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Improving field establishment of safflower in soils infected by *Phytophthora drechsleri* and *Pythium ultimum*

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Abstract

One of the major field constraints to seed production in safflower has proven to be soil born pathogens, *Phytophthora drechsleri* and *Pythium ultimum*. In order to evaluate the efficiency of a field-laboratory selection method to improve resistance of safflower against soil born pathogens, *Ph. drechsleri* and *P. ultimum*, a two-year investigation was conducted. The results showed that selection is an efficient method for increasing resistance to seed and seedling death caused by *Phytophthora* and *Pythium* in safflower. Selection could have improved germination percent, days to 50% germination, percent of undamaged seedlings and index of disease percent when genotypes were faced with both pathogens. Regarding kind of damages, *Pythium* caused more seed rot and *Phytophthora* induced more seedling death. It could be concluded that selection for resistance to *Pythium*, could also increase resistance to *Phytophthora*. Safflower was, however, more susceptible to *Phytophthora* than *Pythium*. Also, genes for resistance to seed rot are different than those controlling resistance to seedling death, so pre-emergence damping off should be considered a completely independent trait from post-emergence damping off in safflower. It was concluded that used field-laboratory selection method could well improve resistance of safflower to pathogens *Phytophthora* and *Pythium* and hence seed yield.

Keywords: Generation; Additive effects; Fungus; Susceptibility.

Introduction

Safflower (*Carthamus tinctorius* L.) is an important oilseed plant and a main crop in arid and semiarid regions of the world. This plant originated

from southwestern Asia, the Mediterranean area and Iran (Knowels, 1969). Safflower seeds contain about 25 to 45 percent oil and 12 to 24 percent protein. Among the 80 usual oilseed plants, safflower has the highest unsaturated fatty acid linoleic, so that it is classified as a high quality edible as well as industrial oil (Dubois et al., 2007; Dajue and Mundel, 1996). In recent years, production of the crop has declined noticeably in Iran because of lack of high production and pest and disease resistant cultivars (Zeinali, 1999). So, breeding new safflower cultivars with enhanced levels of resistance to biotic and abiotic stresses, especially plant disease, could increase the crop production. Cultivation of safflower is severely affected by soil pathogens attacking seeds and young seedlings (Huang et al., 1992). Some of these pathogens cause seed rot, pre- and post- emergence damping off, root and hypocotyl decay (Zeinali, 1999). Pathogens *Phytophthora drechsleri* Tucker and *Pythium ultimum* Trow are the most important causal agent of seed rot, damping off and death of underground parts in safflower and many other plants and are widespread in all arable lands in the world (Huang et al., 1992; Mundel et al., 1997). The first observation of seedling death or damping off in safflower was reported by Classen from Nebraska in 1949 (Cormack and Harper, 1952). This disease has been reported in many countries like Australia, Argentina, India, Mexico, Afghanistan and Iran (Sharifnabi and Saiedi, 2004). In Iran, safflower damping off in cultivar Frio, caused by *Ph. Drechsleri,* was reported for the first time by Al-Agha (1970). *Ph. drechsleri* has been considered as a pathogen for different disease in crop plants that could create symptoms like root rot, damping off, leaf and stem blight and also necrosis in fruit, stem and crown; it usually results in killing the host. *Phytophthora* rotting can damage other economically important plants like tomato and alfalfa as well as safflower (Irwin et al., 1979; Klisiewicz, 1977). *P. ultimum* is also an economically harmful pathogen for a range of crop and non-crop plants (Martin and Loper, 1999). Besides safflower, damping off and seed rot caused by *Pythium* have reported in wheat, canola, common pea, sugar beet and common bean (Higginbotham et al., 2004; Bardin et al., 2004; Ahmadzadeh et al., 2004). The most important tactics that have been proposed and used to reduce the damages of *Phytophthora* and *Pythium* are chemical fungicides, cultural managements and resistant cultivars. Breeding and cultivation of resistant cultivars is considered, by safflower researchers and producers, the most efficient, cost effective and environmentally acceptable means of controlling the disease.

Safflower is a predominantly self-pollinating crop with the genetic potential of outcrossing over 50% depends on environmental conditions (Dajue and Mundel, 1996). The higher outcrossing falls safflower in the category of often cross-pollinated crops and may result in heterogeneity builds up quickly in the populations. This makes the populations suitable for developing superior cultivars by means of selection breeding. Selection involves sorting out and propagating individual genotypes or groups of genotypes from mixed populations or from segregating populations (Poehlman, 1987). If a group of similarly appearing plants are selected and harvested and the seed composited, the resulting mixture is known as a mass selection. Mass selection from fields naturally infested with a multitude of diseases has been used by plant breeders of safflower to develop cultivars with improved resistance to several diseases (Dajue and Mundel, 1996). Sclerotinia head rot (*Sclerotinia sclerotiorum*) resistance was incorporated into the first registered canadian safflower cultivar, Saffire, by performing mass selection in disease nurseries (Mundel et al., 1985). Success in selection depends on the amount of genetic variation in the population and the mode of genetic action of resistance, so that selection will be effective just for traits governed by additive genetic effects.

The presence of genetic variation in Iranian safflower populations has been proven in various studies (Bagheri et al., 2001; Maali-Amiri et al., 2001). Screenings of safflower genotypes to find sources of genetic resistance to the diseases have always been attended by breeders in an experiment done by Heritage and Harrigan (1984) on safflower lines from different region of the world, one line originating from Iran showed satisfactory resistance to lower stem infection in both field and glasshouse screenings. The results of Thomas and Hill (1977) showed that resistance to *Ph. drechsleri* is controlled by one single dominant gene. Some studies also indicated that the non-cultivated species, *Carthamus oxyacanthu*s, is a profitable gene source of resistance to *Fusarium* disease that could be utilized in safflower breeding schemes (Derakhshan et al., 2011). Nikmanesh et al. (2011) showed that one cycle of selection improved percent of emergence of four safflower genotypes in the soil infested by *P. ultimum*. They also declared that their observation of significant response to selection means that genes controlling resistance to the pathogen are governed by additive genetic effects. Evaluation of susceptibility to mixture of *Phytophthora* and *Fusarium* at laboratory and greenhouse conditions showed that there is a considerable difference among safflower genotypes in

reaction to the disease (Nasehi et al., 2010). Since most of agricultural fields in Iran are naturally infested by *Phytophthora* and *Pythium* and since just a few studies have been so far been done for improving resistance to both of these pathogens at the same time, this current study was performed.

With the aim to reduce loss of safflower production in fields infecting to fungi *Phytophthora* and *Pythium* this study was designed. It was conducted (1) to estimate and compare the degree of susceptibility against two pathogens *Phytophthora* and *Pythium* in safflower, (2) to evaluate the effects of selection on the improvement of seed germination and seedling survival in media infested by separate or simultaneous inoculation of the pathogens and (3) to test the hypothesis that selection for resistance to *P. ultimum* could improve resistance to the other pathogen, *Ph. drechsleri.*

Materials and Methods

This study was conducted plant breeding laboratory and research farm of Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran, during 2010 and 2011. The experiment had two parts: the selection of seedlings resistant to the pathogen *P. ultimum* (First year, 2010) and the evaluation of progenies of the seedlings resistant to the pathogens *Ph. drechsleri* and *P. ultimum* (Second year, 2011). Four open-pollinated safflower genotypes including cultivars Aceteria (originated from Canada), LRV (originated from Iran), Arak-2811 (originated from Iran) and breeding line 34072 (unknown origination) were employed in this study. In the first year, a zoospore suspension of *P. ultimum* was applied for infection. The fungus was cultured on corn-meal-agar (CMA) medium, after which square fragments of the culture were poured into the autoclaved water and then kept for four days under fluorescent light to activate zoospores. Then the number of zoospores was counted by a hemocytometer and the suspension was prepared with a final concentration of 10^5 zoospores in one milliliter. The seeds, after having been disinfected with five % bleach (Sodium hypochlorite) for three minutes, were rinsed until clear with distilled water and then immersed into the suspension of *P. ultimum* for ten minutes to make sure that the seeds were inoculated. Fifty inoculated seeds of each genotype were arranged on a paper towel soaked with the pathogen suspension (Kozik et al., 1991; Govindappa et al., 2005). Another paper towel of the same size was put on over each towel to cover the seeds. The paper towels containing seeds were rolled and put in a plastic bag to prevent

loss of water. All towels containing seeds were then placed in a dark incubator at 22±2 degree centigrade for six days; water was added as needed to towels to keep them moist. During the incubating time, apparently healthy seedlings in the infected paper towels were kept, while infected seedlings and ungerminated or rotted seeds were discarded. By the seventh day, surviving healthy seedlings of each genotype were planted in separate rows in the field. To obtain same-year seeds for final evaluation, one row of unselected seeds was also planted for every genotype. Adequate humidity conditions were provided by sprinkler irrigation from the time of planting to the emergence of seedlings above the ground. The plants were totally covered by suitable textile net to prevent insects from obtaining self-pollinated seeds. At maturity time, seeds produced on resistant plants of the genotypes were harvested separately. In the second year, seeds collected from *Pythium*-resistant seedlings in the first year, along with unselected seeds, were used for evaluation. The second year evaluation was performed in four different conditions: infection by suspension of *P. ultimum*, infection by suspension of *Ph. Drechsleri*, infection by mixed suspension of the two pathogens and distilled water as control. All experiment and inoculation conditions were similar to the first year. The concentration of the mixed suspension was also adjusted in 10^5 zoospores in milliliter (the proportions of two pathogens were identical in the suspension).

The germinated seeds, diseased seedlings and healthy seedlings were counted daily for six days; meanwhile, some water was added to the paper towels that needed humidity. Recorded traits consisted of percent of germination, percent of diseased seedlings, percent of healthy seedlings, days to 50% germination and disease index. Percent of germination, percent of diseased seedlings and percent of healthy seedlings was computed by using the proportion of cumulative numbers of germinated seeds, diseased and healthy seedlings to total number of seeds in each experimental unit, respectively and expressed as percent.

Days to 50% germination was computed according to the formula of Hartman et al. (1990) and for disease index the equation 1 was employed.

$$Disease\ index = \frac{Percent\ of\ diseased seedlings}{Percent\ of\ germinated seeds} \times 100 \quad (1)$$

For better understanding of selection effect on the characteristics of each genotype, an index, namely selection effect, was defined as equation 2.

$$Selection\ effect = \frac{\overline{X}_{Selected\ population} - \overline{X}_{Unselected\ population}}{\overline{X}_{Unselected\ population}} \times 100 \quad (2)$$

This index shows the amount of improvement after one cycle of selection in the population.

The experiment was conducted as a completely randomized design (CRD) with unequal replicates for each factorial. The two factors were genotype (with four levels) and selection (with two levels, selected and unselected). Data analysis was performed by SAS software (SAS, 2002). Least significant difference (LSD) at 1% statistical level was done for means comparison.

Results

As might be expected, both percent of germination and days to 50% germination were lower in *Pythium* and *Phytophthora* infected media than no-infected control (distilled water). Also, there was no statistical difference among the genotypes for percent of germination and days to 50% germination when treated with distilled water (Data not shown). This allowed applying data of infected environments in the statistical analysis without any subsequent correction.

Assessment of the selected populations at Pythium-infected media

Analysis of variance showed that the selection, the genotype and selection × genotype interaction had a significant effect on all studied characteristics over the media infected by pathogen *P. ultimum* (Table 1). Means comparison revealed that selection improved percent of germination, percent of diseased seedlings, percent of healthy seedlings, days to 50% germination and disease index of the genotypes in facing with *P. ultimum* (Table 2). The selection effect for each genotype is displayed in Table 2. Percent of germination for selected population of genotype Aceteria was increased 24% relative to before-selection population, but just 7% for genotype 34072 (Table 2). Selection also speeded up seed germination in the studied populations as the days to 50% germination of genotype Arak-2811 were reduced about 36% and from 2.20 reached 1.40 (Table 2). Percent of diseased and healthy seedlings in after-selection population of Aceteria showed an 88% decrease and a 409% increase, respectively.

Table 1. Analysis of variance for seed germination and seedling disease before and after selection populations of safflower in environment infected to pathogen *P. ultimum.*

Source of variation	df	Percent of germination	Days to 50% germination	Percent of diseased seedlings	Percent of healthy seedlings	Disease index (%)
Selection†	1	2300.59**	4.36**	27958**	32226.01**	33527.10**
Genotype	3	172.42**	0.19**	98.84	289.95**	406.52*
Selection×Genotype	3	127.58*	0.26**	233.88	343.24**	405.03*
Error	54	36.52	0.01	99.67	88.77	132.75

* and ** Significant at 5 and 1% level, respectively.
† Selection was done for resistance to *P. ultimum* in the previous generation.

Table 2. Means comparison for seed germination and seedling disease of before and after selection and also selection effect of four open-pollinated safflower genotypes over environment infected to pathogen *P. ultimum.*

Genotype		Percent of germination	Days to 50% germination	Percent of diseased seedlings	Percent of healthy seedlings	Disease index (%)
Before selection†		78.26^{b}	2.17^{a}	59.00^{a}	19.26^{b}	75.66^{a}
After selection		91.81^{a}	1.57^{a}	13.00^{a}	69.06^{a}	24.65^{b}
Before selection	Aceteria	74.28^{b}	2.33^{a}	59.14^{a}	15.14^{a}	80.08^{a}
	34072	86.50^{a}	1.87^{b}	59.75^{a}	26.75^{a}	68.37^{a}
	Arak-2811	78.00^{ab}	2.20^{a}	62.50^{a}	15.50^{a}	80.16^{a}
	LRV	76.00^{ab}	2.17^{a}	53.50	22.50^{a}	70.29^{a}
After selection	Aceteria	92.00^{ab}	1.54^{bc}	7.25^{b}	77.00^{a}	15.72^{c}
	34072	92.25^{ab}	1.58^{b}	8.75^{b}	73.75^{a}	19.54^{bc}
	Arak-2811	95.50^{a}	1.40^{c}	16.00^{a}	63.25^{b}	34.94^{a}
	LRV	87.50^{b}	1.77^{a}	20.00^{a}	62.25^{b}	28.51^{ab}
Selection effect	Aceteria	24	34	88	409	77
	34072	7	16	85	176	72
	Arak-2811	22	36	74	308	56
	LRV	15	18	63	177	59

Means in each sliced column that have at least one common letter has no statistical difference at 1% level.
† Selection was done for resistance to *P. ultimum* in the previous generation.

Disease index presenting the ratio of diseased seedlings to germinated seeds is a proper index for comparing the potential of a genotype to resist the pathogens. Based on the definition, genotypes that have a lower disease index would be considered more resistant. The effect of selection was not identical for all studied genotypes, so that the difference among the four

after-selection populations was more than their difference before selection (Tables 1 and 2). For example, there was no difference among the genotypes for percent of diseased seedlings before selection, whereas after selection the genotypes fell into two distinct groups (Table 2). Also, there was no difference among the genotypes before selection for disease index, but after selection, the difference was significant as the lowest index was observed in genotypes Aceteria, 34072, LRV and Arak-2811, respectively (Table 2). Selection also had a 77% effect on the disease index of Aceteria, decreasing it from 80.08 in unselected population to 15.72 in the after-selection population (Table 2).

Overall the genotypes, selection for one generation improved percent of germination from 78.26 to 91.81, days to 50% germination from 2.17 to 1.57, percent of diseased seedlings from 59.00 to 13.00, percent of healthy seedlings from 19.26 to 69.06 and disease index from 75.66 to 24.65 in the *Pythium*- infected environment (Table 2). Moreover, Aceteria and LRV had the best and the worst after-selection populations when faced with invasion of *P. ultimum*.

The greatest selection effect was observed in the decrease of percent of diseased seedlings and increase of percent of healthy seedlings. Generally, selection for resistance to *P. ultimum* in the first year could improve germination, seedling growth and resistance of the selected progenies against *P. ultimum* in the next year.

Assessment of the selected populations at Phytophthora-infected media

Based on the results of analysis of variance, the selection, the genotype and selection × genotype interaction significantly affected the seed germination, percent of diseased seedlings, percent of healthy seedlings, days to 50% germination and disease index on environment infected with *Ph. drechsleri* (Table 3). Means comparison with the LSD method revealed that selection efficiently raised all studied characteristics except for the percent of germination (Table 4). Amounts of the selection effect for the genotypes are presented in Table 4. Speed of germination was promoted by selection as days to 50% germination in after-selection population of Aceteria dropped 40% relative to the unselected population while this amount had been just 13% for genotype 34072 (Table 4). As a result of

selection, percent of diseased seedlings of genotype Arak-2811 in the presence of pathogen *Ph. drechsleri* was decreased up to 88% and dropped from 70.14 to 8.75% (Table 4). For genotype LRV, the amount of selection effect was 49%, since the percent of diseased seedlings of its population decreased from 76.57 to 38.75%. Percent of healthy seedlings in after-selection population of 34072, showed a 1245% increase from 5.28 to 71.00% (Table 4). As in the media infected with *P. ultimum*, the improving effects of selection on germination and symptoms of disease were not identical for all genotypes in media inoculated with *Ph. drechsleri* however, the nature of this variation differed from the last one as the difference among the four after-selection populations was less than the difference among them before the selection (Table 4). For example, there was a statistical difference among all before-selection populations of the genotypes for days to 50% germination and percent of disease seedlings, but after selection, the genotypes had enough similarity to become just two different groups (Table 4). Selection also changed the kind of genotypes grouping for percent of healthy seedlings and disease index (Table 4). Before the selection, the lowest disease index belonged to the populations of genotypes 34072, LRV and Arak-2811 and the highest was observed in Aceteria, while after selection LRV had the highest disease index and the lowest was observed in other genotypes (Table 4). The selection improved disease index of Aceteria up to 88% at *Ph. drechsleri* infected media and it inversely showed a 45% decrease for LRV.

One generation of selection generally improved days to 50% germination from 2.25 to 1.60, percent of diseased seedlings from 77.00 to 21.75, percent of healthy seedlings from 9.67 to 67.37 and disease index from 88.83 to 24.93 in the *Phytophthora*-infested media (Table 4). No changes were created by selection in the percent of germination of the genotypes, in general. The best and the worst genotypes for improving resistance to *Ph. drechsleri* by selection were Aceteria and LRV, respectively. Selection had the highest effect on the percent of healthy seedlings and the disease index. It could be generally concluded that selection for resistance to *P. ultimum* in the first year could increase seed germination and seedling growth in *Ph. drechsleri* infected environments of the second year.

Table 3. Analysis of variance for seed germination and seedling disease of before and after selection populations of four open-pollinated safflower genotypes over environment infected to *Ph. drechsleri.*

Source of variation	df	Percent of germination	Days to 50% germination	Percent of diseased seedlings	Percent of healthy seedlings	Disease index (%)
Selection†	1	104.73*	8.77**	62161.27**	67788.05**	83156.09**
Genotype	3	127.80**	0.94**	1230.35**	1056.78**	1182.45**
Selection×Genotype	3	268.40**	0.93**	757.38**	1578.11**	1474.62**
Error	54	17.14	0.05	63.74	73.43	86.13

* and ** Significant at 5 and 1% level, respectively.
† Selection was done for resistance to *P. ultimum* in the previous generation.

Table 4. LSD means comparison for seed germination and seedling disease of before and after selection populations and also selection effect of four open-pollinated safflower genotypes over environment infected to *Ph. drechsleri.*

Genotype		Percent of germination	Days to 50% germination	Percent of diseased seedlings	Percent of healthy seedlings	Disease index (%)
Before selection†		86.85^{A}	2.25^{A}	77.00^{A}	9.67^{B}	88.83^{A}
After selection		89.12^{A}	1.60^{B}	21.75^{B}	67.37^{A}	24.93^{B}
Before selection	Aceteria	81.71^{B}	2.65^{A}	70.14^{C}	11.71^{A}	85.66^{B}
	34072	90.85^{A}	1.74^{C}	85.42^{A}	5.28^{B}	94.20^{A}
	Arak-2811	84.57^{B}	2.40^{AB}	75.85^{BC}	8.57^{AB}	89.82^{AB}
	LRV	90.28^{A}	2.22^{B}	76.57^{B}	13.14^{A}	85.64^{B}
After selection	Aceteria	88.75^{AB}	1.58^{B}	8.75^{B}	80.00^{A}	9.91^{B}
	34072	91.00^{A}	1.51^{B}	20.00^{B}	71.00^{A}	21.82^{B}
	Arak-2811	93.50^{A}	1.43^{B}	19.50^{B}	74.00^{A}	21.08^{B}
	LRV	83.25^{B}	1.86^{A}	38.75^{A}	44.50^{B}	46.90^{A}
Selection effect	Aceteria	9	40	88	583	88
	34072	1	13	77	1245	77
	Arak-2811	11	40	74	763	77
	LRV	3	16	49	239	45

Means in each sliced column that have at least one common letter has no statistical difference at 1% level.
† Selection was done for resistance to *P. ultimum* in the previous generation.

Assessment of the selected populations at the both pathogens-infected media

In the environment inoculated with both pathogens *Ph. drechsleri and P. ultimum*, the effect of selection was significant for all studied traits; the effect of genotype was significant for percent of germination, days to 50%

germination and percent of healthy seedlings (Table 5). Seed germination of the selected populations was greater than in unselected populations when cultured on media infected with both pathogens (Table 6). Speed of germination was increased by selection, as days to 50% germination for after-selection population of Arak-2811 and Aceteria showed a 44 and 33% decrease relative to basic population, respectively; and it was 24 and 22% for 34072 and LRV, respectively (Table 6). Percent of diseased seedlings of LRV dropped from 69.14 to 5.57 (a 96% decline) in media inoculated with pathogens *Phytophthora* and *Pythium*, because of the selection (Table 6). For this genotype also, the percent of healthy seedlings improved about 290%, increasing from 22.57 to 88.00% (Table 6). In comparison with environments infected with *Ph. drechsleri* or *P. ultimum*, the selection had more effect on germination and disease resistance in the media that were inoculated with both pathogens (Table 6). For example, the effect of selection on disease index was more than 90% in all evaluated populations (Table 6).

Selection for one generation generally improved percent of germination from 85.46 to 93.39, days to 50% germination from 2.30 to 1.59, percent of diseased seedlings from 67.82 to 5.92, percent of healthy seedlings from 18.21 to 87.46 and disease index from 86.59 to 6.33 in both pathogens-infected media (Table 4). The greatest effect of selection occurred on the percent of diseased and healthy seedlings. In general, it could be said that selection for resistance to *Pythium* can improve seedling growth and resistance in environments infected with both pathogens, *Ph. drechsleri and P. ultimum*, but the amount of this improvement was greater than those that appeared in media that were separately inoculated with these two pathogens.

The selection× genotype interaction effects should be interpreted with figures or writing.

Table 5. Analysis of variance for seed germination and seedling disease of before and after selection populations of four open-pollinated safflower genotypes over environment simultaneously infected to pathogens *Ph. drechsleri* and *P. ultimum.*

Source of variation	df	Percent of germination	Days to 50% germination	Percent of diseased seedlings	Percent of healthy seedlings	Disease index (%)
Selection†	1	1173.42**	9.35**	69214.88**	89517.16**	120249.39**
Genotype	3	235.42**	0.58**	22.82	123.57**	903.01
Selection×Genotype	3	265.49**	0.34**	27.32	168.21**	884.16
Error	54	25.75	0.02	38.47	23.13	2383.18

* and ** Significant at 5 and 1% level, respectively.
† Selection was done for resistance to *P. ultimum* in the previous generation.

Table 6. Means comparison for seed germination and seedling disease of before and after selection populations and also selection effect of four open-pollinated safflower genotypes over environment simultaneously infected to *Ph. drechsleri* and *P. ultimum*.

Genotype		germination (%)	Days to 50% germination	diseased seedlings (%)	healthy seedlings (%)	Disease index (%)
Before selection†		85.46^{A}	2.30^{A}	67.82^{A}	18.21^{B}	86.59^{A}
After selection		93.39^{B}	1.59^{B}	5.92^{B}	87.46^{A}	6.33^{B}
Before selection	Aceteria	79.71^{B}	2.53^{A}	65.57^{A}	12.58^{B}	83.58^{A}
	34072	90.57^{AB}	1.98^{C}	68.14^{A}	22.14^{A}	86.69^{A}
	Arak-2811	80.00^{B}	2.37^{AB}	64.42^{A}	15.28^{B}	80.75^{A}
	LRV	91.57^{A}	2.32^{B}	69.14^{A}	22.57^{A}	75.35^{A}
After selection	Aceteria	97.71^{A}	1.70^{B}	7.00^{A}	90.71^{A}	7.15^{A}
	34072	92.85^{B}	1.51^{C}	5.85^{A}	87.00^{AB}	6.28^{A}
	Arak-2811	89.42^{C}	1.33^{D}	5.28^{A}	84.14^{B}	5.90^{A}
	LRV	93.71^{B}	1.82^{A}	5.57^{A}	88.00^{AB}	5.98^{A}
Selection effect	Aceteria	23	33	89	624	91
	34072	2	24	91	293	93
	Arak-2811	12	44	92	451	93
	LRV	2	22	96	290	92

Means in each sliced column that have at least one common letter has no statistical difference at 1 % level.

† Selection was done for resistance to *P. ultimum* in the previous generation.

Discussion

The studied pathogens caused two kinds of symptoms on seeds and seedlings of the safflower genotypes. The most damage created by pathogen *P. ultimum* in this study was seed rot and prevention of germination while *Ph. drechsleri* caused mostly post-germination seedling infection and decay. Some previous studies also proved that *P. ultimum* is the most widespread and the most pathogenic *Pythium* species that attacks many plant species (Green and Dan, 2000; Marks and Kassaby, 1974). Huang et al. (1992) also showed that safflower along with canola, sugar beet, spinach and cucumber are the most susceptible crops to the pathogen *P. ultimum*. *P. ultimum* invades the hypocotyl and first internode, creates rotting, collapses infected tissues and finally causes death to the seedlings. Brown to black rot of roots and crown and shoot wilting are also the most typical symptoms of *Ph. drechsleri* (Shekari et al., 2006).

From a comparative viewpoint of the pathogenesis, safflower is more susceptible to *Ph. drechsleri* than *P. ultimum*. In regard to damage, *Pythium* caused more seed rot and *Phytophthora* caused more seedling death.

Therefore, it could be concluded that pre-germination pathogenesis is more intensive in the pathogen *P. ultimum* while *Ph. drechsleri* has more pathogenesis in the post-germination process of the seedlings. So, it is very important to determine relative frequency and pathogenesis of these two pathogens in the soils but even more important to create resistance to *Phytophthora* in safflower breeding.

In the present study, seeds produced on *P. ultimum*-resistant seedlings were evaluated for their reaction to media infected with *Ph. drechsleri*, *P. ultimum* and both pathogens. Our results proved that selection for resistance to *Pythium*, besides increasing resistance to this pathogen, could improve resistance to *Phytophthora* and to both pathogens. So, selection is a profitable method for improving resistance to seed rot and seedling damping off in safflower. As we mentioned above, we observed two kinds of symptoms, including pre- and post-germination decay, so it would be interesting if we could compare the role of selection for each pathogen in each of these stages of growth. For pre-germination, selection could have improved seed germination in the presence of *P. ultimum*, but had little effect against *Phytophthora* or both pathogens. This finding was somewhat logical, because selection in an environment inoculated with *Pythium* just could raise the frequency of resistance genes expressed at germination phase. These genes also had no role in facing *Phytophthora* since this pathogen mostly invades the young seedlings and has no effect on germinating seeds. Percent of diseased seedlings was decreased by selection at environments inoculated with *Ph. drechsleri*, *P. ultimum* and both pathogens. However, the reduction effects of selection on diseased seedlings in environments infected with both pathogens were larger than in environments inoculated with one pathogen. The efficiency of selection as a breeding method for improving safflower germination in fungi-infected media has been observed before (Nikmanesh et al., 2011; Palooj, 2010). The proficiency of selection for increasing resistance to pathogens has also been indicated in other crops. In cotton, selection for two consecutive generations could create a significant difference between parent and offspring for resistance to *Pythium* as some offspring showed more resistance than their parents (Johnson and Palmer, 1985). Hollingsworth et al. (2005) investigated brown root rot of alfalfa, caused by *Phoma sclerotioides* and showed that after one cycle of selection, the plants exhibited a reduced level of disease severity compared with the plants of cycle zero. In field pea, it has been proven that resistance to *Mycosphaerella pinodes* can be improved

through progeny selection from crosses of the most resistant lines (Zhang and Gossen, 2007). Results of an investigation on sesame demonstrated that the heritability of resistance to root rot (*Macrophomina phaseolina*) was high enough to perform selection (El-Bramawy and Abdul Wahid, 2006).

The effect of selection was not identical for all investigated genotypes as the highest improvement was observed in the population of genotype Aceteria and the lowest occurred in 34072 and LRV. The same results have been obtained for enhancing seed germination in *P. ultimum*-infected soil by Palooj (2010), as the highest progress was observed in safflower genotype 34040 where the percent of emergence has been developed from 68.12 to 86.75 through selection. Because success in selection depends on genetic variation and additive genetic effects in control of the traits, it can be concluded that resistance to *Ph. drechsleri* and *P. ultimum* is governed by genes indicating additive effects. The existence of additive effects has been reported by other researchers in control of resistance to *Fusarium* in safflower (Sharifnabi and Saiedi, 2004). Nikmanesh et al. (2011) observed the significant response to selection for resistance to *P. ultimum* in a safflower population and concluded that the resistance is governed mostly by genes which have the additive effects. Since selection for resistance to *P. ultimum* also increased resistance to the other pathogen, *Ph. drechsleri*, we conclude that common genes or linkage groups control resistance to both *Ph. drechsleri* and *P. ultimum*. In addition, genes conferring resistance to seed rot are probably independent of genes controlling resistance to seedling death, so pre- and post-germination death can be considered as two different traits from the breeding point of view. Based on these results, this field-laboratory selection method could improve the genetic potential of seeds for performance in soil infested with *Ph. drechsleri* and *P. ultimum* and this reduces the losses due to these pathogens in field conditions and eventually will increase safflower production.

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