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Research Article

Evaluation of peanut genotypes for resistance to Cercospora leaf spot diseases in Iran

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Abstract: The early and late blight are important diseases of peanut. The reactions of eleven peanut genotypes *Arachis hypogea* L. and one local cultivar (cv. Goli) to Cercospora leaf spot (CLS) diseases were evaluated under CLS high incidence field conditions during 2016. The field experiment was conducted in Lasht-e Nesha Agricultural Research Station (north-east of Rasht) in 2016 using randomized complete block design with four replications. The results indicated a possible differential reaction to infection by the fungi *Passalora arachidicola* and *P. personata* among genotypes of peanut. There was a significant variability among peanut genotypes to CLS diseases ($P \leq 0.01$). The differences between susceptible and resistant genotypes were identified by the number and size of spots produced per leaf (leaflets), leaf defoliation, and leaf spot severity percentage. Mean comparisons revealed that Cercospora leaf spot incidence in terms of the number of spots per leaf were greater in the Pn202 (85.1) and Pn220 (75.8) by a factor of about 5- to 8-fold relative to the Pn213 (9.2), Pn170 (10.9), Pn140 (11.6), and Pn204 (15.2) genotypes. When the severity of Cercospora leaf spot disease was considered, this difference was 3-5-fold. The defoliation and severity of CLS percentage on genotypes of peanut were ranged 4.7-60.9% and 11.3-52.5%, respectively. Accordingly, the defoliation and severity of CLS were observed as high as 60.88% and 52.49% on Pn202 peanut genotype, respectively. Our results revealed that with increasing CLS severity from 11.3 to 52.5% (41.2%), defoliation increased from 4.7 to 60.9% (56.2%) while the pod yield decreased from 3809.4 to 1217.6 kg/h (2591.8 kg/h). In total, three genotypes (Pn170, Pn140, and Pn213) were moderately resistant, while genotypes Pn220 and Pn202 were susceptible to CLS diseases. The resistant genotypes indicated favorable agronomic characteristics and had a potential to be released as commercial cultivars or to be used as parents in peanut breeding programs.

Keywords: *Cercospora*, early leaf spot, late leaf spot, peanut, resistance

Introduction

The legume *Arachis hypogaea*, commonly known as peanut or groundnut, is a very important food crop throughout tropical and sub-tropical areas. All species in the genus are unusual among legumes in that they produce their fruit below the ground. Yield losses in peanut cultivars are caused by different factors, with leaf spotting diseases being a major factor, mainly caused by *Cercospora* fungus (Gaikpa *et al*., 2015). The early and late spots are the most common foliage diseases of peanut. Leaf

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spot diseases produced by *Passalora arachidicola* (Hori) U. Braun and *P. personata* (Berk. & M.A. Curtis) S.A. Khan & M. Kamal are present every year on peanuts in Guilan province (Northern Iran). For many years, the intensity of CLS diseases was high in the Astaneh-e Ashrafiyeh region (northern Iran, Guilan province) adversely affecting farmers who live with limited resources. The potential peanut crop losses caused by CLS have been well recognized in many peanut-producing countries. The early and late leaf spots cause severe pod yield losses between 10 and 80% (Grichar *et al*., 1998; McDonald *et al*., 1985; Miller *et al*., 1990; Semangun, 2004; Ambang *et al*., 2011). The *Cercospora* leaf spot is a major severe disease of peanut and could result in yield reduction up to 50% or even further (Thakur *et al.,* 2012). The diseases occur throughout the year, but the disease incidence varies depending on season, location, and year. The disease is significant since it rapidly destroys foliage when the plant is close to pod maturity. Although fungicide application is effective in controlling the disease, its high cost is considered uneconomical in many developing countries. Chiyembekeza *et al*. (1993) indicated that resistant varieties can increase yield, decrease costs of production, and minimalize environmental hazards related to fungicide application. It is believed that the main control strategy is to use resistant (tolerant) cultivars as a better alternative (Zadoks *et al*., 1979). Pereira *et al*. (2009) observed that most of the genotypes of wild species had greater resistance than genotypes of *A. hypogaea*. Li *et al.* (2012) compared field susceptibility of several peanut genotypes to late leaf spot for screening genotypes. They found that six and five accessions were resistant and susceptible, respectively to the disease. The cultivation of resistant (or tolerant) peanut cultivars does not only eliminate the crop losses caused by disease; it also contributes to reduced costs associated with fungicide sprayings and other control methods (Méndez-Natera *et al*., 2016). Pensuk *et al*. (2003) evaluated seven peanut cultivars for their resistances to late leaf spot. The recognition of peanut genotypes tolerant to Cercospora leaf spots and simultaneously having higher production potentials should benefit growers and breeders to choose the proper cultivar for planting or breeding (Gaikpa *et al*., 2015). Chapin *et al*. (2010) conducted field experiments to assess the disease reaction of 47 experimental Virginia-type breeding lines and eight genotypes of peanut. Tallury *et al.* (2009) assessed 26 interspecific hybrid derived breeding lines (IHDBL) with five *Arachis* species in their pedigrees, six resistant *A. hypogaea* controls, and 11 susceptible cultivars for leaf spot resistance in field experiments.

In Iran, no study has been conducted on evaluating resistance (or tolerance) of peanut cultivars (genotypes) to *P. arachidicola* and *P. personata*. The purposes of this study included: 1) to determine the breeding potential of selected peanut genotypes known to vary in CLS resistance; 2) to explore the usefulness of disease incidence (number and size of leaf spot, defoliation) and disease severity as the assessments of resistance for effectively discriminating resistant and susceptible genotypes of peanut; and 3) to determine the effects of leaf spot disease on pod yield and yield components of peanut crop.

Materials and Methods

Peanut genotypes

Eleven peanut genotypes (Pn195, Pn202, Pn208, Pn220, Pn125, Pn1702, Pn2045, Pn127, Pn152, Pn142,Pn213, Pn140) and one local cultivar as control (cv Goli) were evaluated for their reactions to CLS diseases in a randomized complete block design with 4 replications. Field evaluation was conducted in the Fakhr-Abad (Lasht-e Nesha) Agricultural Research station (Guilan province, Northern Iran) from April to October in 2016. The experimental field had a history of high CLS incidence and was predominantly infected by Cercospora leaf spot fungi during the last decades. The plot size for each genotype constituted 8 rows 6m long (100:80:80kg of NPK/ha). The cultural

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practices including mechanical weed control at day 20 post-planting and application of chemical fertilizer (100:80:40 kg of NPK/ha.) were performed during the growing season. However, no foliar fungicide was applied to allow natural infection of CLS diseases.

Disease assessment

Ten plants (third leaf on the main stem of each plant was sampled) in a single plot were scored for disease-resistant parameter evaluation based on 1-9 scale (1 = no symptoms, 2 = 1-5% leaf infection, 3 = 6-10% leaf infection, 4 = 11-20% leaf infection, 5 = 21-30 leaf infection leaf infection, 6 = 31-40% leaf infection, 7 = 41-60% leaf infection, 8 = 61-80% leaf infection, 9 = 81-100% leaf infection) at day 100 after seed planting (Subrahmanyam *et al.*, 1995). Disease incidence (percentage of infected plants), number and size of leaf spots (early and late blight leaf spots), %CLS leaf area of infected leaf, and defoliation were recorded for the incidence and severity of CLS in the plot.

Classification of resistance in genotypes

Observation of CLS severity (%) was obtained according to Subrahmanyam *et al.* (1995) as follows (Table 1).

Table 1 Resistance categories for reactions of peanut genotypes to Cercospora diseases.

No	Leaf spot disease severity (%)	Resistance categories
1	1-10	R
2	11-20	MR
3	21-40	MS

R = resistance, MR = Moderate resistance.
MS = Moderate susceptible, S = susceptible.

Agronomic assessment

For evaluating agronomic characteristics, 20 plants were harvested from each plot at maturity. Peanut pods were dried to approximately seed moisture 8%, with the dry pod weight kg/h), No. of kernel.pod, kernel size (g/100 kernels), and shelling percentage (kernel weight × 100/ pod weight) determined further.

Statistical analysis

Analysis of variance was performed on data from leafspot-resistant parameters and agronomic characteristics. Before analysis of variance, the data of the number and size of leaf spot and number of kernels per pod were log-transformed [log(x + 1)], while defoliation, disease severity, and shelling were arcsin-transformed. The comparisons among means were performed by Duncan's Multiple Range Test ($p < 0.01$).

Results

Disease incidence

Significant differences ($P \leq 0.01$) among genotypes were found at day 100 after the planting (DAP, Table 2). Concerning disease incidence, the mean number of leaf spot ranged from 9.2 to 75.7 for Pn140, Pn213, Pn170, and Pn204 which were the lowest (Table 3). Also, considering disease incidence, the size of leaf spot (mm) was determined at 100 DAP ranging from 2.7 to 6.7 for Pn140, Pn213, Pn142, Pn127, Pn204, Pn125 and Pn195, which were lower than that of the others (Table 3).

Table 2 Mean squares (MS) of some resistance components to Cercospora diseases.

Source of variation	df	Number of spots/leaf	Leaf spot severity (%)	Size of leaf spots (mm)	Defoliation (%)
Treatment	11	1597.49*	267.38**	1.06**	904.56**
Replication	3	116.47	10.53	0.66	81.33
Error	33	103.65	23.43	2.32	62.93
C.V (%)		23.78	18.04	26.81	22.97

*: significant at 5% probability level **: significant at 1% probability level.

Table 3 Mean comparison of Cercospora leaf spot incidence, size and number of leaf spot, defoliation and severity of eleven peanut genotypes and one local check cultivar in Guilan province.

Genotypes	Number of spots/leaf	Size of leaf spots (mm)	Defoliation (%)	Leaf spot severity (%)
Pn195	39.4 b	2.9 c	31.4 bc	34.1 b
Pn202	85.1 a	4.3 b	60.9 a	52.5 a
Pn220	75.7 a	4.9 b	50.5 ab	43.9 a
Pn125	19.9 cd	2.9 c	11.5 cd	23.7 cd
Pn170	10.9 d	6.7 a	4.7 d	11.3 e
Pn204	15.2 d	2.8 c	13.6 cd	23.1 cd
Pn127	23.7 bcd	2.9 c	21.3 cd	27.4 bc
c.v Goli	22.3 bcd	5.8 a	19.6 cd	22.9 cd
Pn152	26.8 bcd	4.8 b	30.5 bc	28.2 bc
Pn142	35.7 bc	2.9 c	30.6 bc	30.4 bc
Pn213	9.2 d	2.7 c	8.9 d	13.2 e
Pn140	11.6 d	2.7 c	9.2 d	12.4 e

Means followed by a different letter in each column are significantly different at 0.05 probability level.

Disease severity

There was a highly significant difference ($P \leq 0.01$) among the peanut genotypes for CLS score (Table 2). The leaf spot severity was rated according to Subrahmanyam *et al.* (1995), using 1-9 scoring scale. Genotypes differed significantly in the mean disease score (Table 2). Leaf spot severity among genotypes varied between 11.3 and 52.5% after 100 DAP (Table 3). The disease severity was able to classify the genotypes into moderately resistant, moderately susceptible, and susceptible categories (Table 4). All groups of genotypes could be separated by disease severity. Pn202 and Pn220 had the highest disease score followed by Pn195, Pn127, Pn152, Pn142, Pn125, Pn204, and c.v Goli, while the genotypes Pn213, Pn140, and Pn170 had the lowest disease scores (Figs. 1, 2, 3). Hence, Pn220 and Pn202 were susceptible, while cv. Goli, Pn125, Pn127, Pn204, Pn142, Pn152, and Pn195 were moderately susceptible, and Pn170, Pn140, and Pn213 were moderately resistant (Table 4). The severity of CLS was higher as large as 52.5% at defoliation of 60.9% and 43.9% at 50.5% on Pn202 and Pn220, respectively. The commercial cultivar Goli (NC2) was moderately susceptible (22.9%). The incidence of CLS (number of leaf spots) was highest as large as 85.1 at severity of 52.5% on genotype Pn202.

Figure 1 The resistant (right and left rows, Pn170 and Pn213) and susceptible (middle row, Pn202) genotypes of peanut to CLs diseases.

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Figure 2 Leaf spot severity and defoliation in susceptible peanut genotype Pn202.

Figure 3 Leaf spot severity in resistant peanut genotype Pn140.

Defoliation

The significant difference among resistant and susceptible genotypes was found by the percentage of defoliation ($P \leq 0.01$) (Table 3). The defoliation ranged from 4.7 % to 60.98 %. The defoliation was higher on genotypes Pn202 (60.9%) and Pn220 (50.5%) when compared to the others (Fig. 2). The local cultivar c.v Goli exhibited 19.6% defoliation among all peanut genotypes. All groups of genotypes could be differentiated by the defoliation%. The Pn202 and Pn220 were considered susceptible, while cv. Goli (19.6%), Pn125 (11.5%), Pn127 (21.3%), Pn204 (13.6), Pn142 (30.6%), Pn152(30.5%), and Pn195 (31.4%) were moderately susceptible. The percentage of defoliation was lowest on Pn170 (4.7%), Pn213 (8.9%), and Pn140 (9.2%) (Fig. 3).

Yield and yield components

Significant differences among resistant and susceptible genotypes were found by the some agronomic characteristics ($P \leq 0.01$) (Table 5). Difference in terms of the number of kernels per pod was not significant between genotypes (Table 5). The results indicated that the tested genotypes responded differently to CLS for all parameters. Genotypes Pn170, Pn140, and Pn213 were the best for several agronomic characteristics (Table 6, Fig. 1) and pod yield was maximum in Pn170 (3809.4 kg/h). Among the new genotypes of peanut producing a higher yield under CLS disease pressure conditions at Fakhr-Abad, Pn125 (3385.5 kg/h), Pn204 (3243.5 kg/h), cv. Goli (3043.5 kg/h), and Pn127 (2648.9 kg/h) yielded higher values (Table 6). The weight of 100 kernels (gr) from randomly selected plants used for CLS score was highest in Pn170 (95.8) and Pn213 (94.8) (Fig. 4).

Table 4 Cercospora leaf spot resistant categories.

No	Genotype	Resistance Categories
1	Pn170-Pn140-Pn213	MR
2	cv. Goli-Pn125-Pn127-Pn204-Pn142-Pn152-Pn195	MS
3	Pn220-Pn202	S

MR = Moderate resistance, MS = Moderate susceptible, S = susceptible.

Table 5 Mean squares (MS) of agronomic characteristics in 11 peanut genotypes and one local cultivar.

Source of variation	df	Number of Kernel/pod	weight of 100 kernels (g)	Shelling (%)	Pod yield (kg/h)
Treatment	11	0.00285657*	470.212**	20.01*	1875344.19**
Replication	3	0.00775278	3.82	4.09	702873.12
Error	33	0.00215278	33.04	16.71	279465.80
C. V (%)		2.434882	7.38	10.53	12.29

*: significant at 5% probability level **: significant at 1% probability level.

Table 6 Mean comparison of yield and agronomic characteristics of 11 peanut genotypes and one local cultivar evaluated under field condition at Fakhr-Abad Agricultural Research station (Lasht-e Nesha, Guilan province).

Genotypes	Number of Kernel/pod	weight of 100 kernels (g)	Shelling (%)	Pod yield (kg/h)
Pn195	1.87 a	69.2 c	36.9 e	2125.2 g
Pn202	1.88 a	53.7 d	34.4 f	1217.6 h
Pn220	1.87 a	75.5 bc	38.6 cd	2013.2 f
Pn125	1.95 a	86.7 ab	40.3 b	3385.5 b
Pn170	1.95 a	95.8 a	42.7 a	3809.4 a
Pn204	1.88 a	77.3 bc	38.8 c	3243.5 bc
Pn127	1.88 a	67.1 c	36.6 e	2648.9 d
Goli	1.91 a	76.8 bc	38.8 c	3043.5 c
Pn152	1.92 a	75.2 bc	37.5 de	2312.3 ef
Pn142	1.98 a	70.8 c	37.2 e	2216 efg
Pn213	1.94 a	94.8 a	42.5 a	3406.4 b
Pn140	1.93 a	82.8 ab	41.9 a	3469.6 b

Means in the same column followed by a different letter are significantly different at 0.05 probability level.

Figure 4 The morphology of kernels in peanut resistant genotypes Pn 170, Pn213 and Pn140.

Discussion

In this study, eleven peanut genotypes were detected showing different levels of resistance to leaf spot caused by *P. arachidicola* and *P. personata*. Genotypes ranging from leaf spot resistant to leaf spot susceptible were observed in a field study. Differences in infected leaf area percentage among peanut genotypes provided useful information. A differential response to infection by the fungal causal agents of early and late blight existed among peanut genotypes with different inherent levels of resistance.

The resistance parameters including the number of leaf spots, size of leaf spots, and infected leaf area percentage could differentiate genotypes into resistant and susceptible groups. The results of this study indicated that genotypes Pn170, Pn140, and Pn213 were consistently tolerant to CLS under field conditions Note that plant resistance to CLS diseases could occur in the structure, biochemistry or both. Further, the inheritance of resistance to CLS is quantitative. A method was used to evaluate the intensity of *Cercospora* disease on peanut, based on severity of leaf area symptoms (disease scores). We indicated that this method was valid because of good repeatability, reproducibility, and accuracy of assessments. With increasing severity of early and late leaf spot diseases, there was a decrease in pod yield and yield components. The shelling percentage was remarkable among genotypes Pn170 (42.7%) and Pn213 (42.5%), where Pn140 (41.95%) seemed to be tolerant to CLS. Izge *et al*. (2007) observed that the peanut tolerant cultivar ICGV-IS 96808 produced a higher kernel yield and shelling percentage. Thakur *et al.* (2012, 2013) evaluated 25 peanut cultivars for resistance to CLS and for yield production. They found highly significant differences among the peanut cultivars for days to 75% flowering, days to maturity, 100 kernel weight, and CLS scores. The genotypes Pn170, Pn140, and Pn213 were identified as a parent in peanut breeding programs. It is believed that resistance to CLS is quantitatively inherited and expressed as reduced disease incidence, disease severity, and defoliation. In our study, partial resistance to late and early leaf spot was observed in tested peanut genotypes, where in all cases the resistance diminished the incidence and severity of the diseases. Fortunately, tolerant genotypes are superior in agronomic performance and possess desirable characteristics including high kernel size, shelling percentage, and pod yield. The desirable agronomic characteristics associated with tolerant genotypes can facilitate the progress in peanut breeding programs aimed at developing promising peanut cultivars resistant to CLS. Genetic resistance to leaf spot diseases is a wanted answer to the issue of yield reduction, but such resistance has typically been related to late maturity and low production (Pixley *et al*., 1990).

Conclusion

This study offers the first report of sources of resistance to CLS in peanut and describes techniques to evaluate resistance to *P. arachidicola* and *P. personatum* in peanut genotypes in Iran. The screening of superior peanut genotypes was conducted under high disease pressure field conditions. The expression of resistance to CLS diseases depended on the geographic location where they were evaluated, since the environment affects the expression of partial resistance in several pathosystems, and could influence stability of resistance to CLS. The disease score (disease severity) was the most influential parameter in discriminating peanut genotypes for resistance to CLS. Therefore, screening of individual plants with low to high CLS scores will be effective indicators. Notably, disease severity was more advantageous than disease incidence. Out of 11 peanuts genotypes tested, three genotypes were moderately resistant (MR), six genotypes and c.v Goli were moderately susceptible (MS), and two genotypes were susceptible (S) against CLS diseases. The sources of resistance identified in this study can be used in peanut breeding programs as transfer CLS resistance to adapted

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peanut genotypes for future cultivar releases to Iran. Also, identification of molecular components of the early phases of the resistance can elucidate the defense mechanisms of peanut and set the grounds for the creation of pathogen-resistant peanut genotypes.

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ارزیابی مقاومت ژنوتیپ‌های بادام زمینی به بیماری‌های لکه سرکوسپورایی در ایران

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چکیده: بیماری‌های بلایت زودرس و دیررس بادام زمینی از بیماری‌های مهم بادام زمینی به‌شمار می‌روند. طی سال زراعی ۹۶-۱۳۹۵ عکس‌العمل یازده ژنوتیپ بادام زمینی و رقم محلی گلی (NC2) نسبت به بیماری‌های لکه‌برگی سرکوسپورایی در شرایط مزرعه با سابقه وقوع بالای بیماری لکه سرکوسپورایی، ارزیابی گردید. آزمایشات مزرعه‌ای در ایستگاه تحقیقات زراعی لشت نشاء به‌صورت طرح بلوک کامل تصادفی با چهار تکرار انجام شد. نتایج نشان داد که، بین ژنوتیپ‌ها از نظر آلودگی به قارچ‌های عامل بلایت زودرس (*Passalora arachidicola*) و دیررس بادام زمینی (*P. personata*) عکس‌العمل متمایزی وجود دارد. به‌طوری‌که، تنوع معنی‌داری بین ژنوتیپ‌های بادام زمینی به بیماری لکه سرکوسپورایی دیده شد ($P \leq 0.01$). تفاوت بین ژنوتیپ‌های حساس با اندازه‌گیری تعداد و اندازه لکه‌های برگی، درصد ریزش برگ و شدت لکه‌برگی تعیین گردید. مقایسه میانگین‌ها نشان داد که، میزان وقوع لکه‌برگی سرکوسپورایی براساس اندازه‌گیری تعداد لکه‌ها در برگ در ژنوتیپ‌های Pn202 (۸۵/۱) و Pn220 (۷۵/۸) به‌میزان ۸-۵ برابر نسبت به ژنوتیپ‌های Pn213 (۹/۲)، Pn170 (۱۰/۹)، Pn140 (۱۱/۶)، و Pn204 (۱۵/۲) بیش‌تر بود. البته وقتی شدت بیماری لکه‌برگی سرکوسپورایی لحاظ شد، این میزان به ۵-۳ برابر رسید. میزان ریزش برگ و شدت لکه‌برگی سرکوسپورایی در ژنوتیپ‌های بادام زمینی به‌ترتیب بین ۶۰/۹-۴/۷ و ۵۲/۵ -۱۱/۳ درصد متغیر بود. به‌طوری‌که، میزان ریزش برگ و لکه‌برگی سرکوسپورایی در ژنوتیپ Pn202 به‌ترتیب تا ۶۰/۸۸ و ۵۲/۴۹ درصد افزایش داشت. نتایج بررسی نشان داد که، با افزایش شدت لکه‌برگی از ۱۱/۳ به ۵۲/۵ درصد میزان ریزش برگ از ۴/۷ به ۶۰/۹ درصد افزایش و عملکرد پیله (غلاف بادام زمینی) از ۳۸۰۹/۴ به ۱۲۱۷/۶ کیلوگرم/هکتار کاهش پیدا کرد (۲۵۹۱/۸ کیلوگرم/هکتار). مجموعاً، سه ژنوتیپ Pn170 ، Pn140 و Pn213 نیمه مقاوم و ژنوتیپ‌های Pn220 و Pn202 حساس به بیماری لکه سرکوسپورایی تشخیص داده شدند. ژنوتیپ‌های مقاوم به لکه سرکوسپورایی خصوصیات زراعی مطلوبی داشتند و از پتانسیل قابل قبولی برای معرفی (آزادسازی رقم) یا استفاده به‌عنوان والد در برنامه‌های اصلاح نباتات برخوردار هستند.

واژگان کلیدی: ایران، بادام زمینی، لکه‌برگی دیررس، لکه‌برگی زودرس، مقاومت به بیماری

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