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RESEARCH NOTE

Genetics of resistance to *Cercospora* leaf spot disease caused by *Cercospora canescens* and *Psuedocercospora cruenta* in yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis*) × grain cowpea (*V. unguiculata* ssp. *unguiculata*) populations

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Abstract. Yardlong bean (*Vigna unguiculata* ssp. *sesquipedalis*), a type of cowpea, is an important vegetable legume of Asia. Cercospora leaf spot (CLS) caused by *Cercospora canescens* and *Psuedocercospora cruenta* is an important phytopathological problem of the yardlong bean grown in tropical regions. The objectives of this study were to (i) determine mode of inheritance of resistance to CLS caused by *C. canescens* and *P. cruenta*, (ii) estimate the heritability of the resistance, (iii) estimate genetic effects on the resistance using six basic populations generated from the cross between the susceptible yardlong bean 'CSR12906' and the resistant grain cowpea (*V.unguiculata* spp. *unguiculata*) 'IT90K-59-120'. Segregation for the resistance to both fungi in the F_2 population fitted both 3 : 1 ratio and 13 : 3 ratio of susceptible:resistant, while that in the BC2 ((CSR12906×IT90K-59-120)×IT90K-59-120) population fitted a 1 : 1 ratio, suggesting one recessive gene or two genes with inhibitory gene action control the resistance. Generation mean analysis showed that a simple additive–dominance model was adequate to explain the genetic control of CLS disease resistance, indicating that a single gene controls the resistance. The average number of major genes (effective factors) controlling the resistance was estimated to be 1.05 and 0.92 for *C. canescens* and *P. cruenta*, respectively. The broad-sense heritability calculated for resistance to both diseases was higher than 0.90. Altogether, these results indicated that the resistance to CLS disease caused by *C. canescens* and *P. cruenta* in grain cowpea IT90K-59-120 is a highly heritable trait governed by a single major recessive gene.

Keywords. cowpea; yardlong bean; *Cercospora* leaf spot; generation mean analysis; *Cercospora canescens*.

Introduction

Vigna unguiculata is one of the most important legume crops of the world. It has four cultivated subspecies of which two are well known, namely *unguiculata* (grain cowpea) and *sesquipedalis* (yardlong bean). Yardlong bean is mostly cultivated in Asia. Young edible pods of this crop are consumed in several ways, both raw and cooked forms. The young pods can be harvested for four to five times beginning at about 45–50 days after sowing.

Cercospora leaf spot (CLS) disease is an important disease of grain cowpea and yardlong bean. This disease is caused by the fungi *Cercospora canescens* and *Pseudocercospora cruenta* resulting in yield loss of up to 40% (Schneider *et al.* 1976; Fery *et al.* 1977). The leaf-spot symptoms of *C. canescens* are circular, while those of *P. cruenta* are angular. Although *C. canescens* is comparatively a weaker parasite than *P. cruenta*, the former has a wider host range under tropical climates than the latter (Fery *et al.* 1977). In Thailand, the disease occurs all year-round where *C. canescens* is the first infector followed by *P. cruenta* (Duangsong *et al.* 2016).

Although there has been no report on the source of resistance to CLS disease in yardlong bean, there are

several genetic studies on the resistance in grain cowpea (Fery *et al.* 1976; Fery and Dukes 1977; Castro *et al.* 2003; Booker and Umaharan 2008; Duangsong *et al.* 2016). Fery *et al.* (1976) found that resistance to *P. cruenta* in grain cowpea breeding lines CR 17-1-34 and Ala. 963.8 are controlled by a single-dominant gene *Cls1* and a single-recessive gene *cls2*, respectively. These two genes are not linked (Fery and Dukes 1977). Castro *et al.* (2003) showed that the resistance to *P. cruenta* in the grain cowpea line L101000-1 is governed by a single-dominant gene. By using four resistant grain cowpea genotypes, Booker and Umaharan (2008) demonstrated that the resistance to *P. cruenta* is controlled by monogene or oligogenes or polygenes depending on the resistant sources. Recently, a major quantitative trait locus (QTL) controlling resistance to *P. cruenta* has been identified (Duangsong *et al.* 2016). Grain cowpea and yardlong bean are cross-compatible, thus grain cowpea can be a gene source for breeding yardlong bean resistance to CLS disease. Nonetheless, there has been no report on genetic basis of the resistance to CLS disease caused by *C. canescens* in cowpea/yardlong bean, although a major QTL for resistance to this pathogen in grain cowpea accession IT90K-59-120 has been tagged with molecular markers (Duangsong *et al.* 2016). IT90K-59-120 showed high resistance to both fungi in Thailand.

Knowledge on genetic controls such as mode of inheritance, number of genes and heritability is necessary for the breeders to choose for efficient breeding procedures. The objective of this study was to determine mode of inheritance of the resistance to CLS caused by *C. canescens* and *C. cruenta* in IT90K-59-120 using qualitative and quantitative analyses.

Materials and methods

Plant materials

Susceptible yardlong bean 'CSR12906' (P_1) was crossed with resistant cowpea 'IT90K-59-120' (P_2) to obtain F_1 seeds. P_1 is a breeding line from Clover Seed Company, Hong Kong. P_2 is a breeding line developed by the International Institute of Tropical Agriculture, Nigeria. P_2 showed high resistance to *C. canescens* in Thailand, while P_1 showed high susceptible to *C. canescens*. P_1, P_2 and F_1 plants were grown, and further an F_1 plant was self-pollinated to produce F_2 populations, while the other F_1 plants were backcrossed with P_1 and P_2 to produce BC1 and BC2 populations, respectively.

Field experimentation

Six generations (populations), namely P_1, P_2, F_1, F_2, BC1 and BC2 were sown in a nonreplicated trial in a farmer field in Rachaburi province, Thailand during March to May 2014. Spacing between rows was 75 cm and between plants was 40 cm. Ten days before sowing the six populations, P_1 was planted surrounding the experimental field to serve as a source of disease inoculum. Artificial inoculation was also carried out at 40 and 50 days after planting (DAP) following the method described by Chankaew *et al.* (2011).

Data collection

Disease scores for symptoms of *C. canescens* and *P. cruenta* recorded on each plant using a rating scale of 1–5. The rating scales were assessed as: 1, no disease symptoms on leaves; 2, 1–25% of leaf area infected; 3, 26–50% of leaf area infected; 4, 51–75% of leaf area infected; 5, 76–100% of leaf area infected. Disease scoring for CLS caused by *C. canescens* and *P. cruenta* was performed every seven days, beginning at 35 DAP until 70 DAP. The score for *C. canescens* were calculated into the area under the disease progress stairs (AUDPS) following Simko and Piepho (2012).

Data analysis

In each population, plants showing the disease score ≤ 2 or AUDPS ≤ 50 were classified as resistant, while those showing the score >2 or AUDPS >50 were classified as susceptible. The numbers of resistant and susceptible plants were subjected to segregation analysis by the chi-square test (χ^2) using the R program 2.14.0 (R Development Core Team 2013).

Variance for disease scores and AUDPS of the six populations was calculated using the R program (R Development Core Team 2013). Mean value and standard error of different generations were tested for the adequacy of additive–dominance model using a joint scaling test (Cavalli 1952). Genetic effects with a three-parameter model involving mean (m), additive (d) and dominance (h) were estimated using weighted least squares following Mather and Jinks (1982). From these estimated parameters, the expected generation means were calculated as follows:

$$\begin{aligned} P_1 &= m - d \\ P_2 &= m + d \\ F_1 &= m + h \\ F_2 &= m + (1/2)\,h \\ BC1 &= m - (1/2)\,d + (1/2)\,h \\ BC2 &= m + (1/2)\,d - (1/2)\,h \end{aligned}$$

This model provides a χ^2 test for the goodness of fit of the model using χ^2. Significance of the gene effects was determined by a t-test.

$\sigma^2_{P_1}$, $\sigma^2_{P_2}$, $\sigma^2_{F_1}$ and $\sigma^2_{F_2}$ are the variances of P_1, P_2, F_1 and F_2 generations, respectively, used to estimate the variation

due to phenotypes (P), environments (E) and genotypes (G) (Warner 1952; Wright 1968) as follows:

$$\sigma_P^2 = \sigma_{F_2}^2$$
$$\sigma_E^2 = \left(\sigma_{P_1}^2 + \sigma_{P_2}^2 + 2\sigma_{F_1}^2\right)/4$$
$$\sigma_G^2 = \sigma_P^2 - \sigma_E^2$$

Then, broad-sense heritability was calculated as σ_G^2/σ_P^2.

The number of effective factors (EF) controlling the CLS resistance was calculated by three methods:

Method I (Wright 1968):

$$EF_1 = \frac{(P_1 - P_2)^2\,[1.5 - 2h\,(1-h)]}{8\left[\sigma_{F_2}^2 - 0.25\left(\sigma_{P_1}^2 + \sigma_{P_2}^2 + 2\sigma_{F_1}^2\right)\right]},$$

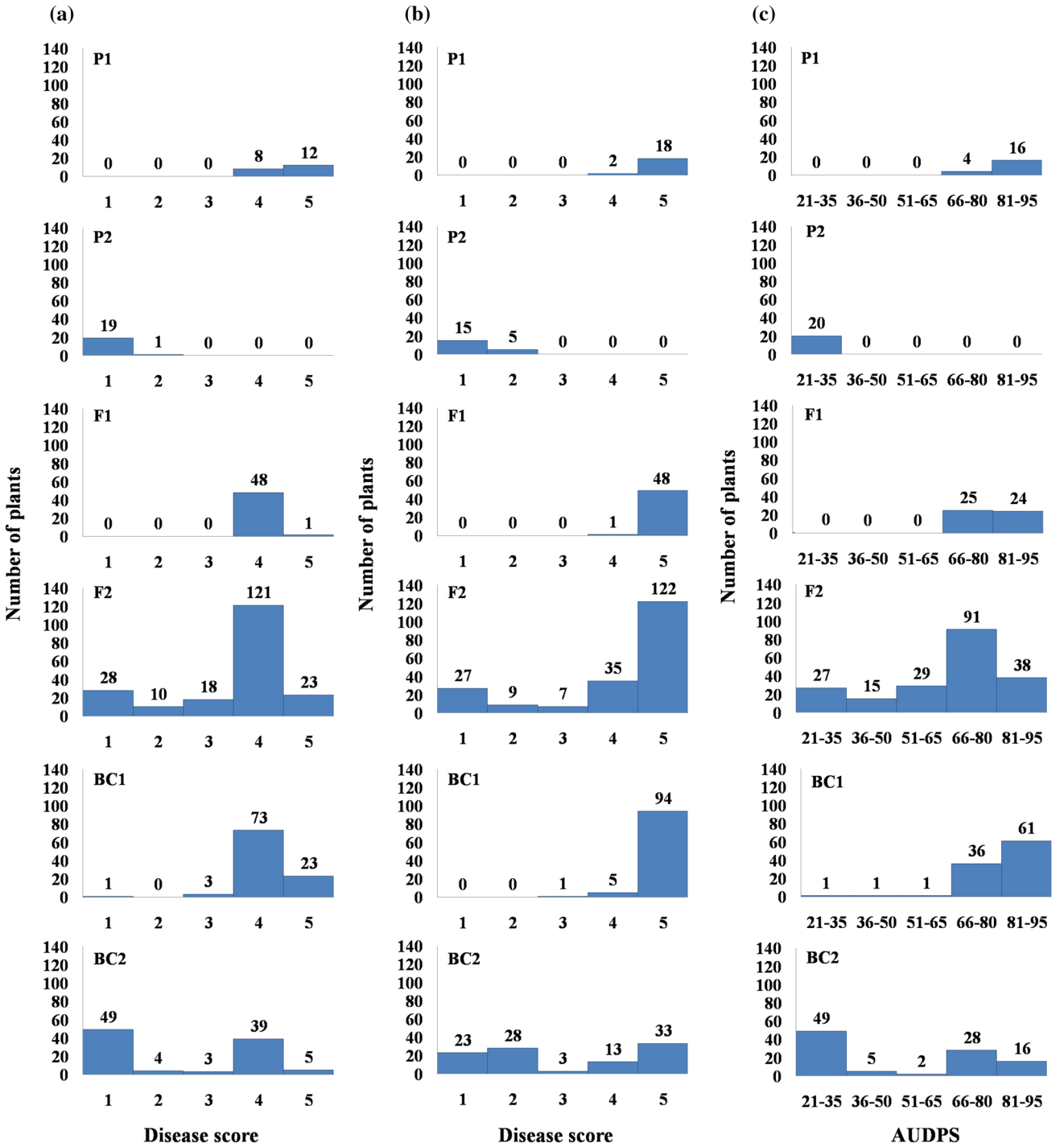

Figure 1. Distribution of the scores for CLS disease caused by *C. canescens* (a) and *P. cruenta* (b), and of AUDPS (c) in six populations derived from the cross between susceptible yardlong bean 'CSR12906' and resistant cowpea 'IT90K-59-120'.

where F_1, P_1 and P_2 are generation means, while $\sigma^2_{P_1}$, $\sigma^2_{P_2}$, $\sigma^2_{F_1}$ and $\sigma^2_{F_2}$ are variances of the disease scores of the respective generations, and $h = (F_1–P_1)/(P_2–P_1)$.

Methods II and III (Lande 1981):

$$EF_2 = \frac{(P_1 - P_2)^2}{8\left[\sigma^2_{F_2} - 0.25\left(\sigma^2_{P_1} + \sigma^2_{P_2} + 2\sigma^2_{F_1}\right)\right]}$$

$$EF_3 = \frac{(P_1 - P_2)^2}{8\left[\sigma^2_{BC1} + \sigma^2_{BC2} - \left(0.5\sigma^2_{P_1} + 0.5\sigma^2_{P_2} + \sigma^2_{F_1}\right)\right]},$$

where generation means and variances have the same meaning as in EF_1 above.

All of the above formulas are based on the assumption that genes segregating for the CLS resistance are from the resistant parent, not linked, having equal effects, without dominance effect and genotype × environment effects (Wright 1968).

Results

Disease variation in six generations

The yardlong bean parent (P_1) was highly susceptible to *C. canescens* (score: 4.28), while the cowpea parent (P_2) was highly resistant (score: 1.03). All F_1 plants were susceptible with score 3.80. In the F_2 population, the scores varied between 1.00 and 5.00 with an average of 3.27. In the BC1 population, all plants were susceptible except one which was found resistant to *C. canescens*. The average scores were of 3.92. In the BC2 population, the scores varied between 1.00 and 5.00 with an average of 2.34. Reactions to *P. cruenta* in the six populations were similar to those to *C. canescens*. The disease scores for *P. cruenta* in P_1 and P_2 were 4.90 and 1.15, respectively. All F_1 plants were susceptible with score 4.67. The scores in the F_2 population varied between 1.00 and 5.00 with an average of 3.88. In the BC1 population, all plants were susceptible except one which was found resistant to *C. canescens* with an average score of 4.58. In the BC2 population, the scores varied between 1.00 and 5.00 with an average of 2.89. In addition, P_1 showed more rapid disease development caused by *C. canescens* than the P_2 parent. AUDPS values of the P_1 and P_2 were 84.52 and 29.52, respectively. The F_1 and BC1 populations were susceptible with an average AUDPS of 76.81 and 81.86, respectively, though two BC1 plants showed resistance. While the F_2 and BC2 populations showed segregation with the average of 64.65 and 51.66, respectively. Frequency distributions of the disease scores caused by *C. canescens* and *P. cruenta* and AUDPS of all populations are shown in figure 1.

Qualitative inheritance

Plants in the populations were classified into two classes (resistant and susceptible) and the numbers of plants in

Table 1. Segregation analysis of resistance:susceptible classes in six generations derived from the cross between susceptible yardlong bean 'CSR12906' and resistant cowpea 'IT90K-59-120'.

	Generation	Observed number		Ratio	Expected number		χ^2	*P* value
		Resistant	Susceptible	R : S	Resistant	Susceptible		
CLS disease caused by *C. canescens*								
CSR12906	P_1	0	20	0 : 1	0	20	0	1
IT90K-59-120	P_2	20	0	1 : 0	20	0	0	1
	F_1	0	49	0 : 1	0	49	0	1
	F_2	38	162	1 : 3	50	150	3.84	0.0501
	F_2	38	162	3 : 13	37.5	162.5	0.0082	0.9278
	BC_1	1	99	0 : 1	0	100	–	–
	BC_2	53	47	1 : 1	50	50	0.36	0.5484
CLS disease caused by *P. cruenta*								
CSR12906	P_1	0	20	0 : 1	0	20	0	1
IT90K-59-120	P_2	20	0	1 : 0	20	0	0	1
	F_1	0	49	0 : 1	0	49	0	1
	F_2	36	164	1 : 3	50	150	5.23	0.0222
	F_2	36	164	3 : 13	37.5	162.5	0.0738	0.7858
	BC_1	0	100	0 : 1	0	100	0	1
	BC_2	51	49	1 : 1	50	50	0.04	0.8410
AUDPS of *C. canescens*								
CSR12906	P_1	0	20	0 : 1	0	20	0	1
IT90K-59-120	P_2	20	0	1 : 0	20	0	0	1
	F_1	0	49	0 : 1	0	49	0	1
	F_2	39	161	1 : 3	50	150	3.23	0.0725
	F_2	36	164	3 : 13	37.5	162.5	0.0738	0.7858
	BC_1	2	98	0 : 1	0	100	–	–
	BC_2	53	47	1 : 1	50	50	0.36	0.5484

Table 2. Joint scaling test and estimated gene effects for CLS disease in six generations derived from the cross between susceptible yardlong bean 'CSR12906' and resistant grain cowpea 'IT90K-59-120'.

Genetic components	Disease scores of *C. canescens*	Disease scores of *P. cruenta*	AUDPS of *C. canescens*
m	2.63***	3.02***	57.01***
d	1.60***	1.86***	27.51***
h	1.16***	1.64***	20.37***
χ^2	3.68^{ns}	4.83^{ns}	5.09^{ns}

***Significantly different at the 0.001% probability level.
^{ns}Not significant.

both classes were subjected to a χ^2 test. The segregation of disease scores and AUDPS for *C. canescens* in the F_2 population fitted both the 3 (susceptible) : 1 (resistant) ratio and the 13 (susceptible) : 3 (resistant) ratio (table 1). Similar to *C. canescens*, segregation of disease scores for *P. cruenta* in the F_2 population fitted both the 3 : 1 ratio and the 13 : 3 ratio (table 1). The results suggested that the resistant to the CLS disease is controlled by either a single-recessive gene or two genes with inhibitory gene actions. The segregation of the disease scores and AUDPS in the BC2 populations fitted the 1 (susceptible) : 1 (resistant) ratio (table 1). This result suggested that the resistance CLS disease is controlled by a single-recessive gene or two genes with inhibitory gene actions, similar to the results of F_2 population.

Quantitative inheritance

Generation mean analysis revealed that a simple additive–dominance model was able to adequately explain the variation among the generations for disease scores and AUDPS (table 2), indicating that a single gene with additive and dominance gene effects controls CLS disease resistance and there was no nonallelic interaction effects for the resistance. Population mean, additive gene effect and dominant gene effect estimated for the disease scores caused by *C. canescens* were 2.63, 1.60 and 1.16, respectively, while those for the disease caused by *P. cruenta* were 3.02, 1.86 and 1.64, respectively. Population mean (*m*), additive gene effect (*d*) and dominant gene effect (*h*) estimated for AUDPS values caused by *C. canescens* were 51.07, 27.51 and 20.37, respectively (table 2).

Heritability and number of effective factors for CLS resistance

Broad-sense heritability (H^2) values calculated for disease scores and AUDPS caused by *C. canescens* were 0.97 and 0.95, respectively. The H^2 calculated for disease scores caused by *P. cruenta* was 0.95. The number of effective factors estimated for the CLS resistance caused by *C. canescens* ranged from 0.58 to 1.45 with an average of 1.05, while those estimated for the resistance caused by *P. cruenta* varied between 0.62 and 1.25 with an average of 0.92. These suggested that the resistance to CLS is conditioned largely by genetic factor(s).

Discussion

Although the results from qualitative analysis by a χ^2 test suggested that CLS resistance caused by *C. canescens* and *P. cruenta* in IT90K-59-120 is conditioned by either a single-recessive gene or two genes with inhibitory gene action (table 1), quantitative analysis by generation mean analysis (table 2) and analysis for number of effective factors consistently revealed that the CLS resistance in IT90K-59-120 is conditioned by a single gene. Therefore, it can be concluded that the CLS resistance caused by *C. canescens* and *P. cruenta* in IT90K-59-120 is controlled by a single-recessive gene. Our findings agreed with those of Fery *et al.* (1976) who reported that resistance to *P. cruenta* in grain cowpea Ala.963.8 is controlled by a single-recessive gene.

High heritability estimated for the resistance (95–97% depending on the resistance parameters) indicated that the resistance is mainly controlled by genetic factor(s). This implies that breeding for resistance cultivars using IT90K-59-120 as the resistant source can be done by any standard selection methods for self-pollinated species or by backcross breeding.

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