42 **	651.86 **	2604.74 **	2947.88 **
Rep. Year	4	0.35	4.65	24.94	11.23
Treat	11	1.38 **	15.19 *	75.02 *	144.83 *
Treat × Year	11	1.38 **	20.63 **	84.50 *	119.35 *
Error	43	0.35	6.29	44.57	63.58
CV%		22.85	33.60	32.58	20.29

*, **, NS= significant at 5%, 1% and not significant probability levels, respectively.

Table 6 - Mean comparison of morphological traits in some Iranian garlic at two years

Treatment	Bulb weight (gr)	Bulb diameter (mm)	Mean clove weight (gr)	Number of bulb skin	Number of cloves per bulb	Leaf number per plant
Gilvan 1	60.13 b	4.96 a	4.98 a	5.83 abc	12.00 cd	6.66 a
Gilvan 2	56.65 b	5.07 a	4.69 a	4.83 c	12.33 bc	6.66 a
Azar shahr	55.08 b	5.05 a	3.15 bcd	7.16 ab	13.16 bc	6.66 a
Hesar	52.77 b	4.77 a	3.85 abc	5.66 bc	8.66 f	7.33 a
Lahijan	51.47 b	4.96 a	3.68 abc	2.33 d	12.00 cd	7.33 a
Khor va biabanak	54.22 b	4.88 a	4.07 ab	2.83 d	11.33 cde	7.33 a
Sojas	61.11 b	5.27 a	2.28 cd	6.66 ab	9.66 def	6.66 a
Dezfol	56.41 b	4.80 a	4.47 ab	2.66 d	14.33 b	7.00 a
Sahneh	79.40 a	5.36 a	5.14 a	6.33 abc	11.50 cde	7.00 a
Khaf	58.45 b	4.10 a	1.97 d	5.83 abc	17.33 a	7.00 a
Chavarzagh	49.84 b	4.93 a	3.89 abc	2.33 d	11.33 cde	7.00 a
Lalejin	60.85 b	5.08 a	3.94 abc	7.33 a	9.33 ef	6.66 a

In each column different letters mean significant differences between samples.

The results of the two-year mean comparison of physiological traits showed that among the studied clones in terms of the leaf surface temperature, the clone 'Gilvan1' had the highest leaf surface temperature (37.98 ℃) while the clone 'Azar shahr' had the lowest one (35.53 ℃). Stomatal conductance was highest in the clone 'Azar shahr' (0.23) while the lowest value was found for the clone 'Gilvan1' (0.15). The clone 'Azar shahr' had the highest photosynthesis rate (13.35) while the clone 'Gilvan1' had the lowest one (8.80). In terms of percentage of the clove dry weight, the highest was related to the clone 'Lahijan' (31.92) and the lowest value was related to the clone 'Azar shahr' (28.11) (Table 7).

The mean comparison of the studied clones in terms of nutrient uptake showed that the highest nitrogen uptake was related to the clones 'Dezful' (4.83) and 'Chavarzagh' (4.77) and the lowest was related to the clones 'Hesar' (4.22) and 'Khaf' (14.32). In terms of phosphorus uptake, the highest uptake was related to the clone 'Lalejin' (0.44) and the lowest was related to the clone 'Sojas' (0.30). In terms of potassium and manganese uptake, the highest uptake was related to the clone 'Dezful' in potassium (4.85) and manganese (42.77), and the lowest was related to the clone 'Lalejin' in potassium (3.62) and manganese (30.89). In terms of zinc and copper uptake, the highest uptake was related to the clone 'Lalejin' in zinc (35.78) and copper (9.21), the lowest zinc uptake was for the clone 'Gilvan1' (24.61) and the lowest copper uptake was for the clone 'Khaf' (7.55) (Table 8).

It is worth noting that all 12 clones were infected with garlic rust, although showing different reaction to the fungus. In two-year mean comparison, it was found that the observation of rust symptoms began from the second half of April, and disease progressed along with the growth of the plants. At the first stage

Table 7 - Mean comparison of physiological traits in some Iranian garlic at two years

Treatment	Leaf surface temperature °C	Sub stomatal CO_2	Stomatal conductance of H_2O	Photo-synthesis	Transpiration	Chlorophyl	Leaf dry weight	Clove dry weight
Gilvan 1	37.98 a	236.33 a	0.15 b	8.80 b	5.96 a	49.45 a	13.33 a	28.83 c
Gilvan 2	36.65 ab	224.75 a	0.20 ab	13.16 a	6.08 a	58.53 a	13.42 a	29.44 bc
Azar shahr	35.53 c	231.83 a	0.23 a	13.35 a	6.37 a	55.76 a	12.17 a	28.11 c
Hesar	36.61 bc	233.50 a	0.22 a	12.85 a	6.46 a	57.26 a	13.05 a	28.66 c
Lahijan	37.03 ab	249.50 a	0.21 ab	10.18 ab	6.31 a	58.70 a	12.38 a	31.92 a
Khor va biabanak	37.30 ab	236.50 a	0.19 ab	11.47 ab	6.29 a	58.96 a	12.55 a	29.39 bc
Sojas	36.98 ab	252 a	0.21 ab	10.78 ab	6.43 a	56.78 a	13.35 a	30.86 ab
Dezfol	35.68 c	247.48 a	0.23 a	12.29 ab	6.28 a	55.45 a	12.32 a	28.76 c
Sahneh	36.60 bc	250 a	0.23 a	12.27 ab	6.61 a	55.26 a	12.60 a	29.62 bc
Khaf	37.65 ab	243.83 a	0.21 ab	11.50 ab	6.74 a	54.23 a	13.91 a	28.75 c
Chavarzagh	37.28 ab	232.25 a	0.22 a	12.36 ab	6.80 a	55.13 a	12.43 a	31.29 a
Lalejin	37.25 ab	238.08 a	0.19 ab	12.35 ab	6.15 a	55.68 a	12.40 a	28.76 c

In each column different letters mean significant differences between samples

Table 8 - Mean comparison of physiological traits in some Iranian garlic at two years

Treatment	Nitrogen	Phosphorus	Potassium	Manganese	Zinc	Copper
Gilvan 1	4.73 ab	0.32 bcd	4.52 ab	42.53 a	24.61 c	8.06 bc
Gilvan 2	4.60 ab	0.35 bc	4.47 abc	37.81 abc	28.69 bc	8.72 ab
Azar shahr	4.67 abc	0.35 bc	4.30 bcd	40.58 ab	31.04 ab	8.52 abc
Hesar	4.22 e	0.35 bc	3.84 ed	36.22 abc	24.80 c	8.71 ab
Lahijan	4.76 ab	0.38 b	4.83 a	35.11 bc	29.03 bc	8.31 abc
Khor va biabanak	4.66 abc	0.35 bc	4.01 cde	40.27 ab	29.48 bc	7.84 abc
Sojas	4.47 cd	0.30 d	3.64 e	36.69 abc	26.63 bc	7.70 bc
Dezfol	4.83 a	0.35 bc	4.85 a	42.77 a	26.98 bc	8.55 abc
Sahneh	4.33 de	0.38 b	3.84 de	37.55 abc	28.53 bc	8.00 bc
Khaf	4.32 de	0.35 bc	3.62 e	36.79 abc	26.71 bc	7.55 c
Chavarzagh	4.77 ab	0.35 bc	4.51 abc	39.83 ab	26.74 bc	8.49 abc
Lalejin	4.48 cd	0.44 a	3.62 e	30.89 c	35.78 a	9.21 a

In each column different letters mean significant differences between samples

of growth, the highest percentage of infection was related to the clone 'Lahijan'. At the second stage, the highest percentage of infection was related to the clone 'Khor va Biabanak and the lowest was related to the clone 'Hesar'. At the third and fourth stages, the clones 'Gilvan1' and 'Lalejin' had the lowest percentage of infection and were identified as resistant to rust. This may indicate that garlic clones behave in different way during seedling and adult plant stages and therefore they have different genetics for resistance to the garlic rust fungus in different growth stages. The most susceptible clone was 'Chavarzagh' showing the highest percentage of infection (Table 9). The first uredospores of garlic rust appeared in the first half of April each year, and the first teliospores appeared two weeks later. The onset and spread of infection completely follow the progressive growth pattern and with the help of ure

Table 10 - Reaction of 12 Iranian garlic clones to rust fungus in a two years field traits under natural infection

Clones	Grouping of resistance			
	Resistant	Semi resistant	Semi susceptible	Susceptible
Gilvan 1	x			
Lalejin	x			
Khaf		x		
Sojas		x		
Sahneh		x		
Hesar		x		
Azar shahr		x		
Gilvan 2			x	
Khor va biabanak			x	
Lahijan			x	
Dezfol				x
Chavarzagh				x

Table 9 - Mean comparison of resistance to rust in some Iranian garlic clones in two year (Difference in days to maturity)

Treatment	Percentage of the disease			
	20/04/2019	05/05/2019	20/05/2019	20/06/2019
Gilvan 1	1.72 b	2.53 bc	3.97 c	5.83 c
Gilvan 2	1.58 b	2.59 bc	4.65 abc	6.42 abc
Azar shahr	1.72 b	2.99 abc	4.66 abc	6.30 bc
Hesar	1.72 b	2.36 c	4.21 abc	6.25 bc
Lahijan	2.01 a	2.86 abc	5.04 a	6.57 abc
Khor va biabanak	1.72 b	3.32 a	4.92 ab	6.52 abc
Sojas	1.72 b	2.45 bc	4.00 bc	5.97 bc
Dezfol	1.72 b	3.00 abc	4.94 a	6.67 ab
Sahneh	1.72 b	2.85 abc	4.29 abc	6.04 bc
Khaf	1.72 b	3.03 ab	4.24 abc	5.95 bc
Chavarzagh	1.72 b	2.88 abc	4.80 abc	7.08 a
Lalejin	1.72 b	2.71 abc	4.55 abc	5.85 c

In each column different letters mean significant differences between samples.

dospores and in the absence of fungicides, the level of plant infection can reach from over 1% to more than 80% within two months.

The results of this study showed that garlic clones respond differently to the rust and are separated into resistant, semi resistant, semi-susceptible and susceptible clones (Table 10). In sum, combined mean comparison of garlic clones in terms of resistance to disease showed that Gilvan1 cultivar had distinct traits compared to other clones. Morphologically, to color pf this clone is violet with code 5, 6-8 skins, side view shape of the compound bulb is broadly ovate, basal plate event, shape of matured bulb is globe and type of bulb structure is regular with two-fan group of cloves. The distinction of this clone with other violet clones is in the number of skins. The next resistant clone, according to the field conditions of the present research, was 'Lalejin' from Hamadan Province, which has white stripes bulb with code 4 and white clove with code 1. The number of skins is 5-8 and side view shape of the compound bulb is broadly ovate, basal plate event, shape of matured bulb is broadly oval, and type of bulb structure is regular multi-shelled.

Traits' correlation

The correlation coefficients between physiological traits and resistance to rust are shown in Table 11. The results of the study showed a positive and significant correlation between the leaf surface temperature, photosynthesis, nitrogen and manganese uptake and percentage of leaf infection at the probability level of 1%. The relationship between the leaf surface temperature and infection frequency indicates that by increasing the leaf temperature, the percentage of infection will be increased. This result is reasonable according to other researchers' reports that the percentage of rust damage has only a direct correlation with the mean temperature. In addition, a positive and significant correlation was observed between percentage of rust infection and photosynthesis and nitrogen, manganese and zinc. Also, a negative and significant correlation was found between percentage of rust infection and transpiration rate at the level of 1%.

The correlation coefficients between morphological traits and resistance to rust are shown in Table 12. A negative and significant correlation was observed between traits such as transpiration rate, bulb weight, bulb diameter, number of bulb skins and type of bulb structure and rust infection percentage at the 1% level. This relationship indicates that garlic rust is effective on the factors affecting the garlic yield, such as the bulb weight, bulb diameter and by increasing the percentage of infection, the value of these traits are reduced.

Results of the infection frequency in four growth stages (Fig. 1) showed that the clones with high infection frequency at the early stages of growth had a higher disease symptomps at the final stages of growth. Therefore, the presence of infection at the early stages reduced the growth, yield and quality of the product. However, those that were resistant to some extent at the early stages of growth had relatively low infection and slow spread of disease, and with better growth of the clones.

Table 11- Correlation coefficients between physiological traits and resistance to rust (*Puccinia allii*) in Iranian garlic clones measured by Pearson's two-domain test

Treat	Percentage of the disease
Percentage of the disease	1
Leaf surface temperature	- 0.602 **
Sub stomatal CO_2	- 0.206
Stomatal conductance of H_2O	0.059
Photosynthesis	0.411 **
Transpiration	- 0.520 **
Chlorophyl	0.217 *
Leaf dry weight	0.059
Clove dry weight	0.160
Nitrogen	0.625 **
Phosphorus	- 0.085
Potassium	0.142
Manganese	0.298 **
Zinc	0.469 **
Copper	0.19

*, **, NS= significant at 5%, 1% and not significant probability levels, respectively.

Table 12 - Correlation coefficients between Morphological traits and resistance to rust (*Puccinia allii*) in Iranian garlic clones measured by Pearson's two-domain test

Treat	Percentage of the disease
Percentage of the disease	1
Bulb weight	-0.462 **
Bulb diameter	-0.285 **
Mean clove weight	0.068
Number of bulb skin	-0.404 **
Number of cloves per bulb	-0.007
Leaf number	0.128
Color bulb	0.183
Color clove	0.009
Color leaf	0.096
Side view of bulb	-0.118
Shape of mature bulb	-0.098
Type of bulb structure	-0.526 **

*, **, NS= significant at 5%, 1% and not significant probability levels, respectively.

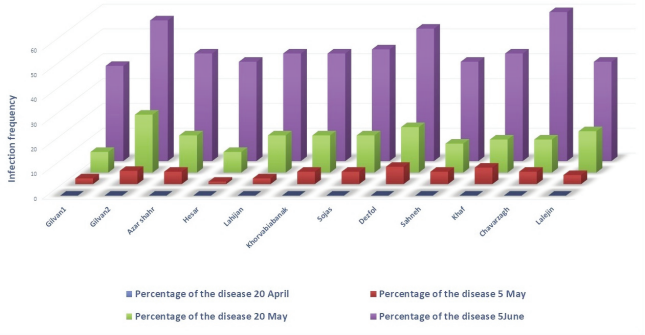

Fig. 1 - Correlation coefficients between Morphological traits and resistance to rust (*Puccinia allii*) in Iranian garlic clones measured by Pearson's two-domain test

4. Discussion and Conclusions

This is the first study on the identification of resistant clones to rust on garlic through the study of morphological and physiological traits collected on

12 Iranian clones from Iran during two crop years. The clones 'Gilvan1' and 'Lalejin' had the lowest number of stomata in the leaf area, because the fungus of the garlic rust through the stomata comes in to the plant, the low number of stomata reduces the possibility of pathogen entry and may be the most important feature of resistance in these clones.

The results of two-year combined analysis of variance of morphological traits are consistent with the research report of Nourbakhshian *et al.* (2007). The results of their research showed that a significant difference was found between the number of cloves in the bulb, clove weight, length and diameter of the clove and amount of dry matter per unit area (Nourbakhshian *et al*., 2007).

Vafaee and colleagues (2009), in a study on the genetic diversity of Iranian garlic clones using morphological traits and AFLP as molecular markers, classified Iranian garlic clones in 6 main groups, while according to morphological traits, the total clones were classified into 4 general groups.

The results of this study showed that garlic clones respond differently to the rust. The results of a study on resistance to garlic rust in Pakistan showed that rust severity of 0.8% was recorded on variety Hazro (Alam *et al*., 2007). A study in the US state of California regarding the susceptibility of the tested clones to garlic rust showed that none of the cultivars including selective later and early clones, and Spanish and Chinese cultivars showed complete resistance and were usually tolerant (Coviello, 2007). Another California breeding program to test the resistance to garlic rust on three genotypes PE493096, PE540315, W12820 showed that all of them were infected with garlic rust and only about 1% of them had less than 26% of the infection (Davis, 2007).

The results of correlation coefficients between morphological traits and resistance to rust are consistent with Nourbakhshian's report on factors affecting garlic yield and bulb weight (Nourbakhshian *et al*., 2008). The results of this study are consistent with the results of other researchers regarding the superior clones' traits (Baghalian *et al*., 2005). Kallo reported that a clove weight, number of cloves and bulb diameter had the most direct effect on garlic yield, and stated that the selection of the clones based on these traits would improve the yield potential of garlic (Kallo, 1988).

The use of commercial clones carrying rust resistance genes is the most efficient, economical and environmental friendly method of rust disease control. Using morphological trait for early screening of the clones for resistance to garlic rust can be very important. The results of the infection frequency showed that the clones 'Gilvan1' and 'Lalejin' had the lowest percentage of infection and were identified as resistant clones to the rust.

We found resistant clones with the introduction of each morphological trait. It should be noted that since the intensity measurements of infection were carried out at four stages, it was found that the earliest symptoms of garlic rust began in early May, and then the growing trend was accompanied by the growth of garlic. Two years of field trails lead us to achieve resistance components and to find resistant clones of garlic to the rust pathogen, however in order to get more in detail on mechanism and genetics of resistance we are following genetics studies on resistance and susceptible clones. By this research we will find the diversity of genes involved in resistance to rust pathogen in garlic and we will explore some more details on mechanisms of resistance in the future.

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