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Improving Late Leaf Spot Resistance of Peanut by Molecular Breeding

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Abstract

Peanut (*Arachis hypogae* L.) is one of the most important foods and oil crops in Vietnam. However, the late leaf spot disease (LLS) is the problematic constraint to reduce peanut production. The objective of this study was to evaluate LLS resistance of the 24 selected peanut lines obtained from the BC_3F_3 populations which were derived from the crosses between the recipient and donor LLS tolerant plants and confirmed by SSR markers. The results showed the lines were potential LLS resistance. Of which 4 lines, namely ĐM1, ĐM2, ĐM3 and ĐM4 were highly resistant to LLS resistance in the artificial infection test. It found that 5 weeks-old leaf, similar to the peanut flowering stage, is the most susceptible disease infection, while the peanut seedling stage is a negligible infection of *P.personata.* Moreover, all lines were confirmed to carry QTLs/genes involved in LLS resistance using SSR markers. Our findings may provide useful information for peanut breeding programs in this country.

Keywords: Peanut, late leaf spot disease, SSR markers, molecular breeding

Introduction

Peanut (*Arachis hypogae* L.) is considered as the groundnut which belongs to the legume family and is widely grown in tropical and subtropical areas in many countries in the world. Peanut is an important oil source with high protein, nutritions, fibers and consumed as a major source of vegetables and protein for human feeds. Moreover, peanut is the key legume crop in this country in terms of

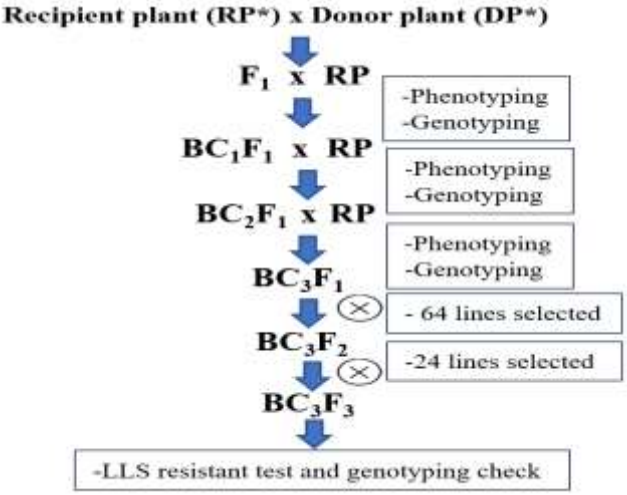
poverty alleviation and sustainable farming systems [1]. Currently, peanut is being cultivated in over 100 countries throughout Asia and Africa [2] and worldwide peanut production in 2019 was approximately 46.0 million tonnes [3].

In Vietnam, peanut is one of the key popular crops and annually produced 0.46 million tonnes [4]. However, peanut production has been significantly reduced due to infection of the late leaf spot disease (LLS), which not only occurs in this country but also is the main constraint to cause yield reduction in many peanut-growing areas throughout the world. LLS disease has been caused by fungi *Phaeoisaraopsis personata* and infested all peanut crops in year-round and severely caused 50%-70% yield loss [2]. Some reports showed to detect LLS and rust disease resistance potential lines [5]. Some major QTLs/genes involved in LLS resistance of cultivated and wild peanut have been reported [6-7]. However, very few studies on evaluating and improving LLS resistant peanuts in this country have been available. Hence, the objective of this study was to evaluate the LLS resistant lines and confirming by SSR markers. The current study may provide useful information for further developing LLS resistant peanut varieties in this country.

Materials and Methods

Material collection

A total of 24 potential peanut lines were previously selected from the BC_3F_3 populations of the crossed combination between the recipient (CNC3) and donor (TN6) plants (Table 1). CNC3 is a high yield and good quality peanut variety but is susceptible with the LLS disease was used as the recipient plant, while TN6 is a traditional variety with low yield but highly resistant to LLS disease [8].



RP: CNC3 variety; DP*:TN6*

Fig 1. Breeding scheme to develop BC_3F_3 and selected 24 potential lines

Table 1. The potential peanut lines were used in this study

No	Line name	Origin	Seed traits	No	Line name	Origin	Seed traits
1	CL4	Vietnam	Pink	12	CL12	Vietnam	Pink
2	CL5	Vietnam	Pink	14	CL13	Vietnam	Pink
3	CL6	Vietnam	Pink	15	CL14	Vietnam	Pink
4	CL7	Vietnam	Pink	16	CL15	Vietnam	Pink
5	CL8	Vietnam	Pink	17	CL16	Vietnam	Pink
6	CL9	Vietnam	Pink	18	CL17	Vietnam	Pink
7	ĐM 1	Vietnam	Pink	19	ĐM 4	Vietnam	Pink
8	CL10	Vietnam	Pink	20	CL18	Vietnam	Pink
9	ĐM 2	Vietnam	Pink	21	CL19	Vietnam	Pink
10	CL11	Vietnam	Pink	22	CL20	Vietnam	Pink
11	ĐM 3	Vietnam	Pink	23	CL21	Vietnam	Pink
12	CL12	Vietnam	Red	24	CL22	Vietnam	Red

Evaluating LLS resistance ability of the selected peanut lines

The leaves infected LLS disease were collected in the peanut field, then washed in distilled water for spore fluid, after that mixed with spore suspension with a density of 106 spores/ml. The groundnut seedlings (24 lines) with 2-3 leaves year-old were grown in the pot. Their leaves were slightly damaged on both leaf sides, then sprayed with the spore suspension. The seedlings after infection were placed in net house 26-28^{o}C, 95% humidity. LLS infection and symptoms were monitored after 7, 14, and 21 days and recorded as rating scores from 1, 3, 5, 7 and 9 following the method of ICRISAT [8-9].The level of infected leaf area was calculated following the standard evaluation of the Crop Protection Institute as follow: Level 1: 1-10% infected leaf areas (high resistance); level 3: 11-25% infected leaf area (resistance); level 5: 26-50% (moderate infection); level 7: 51-75% (infected); level 9: over 75% infected leaf area (high infection). The L14 peanut variety is widely grown in some areas in this country. Hence it was used as the control. The most potential peanut lines ĐM1 and ĐM4 have further examined the effects of their leaves-age on the infection of LLS disease in the artificial conditions [8].

DNA extraction and SSR markers application

The young leaves of 3 weeks of 10 individual plants of each line were collected and intermediately transferred to the laboratory for DNA extraction following the CTAB methods [10]. Ten samples were mixed together as the presentative of one line. The DNA quality was checked by the agarose gel (1%). Six SSR markers included PM179; GM633; GM2301 IPAHM103; Lec1; seq7G02; TC9F10 và GM1760 which related to the LLS disease resistance were previously reported by Cuc et al.[8] were used. The information of the marker in detail was presented in Table 2.

Table 2. The detailed information of SSR markers was used in this study

No	Marker name	Sequences	
		Forward primer	Revert primer
1	PM179	TGAGTTGTGACGGCTTGTGT	CTGATGCATGTTTAGCACACTT
2	GM633	CAAAGTTTGCAGTGATTTTGTTG	AAATTTTCAGGTAAATCATTCTT
3	GM2301	GTAACCACAGCTGGCATGAAC	TCTTCAAGAACCCACCAACAC
4	IPAHM103	GCATTCACCACCATAGTCCA	TCCTCTGACTTTCCTCCATCA
5	TC9F10	ATCACAATCACAGCTCCAACAA	GGCAAGTCTAATCTCCTTTCCA
6	GM1760	TGAAGAGCCATGTCAGATCG	AGGGCCCCAACAAGATAAGT

Statistical Analysis

The data were calculated and statistically analyzed by Excel version 2016 and IRRISTAT 5.0.

Results and Discussion

Evaluation of LLS resistance of the selected lines

A total of 24 lines were selected from the large population of backcross and selfing generations of BC_3F_3, which developed from the recipient and donor plants (Fig 1). These lines have had good agronomic traits such as high yield, good quality and good phenotypic traits [8]. To confirm whether these lines can resist to LLS disease or not, the artificial screening was made. As the results presented in Table 3, 4 lines included ĐM1, ĐM2, ĐM3 and ĐM4 showed the highest number of LLS resistant plants. In the other lines, there were ranging from 13 to 16 plants/20 plants which were infected leaf area less than 1%. Moreover, the lines CL4, CL5 and CL10 show a low rate of LLS infection at rating 1.0 score of 4 to 5 individual plants, and rating 3.0 score of 5 to 6 plants, especially, no individual plant was found to be affected the LLS disease at score 9. The lines CL8, CL11, CL16 and CL17 show a mediate infection at score 5 to 7 for 6 to 10 plants. However, the other line CL6, CL7, CL9, CL12, CL13, CL14, CL15, CL18, CL19, CL20, CL21, CL22, CL23 ad L14 showed LLS infection at score 9 equally 50% LLS affected leaves areas (Table 3).

Table 3. The level of LLS resistant disease of 24 potential lines in artificial conditions

No	Name of line	Number of plant	Level of disease				
			1	3	5	7	9
1	ĐM1	20	15	4	1	0	0
2	ĐM2	20	13	6	1	0	0
3	ĐM3	20	13	5	2	0	0
4	ĐM4	20	16	3	1	0	0
5	CL4	20	4	5	6	5	0
6	CL5	20	4	5	8	3	0
7	CL6	20	3	5	6	4	2
8	CL7	20	3	5	6	5	1
9	CL8	20	3	4	8	5	0

Table 3 (continued). The level of LLS resistant disease of 24 potential lines in artificial conditions

10	CL9	20	4	5	7	2	2
11	CL10	20	5	6	8	1	0
12	CL11	20	3	6	8	3	0
13	CL12	20	3	4	6	5	2
14	CL13	20	2	3	8	5	2
15	CL14	20	2	4	7	6	1
16	CL15	20	2	3	8	5	2
17	CL16	20	4	6	7	3	0
18	CL17	20	3	5	10	2	0
19	CL18	20	1	3	9	5	2
20	CL19	20	1	4	10	3	2
21	CL20	20	0	4	8	5	3
22	CL21	20	0	3	8	7	2
23	CL22	20	0	3	6	7	4
24	CL23	20	0	3	10	5	2
25	L14 (C)	20	1	3	10	5	1

C: control variety

Table 4. Effects of leaf age on pathogenicity of *P.personata* spraying on ĐM1peanut line at the concentration of 5.10^4/spores

Leaf -age	Latent period (day)	Life circle (day)	Spot diameter (mm)	Number of lesions/double leaf (spot)	Level of leaf area infected (%)	Infected frequency (spot/cm^2)
2 weeks	23.33^a	30.56^a	1.41^e	9.50^d	0.64^d	0.42^c
3 weeks	22.06^b	28.22^b	1.76^d	16.44^c	1.53^c	0.62^b
4 weeks	18.22^d	23.28^d	2.51^b	22.39^b	3.63^b	0.74^a
5 weeks	13.33^f	16.50^f	3.52^a	29.44^a	8.42^a	0.85^a
6 weeks	17.17^e	21.39^e	2.82^b	27.05^a	4.19^b	0.67^b
7 week	20.17^c	25.28^c	2.20^c	20.72^b	1.69^c	0.45^c
$LSD_{0,05}$	0.76	1.13	0.38	2.93	0.74	0.11

Means with the same letters in a colums are not significantly different at P<0.05

Effects of leaf-age on pathogenicity of *P. personata* on the peanut line ĐM 1 and ĐM4 peanut lines

In this study, the solution with the spore 5.104 spores/ml was sprayed on the peanut leaves 3 to 8 weeks old. The leaf was daily observed to determine the latent period, the diameter of pathogenicity, number of lesions and the frequency of infected leaves, and was calculated at 28 days after infection. The results showed that leaf-age was greatly infected by the pathogenicity of *P. personate* (Table 4). Specifically, all parameters of leaf age were infested included the latent period, life cycle, incubation time, diameter of pathogenicity which led to infecting the leaf areas and frequency infection leaves. The symptoms of the disease such as spore formation were the earliest observation on the 5 weeks-old- leaves, following the 4 weeks-old-leaves. However, the 7 weeks-old-leaves were observed to be a longer incubation period and longer life cycle of disease symptoms due to the late appearance of lesions and slower spore-forming. The longest incubation time and life cycle of disease of the leaves were from the 2 weeks and 3 weeks-old-leaves. Nevertheless, the 5 weeks-old-leaves were the most susceptible to *P. personata*. The findings are consistent with our practical investigation on the field condition. Specifically, LLS disease often appears when the fruit formation starts. It has been about 7 weeks in the Spring crop and 6 weeks in the autumn crops, respectively. The latent period of spores has occurred about 14 days. The *P. personata* spores were initially infected with the leaves when the plants were about 5 weeks-old in Spring crop and 4 weeks-old for the autumn crop when peanut starts flowering (data not shown). Moreover, the diameter of the spot lesion was depended on the age of leaves, the most infection was 5 weeks-old leaves which was approximately 3.52mm, following by the 4 and 6 weeks-old-leaves were similar infection values by 2.51 and 2.82mm, respectively. However, when infecting the 7 weeks-old leaves, the spot lesion was the least by 2.20 mm. Therefore, the flowing time of peanut is favorable for LLS disease development. Contrarily, the LLS was caused by negligible infection during the seedling stage. For example, spot diameter of 2 and 3 weeks-old leaves was less infected by 1.41 and 1.76 mm, respectively (Table 4).

Similarly, the effects of leaf age on pathogenicity were examined on the ĐM4 peanut line. We found that the highest values of latent period, life cycle, spot diameter, number of lesions and rate of infected areas were at the 5 weeks-old-infected leaves, while the other leaf ages were found to lower infected values (Table 5). Our obtained results have been in agreement with the previous report of Zhang et al [11] who found that artificial infection of *P.personatum* on peanut at 3 weeks-old-plant was lower than 4 and 5 weeks plant infection. Therefore, we conclude that peanut flowering stage is the most susceptible disease infection, while peanut seedling stage is a negligible infection. Therefore, our findings may provide useful information to control LLS disease to improve peanut production effectively.

Table 5. Effects of leaf age on pathogenicity of *P.personata* spraying on ĐM4 peanut line at the concentration of 5.10^4/spores

Latent period (day)	Life circle (day)	Spot diameter (mm)	Number of lesions/double leaves (spot)	Level of leaf area infected (%)	fected frequency (spot/cm^2)	Latent period (day)
2 weeks	23.63^a	30.66^a	1.43^e	9.55^d	0.63^d	0.43^c
3 weeks	22.12^b	28.21^b	1.73^d	16.32^c	1.73^c	0.52^b
4 weeks	19.02^d	23.03^d	2.50^b	23.39^b	3.83^b	0.70^a
5 weeks	13.53^f	16.32^f	3.54^a	29.84^a	8.52^a	0.75^a
6 weeks	17.25^e	21.43^e	2.64^b	28.05^a	4.27^b	0.67^b
7 week	20.37^c	25.38^c	2.23^c	22.72^b	1.71^c	0.45^c
$LSD_{0,05}$	0.87	1.23	0.32	3.02	0.71	0.20

Means with the same letters in a column are not significantly different at P<0.05

Genotyping the groundnut lines LLS tolerance by SSR markers

In this study, 6 SSR markers (PM179; GM633; GM2301 IPAHM103; Lec1; seq7G02; TC9F10 và GM1760) involved in QTLs/genes IP1, IP2, LN1 LN2, and DS and explained phenotypic variation of LLS resistance by25.26%; 12.26%; 19.6%; 12.43% và 865% [8] were used to examine the selected lines. As our previous reports, the recipient variety is the elite groundnut variety and is widely grown in this country but is sensitive to LLS disease, while the donor plant is the low yield and high LLS resistant variety. The crossing was made to develop F_1, then backcrossed to generate BC_1F_1 to BC_3F_1. The individual plants of these generations were genotyped as foreground selection by the above SSR markers to select the individual plants carrying LLS QTLs/genes (heterozygote type). The plants were then made selfing to develop BC_3F_3. At this generation, 24 lines were selected and genotyped by using SSR markers. The results showed that all lines were homozygous type which had a similar band with the donor plant variety, as shown in Figure 2 and Table 6.

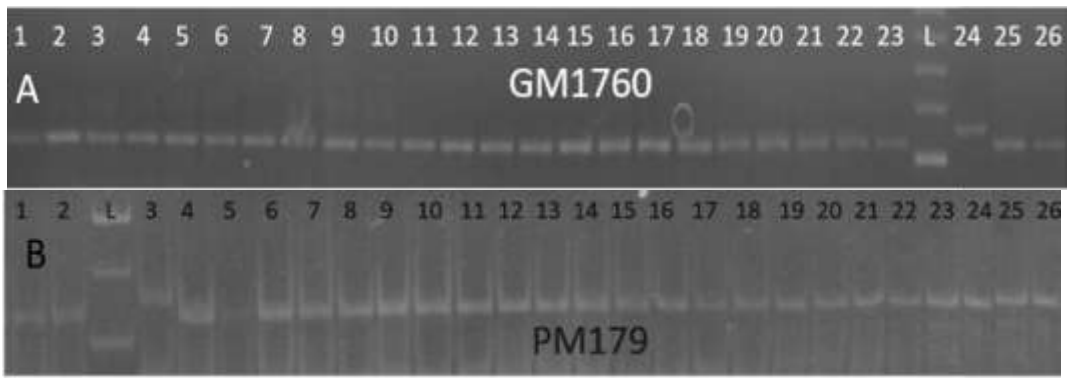


Fig. 2. Some illustrations of electrophoresis of the marker GM1760, PM179 to examine the groundnut lines carrying the QTLs/genes LLS resistance. A: Lane L: Standard Lader; Lane 24: CNC3; Lane 25:TN6; Lane: 1-23, 26 (the potential groundnut lines as the code number shown in Table 1); B: Lane L: Standard Lader; Lane 3: CNC3; Lane 4:TN6; Lane: 1,2, 5- 26 (the potential groundnut lines as the code number shown in Table 1)

Table 6. Summary of the peanut lines had the homozygous which were similar to the donor plant

No	Marker	The lines carrying LLS resistant QTLs/gene
1	PM179	CL4, CL5, CL6, CL7, CL8, CL9, ĐM1, CL10, ĐM2, CL11, ĐM3, CL12, CL13, CL14,CL15, CL16, CL17, DDM4, CL18, CL19, CL20, CL21
2	GM633	CL4, CL5, CL6, CL7, CL8, CL9, ĐM1, CL10, ĐM2, CL11, ĐM3, CL12, CL13, CL14,CL15, CL16, CL17, DDM4, CL18, CL19, CL20, CL21
3	GM2301	CL4, CL5, CL6, CL7, CL8, CL9, ĐM1, CL10, ĐM2, CL11, ĐM3, CL12, CL13, CL14,CL15, CL16, CL17, DDM4, CL18, CL19, CL20, CL21
4	IPAHM103	CL4, CL5, CL6, CL7, CL8, CL9, ĐM1, CL10, ĐM2, CL11, ĐM3, CL12, CL13, CL14,CL15, CL16, CL17, DDM4, CL18, CL19, CL20, CL21
5	TC9F10	CL4, CL5, CL6, CL7, CL8, CL9, ĐM1, CL10, ĐM2, CL11, ĐM3, CL12, CL13, CL14,CL15, CL16, CL17, DDM4, CL18, CL19, CL20, CL21
6	GM1760	CL4, CL5, CL6, CL7, CL8, CL9, ĐM1, CL10, ĐM2, CL11, ĐM3, CL12, CL13, CL14,CL15, CL16, CL17, DDM4, CL18, CL19, CL20, CL21

Conclusions

In summary, the selected 24 peanut lines were obtained from the BC_3F_3 populations which were derived from the crosses between the recipient and donor LLS tolerant plants. The results showed the lines were potential LLS resistance. Of which 4 lines, namely ĐM1, ĐM2, ĐM3 and ĐM4 were highly resistant to LLS resistance in the artificial infection test. We found that peanut flowering stage is the most susceptible disease infection, while peanut seedling stage is a negligible infection of *P.personata* Moreover, all lines were confirmed to carry QTLs/genes involving in LLS resistance by using SSR markers. Our findings may provide useful information for peanut breeding programs in this country.

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