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Inheritance of resistance to *Pythium ultimum* in safflower determined by generation means analysis

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Abstract

Pythium species cause seed rot, damping-off, and root rot of safflower (*Carthamus tinctorius* L.). In this study the genetics of resistance to damping off caused by *Pythium ultimum* was investigated in two different crosses of safflower, using generation means analysis (GMA). Generations P_1, P_2, F_1, F_2, F_3, BC_1 and BC_2 were developed to measure the percentage of un-emerged seeds (PUS), rate of seedling off (RSO), ratio of seedling off to total emerged seedlings (ROE), and disease susceptibility index (DSI). The ANOVA showed that seed emergence was faster in soil infected with the pathogen than in sterilized soil. GMA indicated that resistance was under genetic control with both simple and digenic interaction effects. The relative importance of additive and dominance genetic effects in controlling the resistance to the pathogen varied in two evaluated crosses. Based on the significant additive genetic effects, selection should facilitate the development of safflower cultivars resistant to *Pythium* damping-off. The low R^2 in regression analysis of generations mean showed that selection should be delayed to later generations, since each family can be evaluated in more replications over micro and macro environments. Negative estimates of [d] for DSI and RSO indicate that dominance effects conferred susceptibility.

Keywords: suspension, zoospore, epistasis, selection, heterosis.
Abbreviations : PUS: Percent of un-emerged seeds, RSO: Rate of seedling off , ROE: Ratio of seedling off to total emerged , seedlings, DSI: Disease susceptibility index of a genotype at *Pathogen*-infested soil.

Introduction

Safflower (*Carthamus tinctorius* L.), an increasingly important oilseed, belongs to the family Compositeae. It is a rich source of oil (35 to 40%) and linoleic acid content (75 to 86%). Traditionally, the crop was grown for its flowers, used for coloring and flavoring foods and making dyes, especially before cheaper aniline dyes became available, and in making medicines. The crop is cultivated in Iran, one of the oldest production areas, and in other parts of the world, due to its adaptability to different environmental conditions (Feizi et al., 2010). Currently, production in Iran is estimated at about 500 tons of safflower seed from an area of 1000 ha land (FAO, 2008). Although Iran does not have a big share of the world's production of safflower seed, the areas it has under safflower cultivation have increased in recent years because, in addition to the increased demand for oilseed crops to compensate for the lack of oil, Iran has numerous types of wild and cultivated safflower. Both the increased demand and the variety of types of safflower are of increased interest to researchers. Safflower cultivation suffers severely from soil borne fungal diseases, which reduce plant stands and threatens production due to pre- and post-emergence damping-off. *Pythium ultimum* Trow. has been isolated and identified as the primary causal agent of the damping-off in safflower, not only in Iran (Ahmadi et al., 2008; Ahmadinejad and Okhovat, 1976) but also in Canada (Bardin et al., 2003; Huang et al., 1992), the U.S (Thomas, 1970) and Australia (Stovold, 1973). Huang et al. (1992) identified the causal organism as *Pythium* sp. "group G" a form of *P. ultimum*. The pathogen invades water-absorbed or germinating seeds, the hypocotyls or first internodes tissues of seedlings and causes rotting and collapse of infected tissues and death of the seeds and seedlings in safflower (Kolte, 1985). Safflower was considered highly susceptible to *P. ultimum*, with less than 8 and 16 % survival of seedlings in artificially inoculated and naturally infected soils, respectively (Huang et al., 1992). Ahmadi et al. (2008) showed that *P. ultimum* could rot up to 4-43 % of seeds and kill up to 6-37 % seedlings of different safflower genotypes in irrigated breeding nurseries and experimental fields of Gorgan, in north Iran. Unfortunately, there is no long-term, sustainable option for controlling *Pythium* damping-off in commercial safflower fields. Incorporating genetic resistance into safflower cultivars would create an ideal, effective, and inexpensive method of control for this pathogen. According to the definition, in a resistant genotype, seeds and seedlings have suitable emergence and growth in soils infected with the pathogen and reach their reproductive phase and seed production stage with the least damping off. Although evidences of resistance to *Pythium* have been found in other crops (Rosso et al., 2008; Yang et al., 2005), no reports are available that identify safflower cultivars with complete and durable resistance to *Pythium* spp. Therefore, knowledge of

Table 1. Analysis of variance for rate and percent of emergence in seven generations (P_1, P_2, F_1, F_2, F_3, BC_1 and BC_2) of two safflower crosses evaluated for resistance to *Pythium* at greenhouse condition.

S.V.	df	Aceteria × 34074		LRV5151 × Arak2811	
		Percent of emergence	Rate of emergence	Percent of emergence	Rate of emergence
Inoculation (I)	1	220.998^{ns}	0.000^{ns}	53.971^{ns}	0.010^{ns}
Error	6	56.328	0.005	229.790	0.008
Generation (G)	6	68.166^{ns}	0.005^{**}	349.589^{**}	0.012^{*}
G × I	6	42.107^{ns}	0.003^{*}	33.738^{ns}	0.013^{*}
Error	40	35.188	0.001	63.618	0.006
CV (%)	-	6.68	12.01	9.21	13.09

*, ** and ns: Significant at *P<0.05,* significant at *P<0.01 and* non significant, respectively.

Fig 1. Symptoms of *Pythium ultimum* on seed and germinating seedlings of safflower.

the genetic basis and heritability of resistance is essential for the efficient development of resistant cultivars.There are no reports that offer information regarding genetic control of resistance to *P. ultimum* in safflower, although some reports on similar damping-off pathogens like *Phytophthora, Alternaria* and *Macrophomina* are available for safflower and other crop plants (Kozik et al., 1991; Mundel et al., 1997; Pahlavani et al., 2007). Resistance to *P. aphanidermatum* in the medicinal plant periwinkle appeared to be governed by a single gene with a broad-sense heritability of 85 and 79 %, depending on the date of evaluation (Kulkarni and Baskaran, 2003). In tomato, estimating the effects of genes contributing to *Phytophthora* root rot resistance, by generation means analysis, showed that the additive genetic effects were much greater than dominance genetic effects (Kozik et al., 1991). So they suggested family selection for improving resistance to *Phytophthora* root rot, since the magnitude of the environmental influence may be too high to realize any gain with single plant selections. Rosso et al. (2008) studied the inheritance of resistance to *Pythium* damping-off and root rot of soybean and its linkage with resistance to *Ph. sojae*. Their results showed that a single dominant gene confers resistance to *Pythium* damping-off in soybean caused by *P. aphanidermatum*. Also, resistance to *P. aphanidermatum* was not associated with the *Phytophthora* root rot resistance gene (Rosso et al., 2008). Zhao et al. (2005) made crosses between one resistance line and four moderately susceptible commercial soybean cultivars to *Rhizoctonia solani* and observed that additive gene action was significant in all populations. Based on the results, they suggested that selection for resistant soybean genotypes to *Rhizoctonia* root and hypocotyl rot using the conventional method is possible, but should be more efficient in later generations. Generation means analysis of resistance to root rot in red raspberry, caused by *Ph. fragariae*, indicated that the plant disease index showed an additive genetic variation with additional significant interactions, but the incidence of petiole lesions could be control by the non-additive genetic effects (Pattison et al., 2007). A dominant, two-gene model was shown to be the best fit for the observed segregation ratios when classification for resistance was based on a combination of all criteria measured. Recurrent selection is thus the appropriate approach for the development of new resistant cultivars (Pattison et al., 2007). More recently, sources of resistance to *Pythium* damping-off and seed rot have been identified within genotypes of cultivated safflower (Ahmadi et al., 2008), but no information is available on the inheritance of resistance to the disease in safflower. The main objective of this study was to determine the mode of inheritance of resistance to *Pythium* damping-off and seed rot in two safflower crosses, Aceteria × 34074 and LRV5151 × Arak2811. Specifically, we would determine the type of gene action controlling resistance. This information is essential for the development of Pythium-resistant varieties of safflower.

Materials and methods

This study was conducted at Gorgan University of Agricultural Sciences and Natural Resources (GUASNAR), Gorgan, Iran, during 2007-2010. For each cross, the experimental design was a two factorial model with: (i) generations, including P_1, P_2, F_1, F_2, F_3, BC_1 and BC_2 of each cross, (ii) inoculation including two levels, pathogen-infested and sterilized soils, and (iii) interaction of generation × inoculation (G×I).

Table 2. Generation means for two safflower crosses evaluated at *Pythium*-infested (I) and sterile soil (S) at greenhouse conditions.

Generation	Aceteria × 34074				LRV5151 × Arak2811			
	Percent of emergence		Rate of emergence		Percent of emergence		Rate of emergence	
	I	S	I	S	I	S	I	S
P_1	78.000^{ab}	92.500^{ab}	0.320^{b}	0.284^{b}	89.000^{a}	91.500^{a}	0.345^{a}	0.242^{cd}
P_2	79.500^{b}	92.000^{ab}	0.294^{b}	0.267^{b}	90.500^{a}	86.000^{a}	0.314^{a}	0.323^{bc}
F_1	85.556^{ab}	88.333^{ab}	0.389^{a}	0.277^{b}	86.667^{a}	89.167^{a}	0.524^{a}	0.267^{cd}
F_2	84.500^{ab}	88.667^{ab}	0.294^{b}	0.281^{b}	89.000^{a}	94.000^{a}	0.317^{a}	0.297^{cd}
F_3	93.500^{a}	94.000^{a}	0.300^{b}	0.268^{b}	72.000^{b}	72.000^{b}	0.268^{a}	0.228^{d}
BC_1	87.778^{ab}	85.833^{b}	0.334^{ab}	0.376^{a}	85.000^{ab}	91.667^{a}	0.310^{a}	0.441^{a}
BC_2	90.833^{a}	91.668^{ab}	0.321^{ab}	0.270^{b}	84.167^{ab}	91.667^{a}	0.331^{a}	0.413^{ab}

Means in the column followed by the same letter do not differ significantly at $P < 0.05$ according to LSD test.

Table 3. Analysis of variance for resistance to *Pythium* damping-off and seed rot in seven generations (P_1, P_2, F_1, F_2, F_3, BC_1 and BC_2) of two safflower crosses evaluated at the greenhouse.

S.V.	df	MS							
		Aceteria × 34074				LRV5151 × Arak2811			
		DSI	ROE	PUS	RSO	DSI	ROE	PUS	RSO
Block	3	0.728*†	0.033^{ns}	0.232^{ns}	0.017*	0.398**	0.042^{ns}	0.099^{ns}	0.009**
Generation	6	3.025**	0.128**	3.325**	0.013*	0.234*	0.067*	3.807**	0.013**
Error	13	0.244	0.013	0.489	0.004	0.079	0.022	0.351	0.001
CV (%)	-	21.22	24.14	22.89	41.48	6.75	23.21	15.24	12.63

PUS: Percent of un-emerged seeds; RSO: Rate of seedling off; ROE: Ratio of seedling off to total emerged seedlings; DSI: Disease susceptibility index at *Pythium*-infested soil.
*, ** and ns: Significant at 5*, 1% and* not significant, respectively.
†: $P < 0.07$

Plant materials

The parents used in this study were Aceteria and LRV5151 as *Pythium* damping-off resistant and moderately resistant, respectively; and 34074 and Arak2811 as *Pythium* damping-off susceptible genotypes. These genotypes were chosen as parents based on disease assessments for resistance to *Pythium ultimum* in a previous study (Ahmadi et al., 2008). The parents were purified for at least four generations before use in these studies. The genotypes were crosses derived from two F_1 hybrids viz. Aceteria × 34074 and LRV5151 × Arak2811 Each of F_1s was crossed to their susceptible and resistant parents to get BC_1 and BC_2 generations, respectively. On the same F_1s, F_2 and F_3, seeds were generated by self-pollination. The experimental materials were derived from each of the two crosses made during 2007 and 2009.

Inoculation of the pathogen in greenhouse

Reaction of the generations to *Pythium* was assessed at the research greenhouse (24 ˚C and 14 h days with light intensity approximately 95 µmol m^{-2}s) of the GUASNAR in the spring of 2010. Each experimental unit was a plastic potting tray containing 14000 cm^3 soil (clay loam with pH=7.7 and EC=0.96) that had been autoclaved three successive times. For each generation, 50 seeds were planted in each unit and each experiment was replicated 4 times. 10^5 zoospores ml^{-1} suspension of the pathogen was used for infecting the sterilized soils. Units were irrigated twice with zoospores suspension of the pathogen, first soon after seeding and then 5 days after that. Isolates of *Pythium* were taken from rotted seeds and apparently diseased seedlings in experimental field in 2006. The rotted seeds and 3 to 5 millimeters pieces of root from diseased plants were separated and thoroughly washed with distilled water, transferred to 0.5% sodium hypochlorite for 1 min and washed again in sterile water for 2 min and then cultured on potato dextrose agar (PDA) and corn meal agar (CMA) mediums. The cultures were incubated in 25°C during four days in darkness for isolation by a hyphal type method (Singelton et al., 1992). Pathogens were distinguished, based on zoospore forms, sporangium, zoospore, antridium, oogonium, number and joint between the antridiums and oogonium, according to monographs of Vander Plates-Niternik (1981). Production of zoospore suspension of the pathogen was based on the method of Rahimian and Banihashemi (1979). Briefly, 4 to 5 mm diameter pieces of fully grown agar plates were flooded in 500 ml Erlenmeyer flask containing sterilized distilled water and kept in light conditions for 72 h. These flasks were incubated for 10 minutes at 5°C and then kept for 2 h at room temperature to release zoospores. Zoospores concentration was estimated with a hemacytometer, and the appropriate dilution was made with sterilized distilled water to a final concentration of 10^5 zoospores ml^{-1}.

Pathogenecity studies and data analysis

Rate and percent of seedling emergence in all experimental units and percent of un-emerged seeds (PUS), rate of seedling off (RSO), ratio of seedling off to total emerged seedlings (ROE), and disease susceptibility index (DSI) at *Pythium*-infected units were determined. Seedling emergence was expressed as percent of seedlings that appeared normally on the soil surface per 50 planted seeds. Rate of emergence was determined by daily counting as described by Maguire (1962). Rate of seedling off (RSO) and ratio of seedling off to total emerged seedlings (ROE) were calculated. For the purposes of this study, resistance to the pathogen was viewed only in terms of the *Pythium* susceptibility index, DSI, a standardized ratio of seed emergence at *Pythium*-infected soil

Table 4. Generation means for two safflower crosses evaluated for resistance to *Pythium* damping-off at greenhouse conditions.

	Aceteria × 34074				LRV5151 × Arak2811			
	DSI	ROE	PUS	RSO	DSI	ROE	PUS	RSO
Generation								
P_1	1.717^{c}	0.361^{c}	3.152^{b}	0.130^{bcd}	3.804^{c}	0.560^{bc}	1.955^{e}	0.205^{c}
P_2	3.130^{b}	0.556^{b}	4.510^{a}	0.212^{b}	4.775^{a}	0.940^{a}	3.821^{bcd}	0.382^{a}
F_1	2.568^{bc}	0.520^{bc}	1.302^{c}	0.158^{bc}	4.379^{ab}	0.310^{c}	3.162^{d}	0.173^{cd}
F_2	1.829^{c}	0.361^{c}	3.411^{ab}	0.134^{bcd}	3.900^{c}	0.790^{ab}	3.623^{cd}	0.315^{b}
F_3	4.276^{a}	0.779^{a}	2.464^{bc}	0.338^{a}	3.916^{c}	0.623^{b}	5.261^{a}	0.198^{c}
BC_1	0.475^{d}	0.089^{d}	2.872^{b}	0.045^{d}	4.404^{ab}	0.564^{bc}	4.342^{abc}	0.138^{d}
BC_2	3.377^{ab}	0.649^{ab}	3.325^{b}	0.089^{cd}	4.183^{bc}	0.831^{ab}	4.928^{ab}	0.167^{cd}

PUS: Percent of un-emerged seeds; RSO: Rate of seedling off; ROE: Ratio of seedling off to total emerged seedlings; DSI: Disease susceptibility index at *Pythium*-infested soil. Means in the column followed by the same letter do not differ significantly at $P < 0.05$ according to LSD test.

to no-*Pythium* soil. DSI is defined as follows (Fischer and Maurer, 1978): $DSI=(1- I/S)/ (1- \bar{I}/\bar{S})$ where I and S represent the percent of seed emergence of a specific generation of *Pythium*-infected soil and sterilized soil, respectively, and $\bar{I}$ and $\bar{S}$, refer to mean percent of seed emergence of all generations at *Pythium*-infected soil and sterilized soil, respectively. All analyses were performed with the Statistical Analysis System (SAS, 2004). Prior to analyses, percentage data were transformed by calculating the square root. Data for each cross were subjected to analysis of variance, and means were compared with Fischer's protected LSD ($P < 0.05$). Where the interaction between generation and inoculation (G×I) was significant, slicing of the interaction was done, and LSD comparisons for mean generations were separately performed at each level of the other factor (inoculation). The parents, F_1, F_2, F_3 and backcross means for the PUS, RSO, ROE and DSI were analyzed according to the generation means analysis of Mather and Jinks (1971) to estimate parameters for the genetic model containing additive, dominance and digenic interaction effects. The F_3 generation was employed in the analysis of both crosses, and the new biometrical model was modified from the six parameter model suggested by Mather and Jinks (1971), to obtain the specification matrix to calculate for the seven generations. Each of the generations used in this study was expressed in terms of the following effects: [m] = overall mean; [a] = pooled additive genetic effects; [d] = pooled dominance genetic effects; [aa] = pooled additive by additive genetic effects; [ad] = pooled additive by dominance genetic effects; and [dd] = pooled dominance by dominance epistatic effects. Suitability of genetic models was tested by a least squares regression technique and the goodness-of-fit of each model was tested by a χ^2.

Results

Analysis of variance

The pathogen, *Pythium ultimum,* invaded the seeds, hypocotyls, cotyledons or some parts of germinating seedlings and caused seed rot and damping-off in the infected soils (Fig. 1). The results of the ANOVA and LSD means comparison test revealed that there is a considerable difference between parents and among the other generations for percent of emergence and rate of emergence in cross Aceteria × 34074 and LRV5151 × Arak2811 (Table 1 and 2). Although inoculation (infesting soil with *Pythium*) did not have significant effects on both rate and percent of emergence, the generation × inoculation interaction was significant for rate of emergence in the both crosses (Table 1). As shown in table 2, in cross Aceteria × 34074, the highest rate of emergence in Pythium-infected soil was observed in F_1 but in sterilized soil it belonged to BC_1. For cross LRV5151 × Arak2811, although there is no significant difference in the seven generations for rate of emergence in infected soil, in sterilized soil, the highest and the lowest rate of emergence belonged to BC_1 and P_1, respectively (Table 2). The ANOVAs for the two crosses Aceteria × 34074 and LRV5151 × Arak2811 showed highly significant differences among the generations (P_1, P_2, F_1, F_2, F_3, BC_1 and BC_2) for DSI, ROE, PUS and RSO in soil infected with the pathogen *Pythium ultimum* (Table 3). Table 4 shows LSD means comparison test for DSI, ROE, PUS and RSO of the 7 safflower generations in *Pythium*-infected soil. For disease susceptibility index (DSI) in the Aceteria × 34074 cross, means of the parents were significantly different, 1.717 and 3.130, respectively. The DSI of F_1 (2.568) was intermediate, and BC_1 and BC_2 with DSIs of 0.475 and 3.337 were similar to P_1 and P_2, respectively (Table 4). Also the highest DSI in cross Aceteria × 34074 was observed in F_3 generation (Table 4). In cross LRV5151 × Arak2811, the highest and lowest DSI in *Pythium*-infected soil belonged to P_2 (4.370) and P_1 (3.804). F_1, BC_1 and BC_2 had DSIs that ranged between the two parents (4.379, 4.404 and 4.183, respectively), and DSIs of F_2 and F_3 had no significant difference from P_1 (Table 4). Significant differences between parents in the ratio of seedling off to total emerged seedlings (ROE) were obtained in both crosses. For the cross Aceteria and 34074, the extreme ROEs belonged to F_3 (0.779) and BC_1 (0.089), while the F_1 (0.520) and F_2 (0.361) generations were similar to P_1 (Table 4). However, for the cross LRV5151 × Arak2811, F_2 and BC_2 tended toward the intermediate parents, and F_3 and BC_1 tended toward P_1, the lower ROE parent. The F_1 generation of LRV5151 × Arak2811, had the lowest ROE in a *Pythium*-infected environment (0.310; Table 4). In both crosses, percent of un-emerged seeds (PUS) of the parents in *Pythium*-infected soil were significantly different (Table 4). In the first cross, mean PUS for the parents P_1 and P_2 were 3.152 and 4.510, respectively (Table 4). The generations F_2, F_3, BC_1 and BC_2 had no significant difference from the lower parent P_1. However, PUS for the F_1 generation in this cross was significantly lower than others, including the lower parent (1.302; Table 4). For the LRV5151 × Arak2811, an intermediate value of PUS was found for the F_1 generation (Table 4). In this cross also, the mean PUS of the F_3 (5.261) was significantly higher than other generations, including the higher parent (3.821). Moreover, the means of the F_1, F_2, BC_1 and BC_2 generations were similar to P_2, the higher parent (Table 4). Average of rate of seedling off (RSO) for parental generations in *Pythium*-infected soil were observed to be

Table 5. Genetic effects estimated for resistance to *Pythium ultimum* in seven generations of two safflower crosses (P_1, P_2, F_1, F_2, F_3, BC_1 and BC_2) at greenhouse conditions.

Parameters	Aceteria × 34074				LRV5151 × Arak2811			
	DSI	ROE	PUS	RSO	DSI	ROE	PUS	RSO
[m]	5.787^{**}	1.107^{**}	1.829^{*}	0.585^{**}	3.620^{**}	0.499^{**}	4.816^{**}	0.320^{**}
[a]	0.715^{**}	0.096^{ns}	0.685^{*}	0.040^{ns}	-0.489^{**}	-0.188^{*}	-0.933^{**}	0.086^{*}
[d]	-9.987^{**}	-1.918^{**}	$4.558^{*\dagger}$	-1.284^{**}	1.034^{ns}	0.919^{ns}	1.097^{ns}	-0.339^{ns}
[aa]	-3.301^{**}	-0.638^{**}	1.961^{*}	-0.412^{**}	0.687^{ns}	0.240^{ns}	-1.842^{*}	-0.038^{ns}
[dd]	6.697^{**}	1.319^{**}	-5.053^{*}	0.855^{**}	-0.282^{ns}	-1.101^{*}	-2.808^{ns}	0.198^{ns}
[ad]	4.373^{**}	0.923^{**}	-0.653^{ns}	0.010^{ns}	1.420^{*}	-0.195^{ns}	1.042^{ns}	0.118^{ns}
R^2 (%)	74.97	71.37	69.83	55.77	44.80	61.75	67.88	40.16
χ^2	1.256^{ns}	0.037^{ns}	0.575^{ns}	0.006^{ns}	2.544^{ns}	0.843^{ns}	22.614^{**}	0.038^{ns}

PUS: Percent of un-emerged seeds; RSO: Rate of seedling off; ROE: Ratio of seedling off to total emerged seedlings; DSI: Disease susceptibility index at *Pythium*-infested soil. *, ** and ns: Significant at *P<0.05,* significant at *P<0.01 and* not significant, respectively.
†: P < 0.09

different, although the difference was significant only in the cross LRV5151 × Arak2811 (Table 4). In Aceteria × 34074 the RSO of F_1 was the same as in the parents, and in the other cross, the mean RSO of F_1 (0.173) was the same as of the lower parent (Table 4). The highest RSO in generations of Aceteria × 34074 belonged to F_3 (0.338), and the values of BC_1 (0.045), BC_2 (0.089) and F_2 (0.134) had no significant difference from P_1 (0.130), the lower parent of the cross. In the other cross, LRV5151 × Arak2811, the values of RSO in a pathogen infected environment for the F_1, F_3 and BC_2 generations were as low as those of the lower parent (Table 4). Mean RSO of F_2 (0.315) was in the range of parents, and BC_1 (0.138) generation had extremely lower values than the parents (0.205 for P_1, and 0.382 for P_2; Table 4). After the analysis of generation means using the Mather and Jinks (1971) method, the complete additive-dominance model, including parameters [m], [a], [d], [aa], [dd] and [ad], was tested to assess the importance of each genetic effect on the control of the studied traits in *Pythium*-infected soil. The parameters [m], [a], [d], [aa], [dd] and [ad] represent mean effect, additive genetic effect, dominance (non-additive) genetic effect, additive × additive epistatic genetic effect, dominance × dominance epistatic genetic effect and additive × dominance (and or dominance × additive) epistatic genetic effect, respectively. The adequacy of the model, estimates of parameters and their significance are presented in Table 5.Regression analysis indicated that over 75 % of generations, variation for disease susceptibility index in *Pythium*-infected soil (DSI) was explained by the six parameter model (Table 5). The non-significant χ^2 showed that the complete additive-dominance model could adequately account for all of the variations observed in these seven generations of cross Aceteria × 34074 (Table 5). The mean effect [m] of crosses Aceteria × 34074 and LRV5151 × Arak2811 for DSI was 5.787 and 3.620 %, respectively (Table 5). In cross Aceteria × 34074, single effects including additive [a] and dominance [d], had a significant role in control of DSI in these generations. Dominance effect showing negative sign (toward the lower or resistant parent, P_1) had the greatest magnitude among all the single and epistatic parameters. In comparison to dominance and epistatic parameters, additive effects [a] were small in magnitude (Table 5). All epistatic effects in the model of this cross, including additive × additive [aa], dominance × dominance [dd] and additive × dominance [ad] were significant (Table 5). However, negative effects of [d] were reduced by positive [dd], and positive [a] was compensated by negative [aa], and the sum of the [d]+[dd] was greater than the sum of [a]+[aa] effects for DSI in cross Aceteria × 34074. In the other cross, LRV5151 × Arak2811, although the six additive-dominance parameter model was adequate (χ^2=2.544, P>0.05), just [a] and [ad] genetic parameters had significant effects on control of DSI in these generations (Table 5). Over 44 % of variation among generations mean was explained by a regression model with the six parameters (Table 5). In contrast to cross Aceteria × 34074, additive genetic effect [a] was negative but dominance effect [d] was positive. The regression analysis for ratio of seedling off to total emerged seedlings (ROE) in *Pythium*-infected soil showed that the six parameter model explained about 71 and 62 % of variation, respectively, for crosses Aceteria × 34074 and LRV5151 × Arak2811; and the chi-square goodness-of-fit test for the single and epistatic effects was not significant in both crosses. Hence, the model used was adequate for explaining the variation for ROE in both crosses. The mean effect [m] of ROE for the crosses Aceteria × 34074 and LRV5151 × Arak2811 was 1.107 and 0.499, respectively (Table 5). In contrast to the cross Aceteria × 34074, in which DSI was significantly controlled by dominance and epistatic effects ([d], [aa], [dd] and [ad]), in the cross LRV5151 × Arak2811, the trait was governed by additive [a] and additive × dominance [ad] genetic effects (Table 5). The sign of dominance effects [d] for ROE in cross Aceteria × 34074 was negative (toward the higher or resistant parent). The signs of [a] and [d] in the genetic model of ROE were different for each crosses (Table 5).Regression analysis indicated that the six parameter model explained greater than 70 and 68 % of the total genetic variation of percent of un-emerged seeds (PUS) in *Pythium*-infected soil among generations in Aceteria × 34074 and LRV5151 × Arak2811, respectively (Table 5). An additive-dominance model was found to be adequate for PUS just in cross Aceteria × 34074 (χ^2 = 0.575, P>0.05; Table 5). The results of generation means analysis showed that significant effect was found for the all genetic parameters in the model of Aceteria × 34074 except for additive × dominance [ad] epistatic effect. Great and significant values of dominance [d] and dominance × dominance genetic effect [dd] were more important than the other genetic effects in this model. For cross LRV5151 × Arak2811 significant χ^2 revealed that an additive-dominance model could not account adequately for the variation present for PUS. However, additive [a] and additive × additive [aa] epistatic effect had a significant negative role in control of PUS in generations of this cross (Table 5). In the populations of crosses Aceteria × 34074 and LRV5151 × Arak2811, an additive-dominance model could explain about 56 and 41 %, respectively, of total observed variation of RSO in *Pythium*-infected soil. The adequacy of

these models was also tested by predicting the seven family means from the estimates of the six parameters and calculating χ^2. The value of the chi-square test (χ^2= 0.006 and 0.038) indicated that the models were adequate for a genetic explanation of RSO in both studied crosses (Table 5). Additional analysis revealed significant [m] effect for RSO in both Aceteria × 34074 and LRV5151 × Arak2811 (Table 5). For the cross Aceteria × 34074, [d], [aa] and [dd] had a significant role in controlling mean RSO in the studied generations. However, in the other cross, LRV5151 × Arak2811, no dominance nor epistatis effects ([d], [aa], [dd] and [ad]) were observed for RSO, and [a] was the only significant effect in this model.

Discussion

Significant mean squares for percent of emergence, rate of emergence, DSI, ROE, PUS and RSO in both crosses confirmed that heritable factors had a considerable role in controlling variations among means of these generations. So, the breeding method could be used for improving resistance to *P. ultimum* in safflower. Significant genetic variation for resistance against *Pythium* and other plant pathogens over different generations have been revealed by earlier researchers (Rosso et al., 2008; Zhao et al., 2005; Liu et al., 2005; Bokmeyer et al., 2009). Of the four parental genotypes evaluated in this study, Aceteria and Arak2811 appear to have the lowest and highest levels of susceptibility to *Pythium* infection when measuring DSI. Among the non-parental generations, BC_1 of both crosses were the least and most susceptible generations, respectively. Generally, the means of the parents (Aceteria and 34074) tended to show more extreme contrasts than the means of the F_1 and F_2 generations for all traits in *Pythium*-infected soil. The least significant comparisons test also demonstrated that differences between the parents of each crosses were indeed real and significant. As expected, the backcrosses (BC_1 and BC_2) showed means that tended to be located close to those of their respective recurrent parents (P_1 and P_2, respectively). These results confirmed the choice of parents for the present study for contrast, which is a prerequisite for generation means analysis as proposed by Mather and Jinks (1971). Significance of mean squares for generations by inoculation interaction shows that rate of emergence of the evaluated generations in the both crosses varied between *Pythium*-free and *Pythium*-infected soils. It means that the rate of emergence of these seven generations is significantly affected by the presence or lack of the pathogen in soil. Therefore, different genetic factors control the rate of emergence of these safflower generations in sterile soil and soil infected with *P. ultimum*, and it also means that different breeding methods are probably needed for improving the rate of emergence in each type of soil. Kulkarni and Baskaran (2003), in a study on *Pythium* dieback in the medicinal plant periwinkle, and Kozik et al. (1991), in a study on *Phytophthora* Root Rot in tomato, also observed that there were clear-cut differences between mean disease ratings in plants from basic genetic generations in pathogen-infected and sterile soil. In contrast to the rate of emergence, the mean squares of generations by inoculation interaction for percent of emergence did not differ significantly either in Aceteria × 34074 or in LRV5151 × Arak2811. This finding shows that negative effects of the pathogen on safflower early growth are more a reduction in the speed of emergence than in the number of emerged seeds. The higher rate of emergence of all studied generations in *Pythium*-infected soil in relation to *Pythium*-free soil confirms these results. These results agree with Mundel et al. (1995) who found that *Pythium*-infected soil reduced speed of emergence in safflower. Complete dominance was suggested for disease susceptibility index (DSI) and ratio of seedling off to total emerged seedlings (ROE) in *Pythium*-infected soil in both crosses, because, despite significant differences between the parents, there were no significant differences between parents, backcrosses and F_1 generations. The mean of the F_1 generation for percent of un-emerged seeds (PUS) in cross Aceteria × 34074 was out of the both parents' mean, suggesting over dominance. It is interesting that there was complete dominance for PUS in the other cross, LRV5151 × Arak2811. These results agree with those of Kulkarni and Baskaran (2003) and Pattison et al. (2007) which indicated that dominance genes are involved in the expression of resistance to *Pythium* dieback in medicinal plant periwinkle and *Phytophthora* root rot resistance in red raspberry, respectively. In both crosses, almost for all traits including DSI, ROE, PUS and RSO in *Pythium*-infected soil, if the mean F_1 was more similar to resistant parent, the mean F_2 tended to the susceptible parent, and mean F_3 tended to the resistant parent again, and vice versa. This is in agreement with Falconer and Mackay (1996) who mentioned that heterosis and inbreeding depression are two opposite genetic phenomena controlling by genes showing dominance effects. Heterosis was observed for resistance to *Mycosphaerella pinodes* in *Pisum sativum* (Zhang et al., 2007), resistance to *Ph. infestans* in tomato (Abreu et al., 2008) and resistance to leaf and stem blight in cucumber (Amand and Wehner, 2001), but reports about inbreeding depression of resistance to fungi pathogens in plants are rare. The effects of genes controlling resistance to *P. ultimum* damping off and emergence speed of safflower in pathogen-infected soil were determined by a generation means analysis. The resistance to the pathogen and rate (speed) of emergence in *Pythium*-infected soil were measured by DSI and rate of seedling off (RSO), respectively. The results of the present study clearly show that both additive and dominance genetic effects play a significant role in control of these traits. However, in cross Aceteria × 34074, the dominance effects were much greater than additive genetic effects, and in the other cross, LRV5151 × Arak2811, the additive effects were significant in relation to dominance genetic effects. The present study also revealed that epistasis may be either present or absent, and the significance of additive, dominance, and epistatic gene effects may vary a little over the two evaluated crosses. Some earlier studies provided evidence that in a crop species, epistasis might occur in some of evaluated crosses and be absent in some others (Snijders, 1990). It refers to the specific combining ability of the parents and means that choosing the parents is important in the processes of crossing and hybrid seed production. The observation of different genetic control in various crosses is in agreement with those reported by Bai et al. (2000) for resistance to *Fusarium graminearum* in wheat and Amand and Wehner (2001) for resistance to gummy blight (*Didymella bryoine*) in cucumber. Negative estimates of dominance [d] and additive [a] gene effects for DSI and RSO in these crosses indicate that dominance and additive effects contribute more to susceptibility than to resistance. Bai et al. (2000) evaluated eleven *Fusarium* resistant × susceptible crosses in wheat and concluded that dominance conferred susceptibility in some of the crosses. The observation of statistically significant non-allelic epistasis effects ([ad]) indicated that more than one gene is involved in control of DSI in *Pythium*-infected soil. In common bean, resistance to *Pythium ultimum* has also been proved to have a polygenic nature (Campa et al., 2010).

Bruehl (1983) postulated that resistance to nonspecific pathogens, such as *Pythium*, is generally nonspecific and is expected to be durable; the durability of the nonspecific resistance is believed to be the results of polygenic nature (Johnson, 1983). Campa et al. (2010) evaluated QTL for resistance to *Pythium ultimum* in common bean by studying 97 F_7 recombinant inbred lines (RIL) developed from the cross of Xana, a highly susceptible variety, to Cornell 49242, a resistant line. Their results suggested both qualitative and quantitative modes of genetic control of resistance to the pathogen involving three different genomic regions. Finding a not-so-high R^2 in regression analysis for all traits in both crosses shows that magnitude of the environmental influences may be too high. It clearly shows that numerous genes control the resistance to *P. ultimum* in these safflower populations. So, realizing any gain with single plant selections is hardly possible. Therefore, selection should be delayed to the next generations, since families can be replicated in field and greenhouse studies. Kozik et al. (1991) also reported that family selection would perform for *Phytophthora* root rot in tomato because the environment had huge effects on the response of plants to the pathogen. The considerable impact of environmental factors on the incidence of *Pythium* damping-off in safflower and other crops has been proved by others researchers (Pahlavani et al. 2009; Ben-Yephet and Nelson, 1999; Mundel et al., 1995).

Conclusion

The general results of this study are promising for safflower resistance to *Pythium* damping off improvement because the additive effects are useful in breeding self-pollinating crops. Generation means analysis indicated that a simple additive-dominance model accounted for most of the genetic resistance in these two safflower populations. These results will help to further our understanding of the genetics of resistance to *P. ultimum* in safflower. Also, safflower cultivars with satisfying resistance to *P. ultimum*, the causal agent of damping off and seed rot, may be developed through crossing two adapted but moderately to highly resistant genotypes, because dominance genetic effects were great and significant. The genetic features described here that confer damping off resistance indicate that the development of resistant F_1 hybrids will be a long term effort requiring a good combining ability and resistance in both parental lines.

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