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Field screening of safflower genotypes for resistance to charcoal rot disease

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Abstract

Nineteen safflower genotypes (*Carthamus tinctorius* L.) that originated from different geographical regions were screening for their response to infection with *Macrophomina phaseolina*, the charcoal rot pathogen at the research farm of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, in 2005. The plants were evaluated for length and width of necrotic lesion at the entry point of inoculum at flowering (LNF and WNF, respectively) and maturity stages (LNM and WNM, respectively), and penetration depth of necrosis in the stem (PDN). Some morphological characteristics including plant height, number of days to maturity, diameter of lower stem (DLS), diameter of vascular bundle (DVS) and relative water content of lower stems (SRWC) were also measured. Analysis of disease symptoms by clustering method revealed that there were four moderately resistance, ten susceptible and five moderately susceptible genotypes. However, no completely resistant genotype was found. DLS had a positive and significant correlation with all disease related traits including LNF, WNF, LNM, WNM and PDN. Therefore, this trait may be used as an index for indirect selection of resistant genotypes in safflower. The moderately resistant genotypes IUT-K115, GUA-Val6, CW-74 and AC-Stirling can be used in breeding programs to improve resistant safflower genotypes.

Keywords: Charcoal rot; *Macrophomina phaseolina;* Resistance; Safflower.

Introduction

Safflower (*Carthamus tinctorius* L.) is an oilseed crop of increasing importance in the world. This crop has been grown for its flowers for many years in Iran, which is one of the centers of safflower culture in the old world (Knowels, 1969). In recent years due to an increasing demand for vegetable oil for the human uses, its production as an oilseed crop has received a great deal of attention. Growth of the crop is severely affected by many seed-borne fungal diseases such as Fsarium and Verticillium wilt, Phythophthora and stem rot, rust, Alternaria leaf spot (Dajue and Mundel, 1996). Recently, charcoal rot caused by *Macrophomina phaseolina* has been considered as a relatively important disease in

safflower. The first report of charcoal rot disease on safflower growth in Iran was in northeastern Golestan Province in the summer of 2002 (Razavi and Pahlavani, 2004).

M. phaseolina, the causal agent of seedling blight, root rot and charcoal rot of more than 500 crop and noncrop species; primarily is a soil-borne fungus (Smith and Carvil, 1997). Although initial infections occur at the seedling stage, they remain latent until the safflower plant approaches flowering or maturity. The first symptom is general wilting of the plant during the middle of hot days followed by a recovery in the evening as temperature declines. The stems of infected plants eventually take on a gray discoloration at the base and finally, the vascular bundles may become covered with microsclerotia of the fungus. Since charcoal rot restricts the flow of water and nutrients to the upper parts, reduced seed sizes usually occur. Disease incidence and severity are often greatest when maturing plants are stressed by drought and high temperature which leads to premature plant death. In some cases, this pathogen kills up to 25% of the plants in commercial fields of safflower (Govindappa et al., 2005).

Similar to other crops, management strategies to control charcoal rot in safflower include crop rotation, lower plant density and scheduling planting date and irrigation to reduce the effect of mid-season drought stress (Smith and Carvil, 1997). Planting resistant cultivars is the most permanent and practical way for the control of the disease, because above mentioned strategies fail to provide adequate control. Although, responses of different genotypes to the charcoal rot disease caused by *M. phaseolina* have been reported many times in other crops such as and soybean (Pearson et al., 1984) and alfalfa (Pratt et al., 1998) such information are not available in the literature for safflower. Therefore, the objective of this study was to screen some genotypes of safflower under field conditions for resistance to charcoal rot disease. We further report those traits that are correlated to the resistance for indirect selection programs.

Materials and Method

Plant materials

Nineteen different genotypes of safflower (cultivars, accessions and advanced breeding lines) originating from diverse regions and without former information on their response to *M. phaseolina* were selected (Table 1). All were provided by the Iranian Seed Production Research Center located at Gorgan, Iran. The geographical origin of the genotypes included in the study is listed in Table 1. The field experiment was conducted at the research farm of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan (36º51' N, 54º16' E, 100 m asl and annual rainfall 657 mm), Iran in 2005. The soil was deep silty clay (fine-silty, mixed, active, thermic, typic Calcixerolls). Seeds of each genotype were planted as 20 seeds per m of 7.5 m rows which were spaced 0.75 m apart, and then 4 weeks later seedlings thinned to finally have 5 plants per each m of rows. In order to increase disease incidence, the crop was raised according to recommended cultural practices under no-irrigation conditions (Zeinali, 1999). The Experimental plots were hand weeded as needed during the growing season.

Table 1. Origin of germplasm and size of lesions caused by *M. phaseolina* and some morphological characteristics of 19 safflower genotypes.

Genotype	Origin	LNF (cm)	WNF (cm)	LNM (cm)	WNM (cm)	PDN (%)	DLS (mm)	DVS (mm)	Height (cm)	Days to maturity	SRWC (%)
Arak-2811	Iran	2.2 ± 0.50	1.9 ± 0.40	6.7 ± 1.12	2.7 ± 0.08	38.9 ± 5.94	8.5 ± 0.42	2.1 ± 0.13	101.9 ± 1.65	99	57.2
Isfahan	Iran	6.7 ± 0.79	4.0 ± 0.07	12.7 ± 0.53	4.1 ± 0.14	40.7 ± 3.36	10.6 ± 0.23	2.6 ± 0.17	116.0 ± 1.09	100	57.8
LRV-55295	Iran	6.0 ± 0.44	3.9 ± 0.00	6.3 ± 0.65	3.9 ± 0.40	32.6 ± 4.10	9.9 ± 0.33	2.2 ± 0.17	103.2 ± 1.10	99	61.6
IL-111	Iran	3.6 ± 0.34	2.6 ± 0.48	8.5 ± 0.34	3.0 ± 0.41	29.9 ± 4.25	8.8 ± 0.48	1.5 ± 0.10	92.1 ± 0.62	87	49.4
GUA-101	Iran	3.5 ± 1.01	3.4 ± 0.37	8.71 ± 0.97	3.6 ± 0.58	42.9 ± 7.21	9.5 ± 0.87	1.7 ± 0.15	92.5 ± 2.01	95	58.4
GUA-Val6	Iran	3.4 ± 0.89	3.4 ± 0.41	3.3 ± 0.78	3.6 ± 0.12	12.9 ± 4.79	8.9 ± 0.23	1.8 ± 0.14	100.5 ± 0.95	101	59.6
IUT-M21	Iran	3.4 ± 0.65	2.9 ± 0.43	13.9 ± 1.22	3.2 ± 0.31	50.1 ± 8.29	10.2 ± 0.26	1.6 ± 0.09	100.7 ± 1.76	101	57.6
IUT-K23	Iran	3.9 ± 0.70	3.7 ± 0.24	13.7 ± 1.01	3.9 ± 0.09	58.0 ± 6.34	10.1 ± 0.67	1.5 ± 0.11	98.2 ± 1.35	105	58.4
IUT-K115	Iran	5.7 ± 0.54	3.0 ± 0.14	10.2 ± 0.90	3.6 ± 0.39	5.3 ± 1.00	9.3 ± 0.44	1.8 ± 0.11	82.3 ± 1.46	115	66.5
Miandoab	Iran	3.2 ± 0.73	2.0 ± 0.31	6.4 ± 0.71	4.0 ± 0.00	51.1 ± 9.97	8.9 ± 0.31	2.4 ± 0.24	93.6 ± 1.32	101	60.2
Lesaf	Canada	5.5 ± 0.75	3.6 ± 0.30	14.5 ± 1.24	3.6 ± 0.20	44.4 ± 6.69	10.4 ± 0.07	2.0 ± 0.13	97.9 ± 0.76	99	59.1
AC-Stirling	Canada	3.9 ± 0.73	2.8 ± 0.30	7.1 ± 1.14	2.5 ± 0.46	2.9 ± 0.36	8.2 ± 0.37	1.4 ± 0.07	70.8 ± 1.81	96	49.2
Hartman	USA	6.5 ± 0.34	3.6 ± 0.10	8.9 ± 1.16	3.7 ± 0.18	34.9 ± 5.31	10.4 ± 0.11	2.1 ± 0.10	101.8 ± 0.75	98	54.9
CW-74	USA	5.5 ± 0.74	3.6 ± 0.30	8.8 ± 0.94	3.7 ± 0.38	9.8 ± 2.97	9.8 ± 0.52	1.7 ± 0.11	71.4 ± 0.55	94	50.1
Syrian	Syria	6.6 ± 0.60	3.6 ± 0.20	11.2 ± 0.90	3.7 ± 0.18	35.6 ± 6.30	10.0 ± 0.28	1.9 ± 0.13	119.3 ± 2.10	129	58.0
PI-34070	Unknown	4.5 ± 0.71	3.9 ± 0.14	13.6 ± 0.69	4.0 ± 0.00	32.4 ± 4.87	10.0 ± 0.18	1.9 ± 0.10	106.1 ± 1.12	98	53.1
PI-250537	Unknown	6.4 ± 0.40	3.1 ± 0.12	7.8 ± 0.49	3.3 ± 0.18	51.2 ± 4.46	10.4 ± 0.17	2.0 ± 0.14	95.2 ±0.78	96	54.5
PI-199877	Unknown	4.5 ± 1.04	3.1 ± 0.46	11.9 ± 0.84	4.0 ± 0.00	26.1 ± 5.30	10.1 ± 0.25	1.7 ± 0.15	107.5 ±0.91	100	60.7
PI-198290	Unknown	5.2 ± 0.67	3.3 ± 0.36	13.4 ± 1.13	4.0 ± 0.00	53.4 ± 5.22	10.1 ± 1.37	2.2 ± 0.14	105.9 ± 2.35	95	50.7
Mean	—	4.7	3.2	9.8	3.6	34.4	9.7	1.89	97.7	100.4	56.7

Preparation of inoculum and artificial inoculation

To prepare inoculum, stems, crowns and roots of diseased plants were collected from the research farm in summer of 2004. Each section was thoroughly washed with distilled water, transferred to 0.5 % sodium hypochlorite for 2 min and washed in sterile water for 1 min and then cultured on potato dextrose agar (PDA) for isolation by a hyphal type method (Singleton et al., 1992). The cultures were incubated at 25 ºC in the darkness, then a 5 mm diameter piece of 10 days colonies grown on agar that prepared according to stem-puncture method used as inoculum (Young and Alcorn, 1982). Before inoculation each piece was

evaluated for microsclerotia density and each stem piece used contained at least 6×10^4 microsclerotia. In the field, crowns and lower stem of 20 to 30 cm plants (approximately in early stem elongation stage) in middle row of each plot were carefully washed to be free of soil. For inoculation, first a small scratch was made with a razor blade in the crown of the plants, around 23 April 2005. For inoculation, a pace of inoculum was placed on the scratch. Immediately following the inoculation, the inoculated area of the stem was wrapped in Parafilm M (American Can Corp., Greenwich, CT). Diseased plant tissue was grown on PDA for reisolation of the pathogen to provide an assurance that the disease was caused by our inoculations by *M. phaseolina.*

Data recording and reaction to disease

Symptoms due to infection with *M. phaseolina* were evaluated at flowering (June 2005) and again at maturity (August 2005). At each stage, the length and width of the necrotic lesion at the inoculation point, identified by the degree of discoloration, were measured and denoted by LNF and WNF in flowering, and LNM and WNM in maturity, respectively. The infected stems were bisected through entrance point of inoculation, and the penetration depth of necrosis in the stem was measured and calculated as percentage of stem diameter (PDN).

Data were recorded on plant height, number of days to maturity, diameter of lower stem (DLS), where stems had the greatest thickness, and diameter of vascular bundle (DVS), including phloem and xylem tissues in width cutting of lower stem. Also, relative water content of lower stems (SRWC) were calculated based on stem dry matter as described by Bryla and Duniway (1997) by drying at 70 ºC for 72 h. All the above mentioned measurements were made on seven uninfected plants of the middle row of each plot and their mean were used in further analyses.

Statistical analysis

Data evaluation and Pearson's correlation analysis among the measured traits were performed for the 19 genotypes with the SAS (Statistical Analysis System Institute Inc, 1996). Following the correlation analysis the relationship between diameter of lower stem (DLS) and disease related traits (LNF, WNF, LNM. WNM and PDN) were also determined by performing a regression analysis. For grouping the genotypes on the basis of resistance or susceptibility reaction to *M. phaseolina* infection, a cluster analysis was made with five diseases related traits including LNF, WNF, LNM, WNM and PDN. The cluster analysis was carried by using of squared Euclidean distance to construct an Unweighted Pair Group Method with Arithmetic mean (UPGMA) dendrogram according to Johnson and Wichern (1988). The cluster analysis and plotting dendrogram were processed using SPSS software (Statistical Package for Social Sciences, Inc. Release 10.0.5, 1999).

Results

There was considerable variation among the evaluated genotypes for reaction to infection measured at flowering (LNF and WNF) and maturity (LNM, WNM and PDN)

stage and also for plant height, number of days to maturity, diameter of stem (DLS), diameter of vascular bundle (DVS) and stem relative water content (SRWC) (Table 1). The lowest mean of both LNF and WNF were seen in Arak-2811, and the highest were seen in Isfahan. Similarly, GUA-Val6 and AC-Stirling had the smallest LNM and WNM, respectively (Table 1). Penetration depth of necrosis in stem (PDN) ranged from 2.9 to 58.0 percent of stem diameter, observed in AC-Stirling and IUT-K23, respectively (Table 1). There was no genotype without infection symptoms; therefore, none could be considered completely resistant.

Among the genotypes, plant height, number of days to maturity, and diameter of stem (DLS) ranged from 70.8 to 119.3 cm, 87 to 129 days and 8.2 to 10.6 mm, respectively (Table 1). The genotypes Isfahan and AC-Stirling had the highest and lowest thickness vascular bundle in their stem, with DVS of 2.6 and 1.4%, respectively (Table 1). SRWC among the evaluated genotypes ranged between 49.2 to 66.5% in AC-Stirling and IUT-K115, respectively (Table 1).

For better understanding of reaction of the genotypes to artificial infection with *M. phaseolina*, clustering analyses were used to group genotypes into resistant and susceptible classes using the diseases related traits LNF, WNF, LNM, WNM and PDN. Figure 1 shows the UPGMA dendrogram with two well-differentiated clusters. The first cluster included fourteen safflower genotypes that almost had high mean of LNF, WNF, LNM, WNM and PDN. In this cluster ten genotypes together constructed sub-cluster 1_A and the rest included PI-250537, Miandoab, PI-198290, IUT-M21 and IUT-K23 formed sub-cluster 1_B (Figure 1). Therefore, the all genotypes grouped in cluster 1 were generally considered susceptible. Although, it is might be better to consider genotypes belonged to sub-cluster 1_A as susceptible and the genotypes located in sub-cluster 1_B as moderately susceptible. The second cluster included four genotypes that had lower means of LNF, WNF, LNM, WNM and PDN (Figure 1). Therefore, CW-74, IUT-K115, AC-Stirling and GUA-Val6 that were located in cluster 2 were considered moderately resistant genotypes (Figure 1).

There was a significant and positive correlation between length of necrotic lesion at flowering (LNF) and at maturity stage (LNM) (Table 2). The penetration depth of necrosis (PDN) correlated significantly with WNF (r=0.46*). The traits LNF (r=0.68**), WNF (r=0.71**), LNM (r=0.70**), WNM (r=0.64**) and PDN (r=0.48*) had a significant and positive correlation with DLS (Table 2). Correlations between plant height and WNM, PDN, DLS and DVS were also positive and significant (Table 2).

To better understand the relationship between diameters of lower stem (DLS) and disease related traits (LNF, WNF, LNM. WNM and PDN), a regression analysis were made on data (Figure 2). Results of regression analysis showed that DLS is good predictors of disease related traits (LNF, WNF, LNM, WNM and PDN), and there was also a significant positive relationship between DLS and others (Figure 2). In the other word, increase of diameter of lower stem (DLS) in the studied genotypes significantly increased symptoms of the disease.

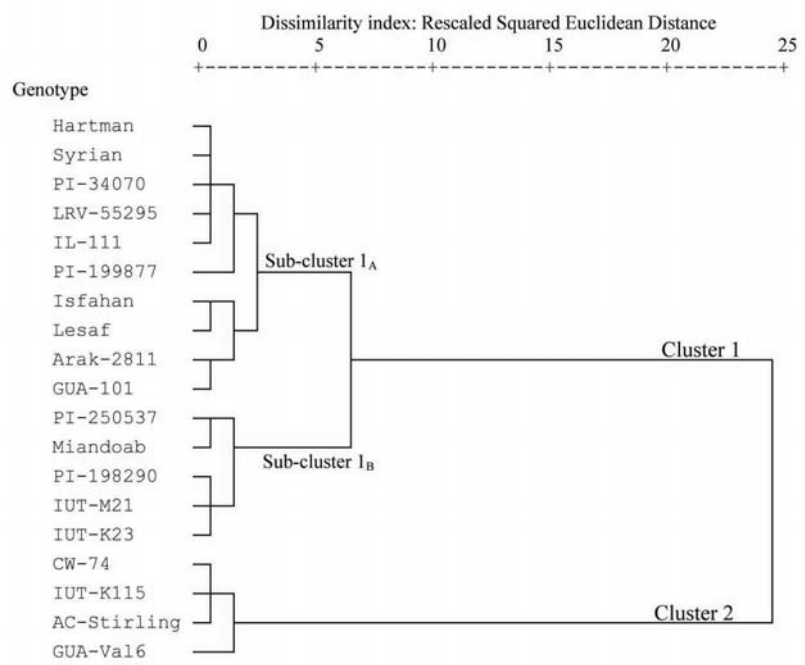

Figure 1. Dendrogram constructed with UPGMA clustering method for 19 safflower genotypes. Dissimilarities were computed from five diseases related traits including length and width of necrotic lesion in flowering (LNF and WNF) and in maturity (LNM and WNM) and also penetration depth of necrosis in the stem (PDN). The scale in the dendrogram is the Rescaled Squared Euclidean Distance. The genotypes located in sub-cluster 1_A, sub-cluster 1_B and cluster 2 were considered susceptible, moderately susceptible and moderately resistant, respectively.

Table 2. Coefficient of Pearson' correlation among some disease related traits and morphological characteristics of 19 safflower genotypes.

Traits	LNF (cm)	WNF (cm)	LNM (cm)	WNM (cm)	PDN (%)	DLS (mm)	DVS (mm)	Height (cm)	Maturity
WNF	0.27								
LNM	0.66**	0.39							
WNM	0.43	0.42	0.56**						
PDN	-0.03	0.46*	-0.01	0.29					
DLS	0.68**	0.71**	0.70**	0.64**	0.48*				
DVS	0.33	0.03	0.08	0.49*	0.37	0.31			
Height	0.24	0.40	0.30	0.47*	0.53**	0.52*	0.51*		
Maturity	0.32	0.17	0.14	0.22	-0.06	0.13	0.06	0.35	
SRWC	0.05	0.03	0.04	0.35	0.04	0.13	0.27	0.27	0.54**

* and **: Significant at 5 and 1 % probability level, respectively.

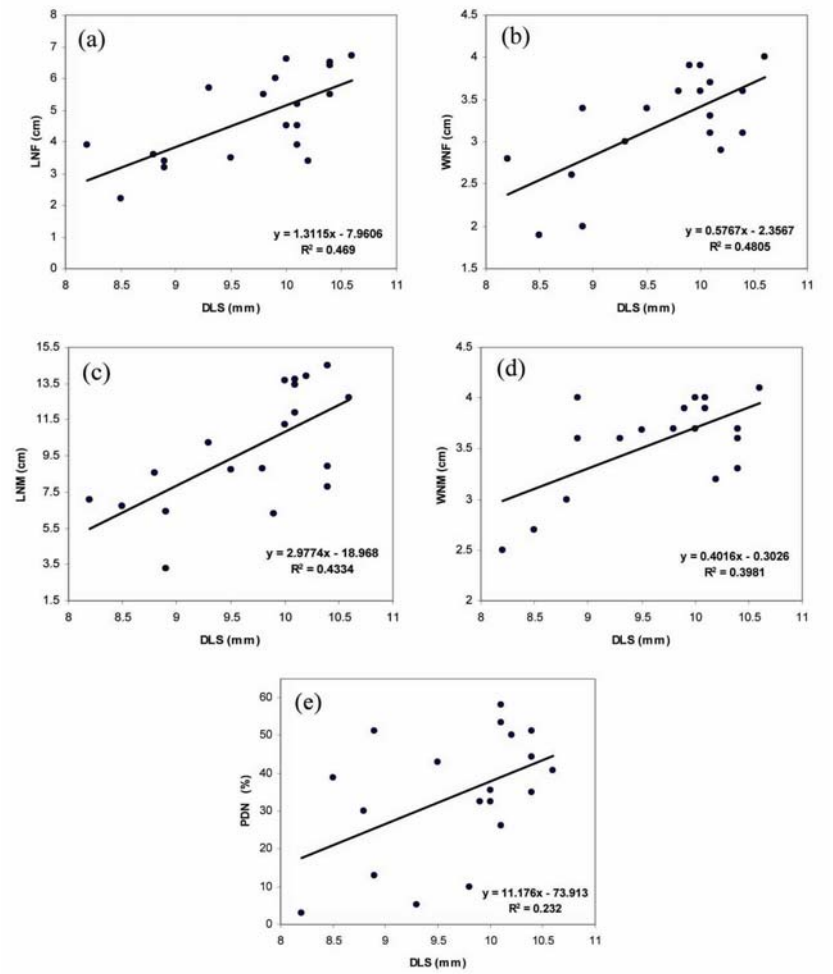

Figure 2. Relationship between mean diameter of lower stem (DLS) and mean values of five diseases related traits. (a) Length of necrotic lesion in flowering (LNF; $P = 0.0018$); (b) width of necrotic lesion in flowering (WNF; $P = 0.0013$); (C) Length of necrotic lesion in maturity (LNM; $P = 0.0034$); (d) width of necrotic lesion in maturity (WNM; $P = 0.0059$); (e) penetration depth of necrosis in the stem (PDN; $P = 0.0514$).

Discussion

This is the first work to study the reaction of safflower genotypes to charcoal rot disease caused by *M. phaseolina* in Iran. The results showed considerable genetic diversity among the studied genotypes for response to infection. The genotypes were grouped in different clusters in relation to their resistance. These results indicate that the North American cultivars CW-74 and AC-Stirling as well as the Iranian breeding lines IUT-K115 and GUA-Val6 were moderately resistant to charcoal rot disease. Therefore, these lines could be used as the basis of developing resistant safflower cultivars in the future. The discovery of a

moderate level of resistance to *M. phaseolina* in this study indicates that resistance to disease is present in current safflower germplasm sources, and this offers promise that future commercial cultivars with enhanced resistance can be developed.

Resistance to *M. phaseolina* in safflower may be associated with resistance to drought stress as reported in grain sorghum and soybean (Pratt et al., 1998). Although response to drought stress and also seed yield of the genotypes were not investigated in this study, CW-74 and AC-Stirling that had high yield and other suitable agronomic characters, and have been released for cultivation in rainfed area (Mundel et al., 1993). So these genotypes can be used for the crossing with Iranian genotypes to develop charcoal rot resistant genotypes. For better understanding of resistance in safflower genotypes to *M. phaseolina*, it is recommended that level of stress-related free amino acids to be measured in the next studies. Reduced growth of the pathogen within host tissues that was observed by length and width of necrotic lesion at flowering and maturity stages and penetration depth of the necrosis may be due to lower levels of the stress-related free amino acids proline and asparagine in resistant genotypes than susceptible genotypes as reported in soybean (Pearson et al., 1987).

Since field study of plant reaction to the pathogen is difficult and time consuming, breeders often search for easily and rapidly evaluated traits that are correlated to resistance. Results of this study indicated that due to the high positive and significant relationship between diameter of lower stem (DLS) and disease symptoms in the field, DLS might be a suitable trait for indirect selection among the materials to increase resistance to the charcoal rot disease in safflower. Therefore, this trait could be used as a selection index for improving resistant and/or tolerant genotypes to the charcoal rot pathogen in safflower.

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