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Inheritance Characteristics of F₁ and F₂ Offsprings Obtained from Full Diallele Crosses of Five Cowpea Cultivars (*Vigna unguiculata* L. Walp) Against *Pseudocercospora* Leaf Spot Disease

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Abstract: The experiment was carried out at the Experimental Farm, Department of Agricultural Technology, Maharakham University, Maharakham Province in the 2005 (February to April) for F₁ offsprings and also at the Experimental Farm, Khon Kaen University, Khon Kaen Province, Northeast Thailand for F₂ offsprings (July to October), i.e., four experiments were carried out, each location had two experiments. The research aimed to investigate inheritance characteristics of F₁ and F₂ offsprings derived from full diallele crosses of five cowpea cultivars (20 pairs of crosses) against a pathogenic disease of *Pseudocercospora cruenta* (Sacc.) Deighton. The first two experiments were carried out under field and glasshouse conditions at Maharakham University for F₁ offsprings and the other two experiments were carried out at Khon Kaen University for F₂ offsprings. A Chi square test method was used to justify dominant genes on the infection of the disease. The results showed that the cowpea plants of F₁ and F₂ offsprings gave only one pair of dominant gene that had its complete dominant effect over recessive gene of the cowpea offsprings.

Key words: Complete dominant gene, cowpea cultivars, detached leaf technique, inheritance characteristics, recessive gene

INTRODUCTION

Cowpea crop (*Vigna unguiculata* L. Walp) is one of many important legume crops being cultivated for man daily consumption in most of the tropical and subtropical countries around the world, particularly in African countries, e.g., Nigeria, Niger and others (approximately 68% of world production). The world records on its annual seed production reached an amount of approximately 3.3 million tonnes in the 2000 where majority of this legume seed production were produced in the African countries (http://www.fao.org/inpho/content/compend/text/ch32_01.htm). Within this decade, the cultivation of cowpea cultivars in Thailand has been facing with many problems such as poor soil fertility, acidic soil conditions, diseases and many others, particularly the problem due to fungous diseases damaging the growth and crop yields, especially during its rapid growth period (Sinsiri *et al.*, 2006). It is well advocated that a fungous disease of *Pseudocercospora* leaf spot caused by pathogenic disease of *Pseudocercospora cruenta* (Sacc.) Deighton has its enormous damages to annual crop yields of cowpea

cultivars. This kind of fungous diseases when infested in leaves of the crop plants it obviously produces chlorotic or necrotic spots on uppermost area of leaves where it produces conidiophores and conidia with grayish appearances (Emechebe and Lagoke, 2003). This pathogenic disease could infect a large number of leguminous crops (Allen *et al.*, 1998) including *Hibiscus esculentus* vegetable crop where this crop plant has also been recognized as a host plant of the disease (Joseph *et al.*, 1998). Spores development of this pathogenic disease could develop most rapidly at 25°C under daylight conditions but not in the dark. A rapid development of the spores could be largely achieved from additional amount of cowpea extract solution derived from New Era cowpea cultivar added to nutrient solution rather than the use of nutrient solution of distilled water alone (Ekpo and Esuruoso, 1977). Pandey and Pandey (2002) carried out experiment in India they reported that *P. cruenta* could develop most rapidly when the plants of Kala Jhamala cowpea cultivar were grown under relative humidity of 81 to 86% with day-length of 7.5-8.5 h and the maximum amount of infection was found at 57-65 days after emergence.

The search for dominant genes against diseases had been carried out by a number of plant breeders, e.g., Taiwo *et al.* (1981), they showed that offsprings derived from F₁, F₂ and a backcross of cowpea plants produced only one pair of recessive genes that possessed a high resistant feature against *Black eye mosaic virus*. The results of a similar breeding process of cowpea cultivars of Ouattara and Chambliss (1991) confirmed this finding. Ogundiwin *et al.* (2002) also reported that only a single pair of genes derived from F₁, F₂ and a backcross of cowpea cultivars possessed high resistant feature against Mottle Carom virus. With the research of Castro *et al.* (2003) on cowpea cultivars of F₁, F₂ and F₃ offsprings against *Cercospora* leaf spot of *Cercospora cruenta* (Sacc.) pathogenic disease. The results revealed that after the completion of the experiment they found only one pair of a dominant gene of the crop plants manifested a high resistant feature against the disease. Whilst other previous published data also have shown a similar result to those latest ones such as Fery *et al.* (1975), Williams (1977), Lin *et al.* (1995) and Fery and Dukes (1995, 1996) and others. From these published data, it may be of important value to carry out some further breeding activities and carrying out experiments with the use of cowpea offsprings where the experiment aimed to search for some possible outstanding genes with a high resistant feature against diseases in cowpea plants, particularly, the genes that possess high resistant property against *Pseudocercospora cruenta* (Sacc.) Deighton pathogenic disease since published data on this respect in Thailand has been relatively limited (Sinsiri *et al.*, 2006).

MATERIALS AND METHODS

For F₁ field experiment, the research was carried out at the Experimental Farm, Department of Agricultural Technology, Maharakham University (Feb-April, 2005 for F₁) and also the Experimental Farm, Khon Kaen University (July-October 2005 for F₂), five cowpea cultivars were used for a full diallele mating. The five cultivars were chosen from the results of the previous experiments (Sinsiri *et al.*, 2006). They include KVC7 and IT 84E-1-108 (immune cultivars); IT81D-1228-14-1 (resistant cultivar); KKV25 and MSU1 (susceptible

cultivars). These five cowpea cultivars were used in a full diallele mating of 20 crosses for F₁ and F₂ offsprings (Table 1). The F₁ and F₂ cowpea plants were allowed to grow under field conditions aiming to search for pairs of genes against *Pseudocercospora cruenta* (Sacc.) Deighton pathogenic disease where a statistical analysis of Chi-square test was used. For F₁ field experiment, the experiment consisted of 20 pairs of diallele mating and each pair has 64 plants, i.e., 1,280 plants altogether plus five parental cultivars and each parental cultivar has 64 plants, i.e., altogether 1600 individual plants. These plants were allowed to grow in a plot with an area of 400.00 m² where the plot was surrounded by two infector rows of KKV25 cultivar (susceptible cultivar). Seeds of KKV25 being used as infector rows were sown by hand 15 days ahead before the sowing of seeds in main plots. The sowing distance used was a 50×50 cm between rows and within rows, respectively. A few seeds drill⁻¹ of each pair together with seeds of the five parental cultivars were sown by hand directly into the soil. Twenty days after emergence, seedlings were thinned out leaving only one seedling drill⁻¹ and at the same time weeding was carried out by mechanical means. After weeding was taken place, a complete chemical fertiliser at a rate of 312.50 kg ha⁻¹ was evenly applied by hand into the plot. The 64 plants of each pair of diallele mating were sown at random starting from group No. 1 up to No. 20 and all of the groups were labeled with their appropriate numbers for data collection. During the early growth period, Lannate (Methomy), an insecticide was spraying twice at days 10 and 20 after emergence to prevent damages caused by insect pests. The recommended rate of the chemical company (EI. Dupont Thailand Co. Ltd.) was used. At day 26 after emergence, leaf numbers 4 and 5 (being counted from top) of 32 plants of each mating pair were collected at random for a further work on detached leaf experiment where the experiment was carried out under glasshouse conditions with the use of the method of Sinsiri and Laohasiriwong (2007) was used. At day 30 after emergence, a foliage spray of *Pseudocercospora cruenta* (Sacc.) Deighton to plant leaves at a rate of 5×10⁴ colony forming units mL⁻¹ was carried out for both field and detached leaf experiments and each mating pair received the spraying of the disease at a rate of 200 mL pair⁻¹ then examinations on

Table 1: An illustration of a full diallele crosses with the use of five cowpea cultivars, the study was carried out at the Experimental Farm of Maharakham University, Northeast Thailand during the rainy season of the 2003

Parental cultivars	MSU1 (S)	KKV25 (S)	KVC7 (I)	IT81D1228-14-1 (R)	IT84E-1-108 (I)
MSU1 (S)	-	X	X	X	X
KKV25 (S)	X	-	X	X	X
KVC7 (I)	X	X	-	X	X
IT81D1228-14-1 (R)	X	X	X	-	X
IT84E-1-108 (I)	X	X	X	X	-

I = Immune cultivars, R = Resistant cultivar, S = Susceptible cultivars

the appearances of the disease were carried out at days 37, 44, 51 and 65 after emergence. However, of the four sampling periods only data at day 65 where the infection was most severed are presented in this work. Scores on disease incidences were carried out with the use of the method described by Adejumo *et al.* (2001) and Sinsiri *et al.* (2006).

For F₂ experiment (carried out at Khon Kaen University), seeds collected from F₁ were sown again in a plot similar to that of F₁ experiment, i.e., the method used was a repeat of F₁ experiment where the plants of 20 pairs of diallel mating together with 5 parental cultivars were used. K KU 25 was used as infector rows as did with F₁ experiment. The method being used with the detached leaf experiment carried out under glasshouse conditions of F₁ experiment was repeated again for F₂ experiment. Data collection was carried out as did with the F₁ experiment. The collected results were subjected to a Chi-square test for the determinations of gene characteristics against the pathogenic disease of *Pseudocercospora cruenta* (Sacc.) Deighton.

RESULTS

Infestation of disease in parental plants and F₁ offsprings:

With initial field experiment, the results on disease

infection of F₁ cowpea plants showed that there were only two pairs of diallele crosses infected, i.e., MSU x K KU25 (S x S) and K KU25 x MSU1 (S x I) with values of disease incidences of 44.41 and 56.65% with severity levels of 5 and 5, respectively (Table 2). A severe infection of the disease was attained with both cowpea parents, i.e., MSU1 (S) and K KU25 (S) where both cultivars gave values of disease incidences of 61.5 and 100% with severity levels of 5 and 5, respectively. Other crosses did not show any sign of infection (Table 2). For detached leaf technique, the results of a similar trend was attained, i.e., the two pairs of crosses of MSU x K KU25 (S x S) and K KU25 x MSU1 (S x I) gave disease incidences of 36.20 and 48.60% with severity levels of 5 and 5, respectively. A similar result to field experiment was also found with MSU1 (S) and K KU25 (S) cultivars where both cultivars gave disease incidences of 59.8 and 100% with severity values of 5 and 5, respectively. Other cowpea plants derived from diallele crosses together with parental cultivars did not show any sign of infection.

Infestation of disease in parental plants and F₂ offsprings:

The results showed that offsprings of F₂ generation derived from crosses of S x S parents, i.e., K KU25 x MSU1 and MSU1 x K KU25 gave disease severity incidences of 5 and 4.5, respectively (Table 3). Whilst offsprings of F₂

Table 2: Cowpea plants of twenty pairs of diallele crosses (F₁) plus five parental cowpea cultivars as affected by *Pseudocercospora* leaf spot disease where the results were of both field growth conditions and detached leaf technique. The experiments were carried out at Mahasarakham University, Northeast Thailand

20 pairs of diallele crosses plus five parental cultivars	Field experiment				Detached leaf technique			
	No. of plants		Incidence (%)	Severity (1-5)	No. of leaves		Incidence (%)	Severity (1-5)
	Total	Infected			Total	Infected		
MSU1 x K KU25 (S x S)	61	54	44.41	5.0	32	27	36.2	5.0
MSU1 x KVC7 (S x I)	59	0	0.00	1.0	28	0	0.0	1.0
MSU1 x IT81D-1228-14-1 (S x R)	64	0	0.00	1.0	29	0	0.0	1.0
MSU1 x IT84E-1-108 (S x I)	64	0	0.00	1.0	32	0	0.0	1.0
K KU25 x MSU1 (S x S)	56	50	56.65	5.0	31	26	48.6	5.0
K KU25 x KVC7 (S x I)	49	0	0.00	1.0	32	0	0.0	1.0
K KU25 x IT81D-1228-14-1 (S x R)	60	0	0.00	1.0	28	0	0.0	1.0
K KU25 x IT84E-1-108 (S x I)	64	0	0.00	1.0	29	0	0.0	1.0
KVC7 x MSU1 (I x S)	57	0	0.00	1.0	29	0	0.0	1.0
KVC7 x K KU25 (I x S)	54	0	0.00	1.0	32	0	0.0	1.0
KVC7 x IT81D-1228-14-1 (I x R)	64	0	0.00	1.0	31	0	0.0	1.0
KVC7 x IT84E-1-108 (I x I)	64	0	0.00	1.0	32	0	0.0	1.0
IT81D-1228-14-1 x MSU1 (R x S)	61	0	0.00	1.0	32	0	0.0	1.0
IT81D-1228-14-1 x K KU25 (R x S)	49	0	0.00	1.0	30	0	0.0	1.0
IT81D-1228-14-1 x KVC7 (R x I)	55	0	0.00	1.0	27	0	0.0	1.0
IT81D-1228-14-1 x IT84E-1-108 (R x I)	48	0	0.00	1.0	29	0	0.0	1.0
IT84E-1-108 x MSU1 (I x S)	59	0	0.00	1.0	32	0	0.0	1.0
IT84E-1-108 x K KU25 (I x S)	64	0	0.00	1.0	32	0	0.0	1.0
IT84E-1-108 x KVC7 (I x I)	61	0	0.00	1.0	32	0	0.0	1.0
IT84E-1-108 x IT81D-1228-14-1 (I x R)	63	0	0.00	1.0	32	0	0.0	1.0
MSU1 (S)	62	57	61.50	5.0	31	31	59.8	5.0
K KU 25 (S)	61	61	100.00	5.0	30	30	100.0	5.0
KVC7 (I)	64	0	0.00	1.0	32	0	0.0	1.0
IT81D-1228-14-1 (R)	64	0	0.00	1.0	32	0	0.0	1.0
IT84E-1-108 (I)	58	0	0.00	1.0	30	0	0.0	1.0

S = Susceptible cultivars, R = Resistant cultivars, I = Immune cultivars

Table 3: Cowpea plants of twenty pairs of diallele mating (F₂) plus five parental cowpea cultivars as affected by *Pseudocercospora* leaf spot disease where the results were of both field growth conditions and detached leaf technique. The experiments were carried out at Khon Kaen University, Northeast Thailand

Twenty pairs of diallele mating plus five parental cultivars	Field conditions					Detached leaf technique				
	No. of plants			Incidence (%)	Severity levels (1-5)	No. of leaves			Incidence (%)	Severity (1-5)
	Total	Infected	χ^2			Total	Infected	χ^2		
MSU1 x K KU25 (S x S)	149	141	-	25.47	5.00	32	28	-	28.09	5.00
MSU1 x KVC7 (S x I)	142	26	3.39	29.51	5.00	27	3	2.78	30.09	4.50
MSU1 x IT81D-1228-14-1 (S x R)	113	20	3.21	88.42	4.50	28	4	1.71	92.64	4.50
MSU1 x IT84E-1-108 (S x I)	147	28	2.72	44.31	4.75	32	4	2.67	49.71	4.25
K KU25 x MSU1 (S x S)	196	188	-	33.09	4.50	32	28	-	49.08	5.00
K KU25 x KVC7 (S x I)	159	44	0.71	49.71	5.00	32	5	1.50	50.63	4.25
K KU25 x IT81D-1228-14-1 (S x R)	174	39	0.62	58.46	5.00	32	7	0.17	77.46	4.33
K KU25 x IT84E-1-108 (S x I)	160	34	1.20	41.32	5.00	32	5	1.50	43.38	4.75
KVC7 x MSU1 (I x S)	140	22	6.44*	28.41	4.00	27	2	4.46*	36.15	5.00
KVC7 x K KU25 (I x S)	196	40	2.20	46.18	4.75	32	6	0.67	69.23	4.66
KVC7 x IT81D-1228-14-1 (I x R)	113	-	-	0.00	1.00	32	-	-	0.00	1.00
KVC7 x IT84E-1-108 (I x I)	160	-	-	0.00	1.00	32	-	-	0.00	1.00
IT81D-1228-14-1 x MSU1 (R x S)	121	21	3.77	35.16	4.75	28	3	3.05	42.21	5.00
IT81D-1228-14-1 x K KU25 (R x S)	190	55	1.58	45.39	5.00	32	4	2.67	58.75	4.75
IT81D-1228-14-1 x KVC7 (R x I)	113	-	-	0.00	1.00	32	-	-	0.00	1.00
IT81D-1228-14-1 x IT84E-1-108 (R x I)	156	-	-	0.00	1.00	32	-	-	0.00	1.00
IT84E-1-108 x MSU1 (I x S)	200	43	1.31	66.31	5.00	28	3	3.05	90.38	3.00
IT84E-1-108 x K KU25 (I x S)	131	28	0.69	49.18	4.75	32	5	1.50	65.07	4.33
IT84E-1-108 x KVC7 (I x I)	144	-	-	0.00	1.00	32	-	-	0.00	1.00
IT84E-1-108 x IT81D-1228-14-1 (I x R)	185	-	-	0.00	1.00	29	-	-	0.00	1.00
MSU1 (S)	189	183	-	40.61	5.00	32	27	-	47.46	4.50
K KU25 (S)	200	200	-	100.00	5.00	31	31	-	100.40	5.00
KVC7 (I)	130	-	-	0.00	1.00	32	-	-	0.00	1.00
IT81D-1228-14-1 (R)	165	-	-	0.00	1.00	32	1	-	18.08	2.00
IT84E-1-108 (I)	200	-	-	0.00	1.00	32	-	-	0.00	1.00

$\chi^2 < 3.841$ = acceptable, Ho ratio of 3:1 at $p \leq 0.05$; * = acceptable, Ha at $p \leq 0.05$; S = Susceptible cultivars, R = Resistant cultivars, I = Immune cultivars

derived from crosses of resistant cultivars (S) and immune cultivars (I) of both field and detached leaf experiments where the crosses include several pairs of crosses, i.e., KVC7 (I) x IT81D-1228-14-1 (R), KVC7 (I) x IT84E-1-108 (I), IT81D-1228-14-1 (R) x KVC7 (I), IT81D-1228-14-1 (R) x IT84E-1-108 (I), IT84E-1-108 (I) x KVC7 (I) and IT84E-1-108 (I) x IT81D-1228-14-1 (R) gave no sign of disease infection. With the crosses derived from susceptible (S) and resistant (R) cultivars, i.e., the two crosses of K KU25 (S) x IT81D-1228-14-1 (R) and IT81D-1228-14-1 (R) x MSU1 (S) gave a ratio between uninfected and infected of 3:1 under field experiment with Chi square values within a range of 0.62 to 3.77. The cowpea plants of the latter crosses also gave a ratio between uninfected and infected of 3:1 with Chi square range values of 0.17 to 3.05 except crosses between KVC7 x MSU1 under field experiment where they gave Chi square values within a range of 6.44 to 4.46 whereas detached leaf experiment did not turn up a similar ratio between uninfected and infected plants.

DISCUSSION

With the results of F₁ offsprings where the results derived from a full diallele mating of the five parental cowpea cultivars of the initial experiments, i.e., a field experiment and a detached leaf technique carried out

under glasshouse conditions, the results attained from field experiment showed that out of the offsprings derived from 20 pairs of crosses only 2 pairs of the crosses, i.e., MSU1 (S) x K KU25 (S) and K KU25 x MSU1 gave the highest disease incidences of 44.41 and 56.63 with severity levels of 5 and 5, respectively. The results evidently showed that offsprings derived from these two pairs of crosses failed to attain any immunity or resistance against the pathogenic disease of *Pseudocercospora cruenta* and a similar trend was also attained with the detached leaf technique experiment. Therefore, the crosses among the cultivars of poor resistance against the disease have proved to provide information of some disadvantages. The results of the cowpea plants derived from both parents [MSU1 (S) and K KU25 (S)] also provided a similar trend to those of the F₁ offsprings. However, the results of other crosses have proved to attain a successful diallele mating, i.e., out of 20 crosses, only 2 crosses failed to provide any advantage. Thus the F₂ cowpea plants derived from the F₁ generation of the 18 crosses may be of significant value for further works in order to attain further information on the infection of the disease. Castro *et al.* (2003) reported a similar finding. Therefore, the crosses between the cultivars of S symbol, i.e., S x S may not be of advantage whereas the S cultivars gave an outstanding result when mating with other

symbols of disease features of cowpea cultivars such as R and I then the offsprings gave no disease incidences. The results indicated an inherited characteristic of genes of both R and I features against the disease where dominant genes have possessed its outstanding properties.

For the results of F_2 offsprings, i.e., the cowpea plants derived from F_1 generation. The results revealed that offspring derived from the crosses between S x S (MSU1 x KKV25) and between S x S (KKU25 x MSU1) did not show any resistant feature against the disease, i.e., all plants were totally infected, whilst crosses of several pairs, i.e., I x R (KVC7 x IT81D-1228-14-1); I x I (KVC7 x IT84E-1-108); R x I (IT81D-1228-14-1 x KVC7); R x I (IT81D-1228-14-1 x IT84E-1-108); I x I (IT84E-1-108 x KVC7); I x R (IT84E-1-108 x IT81D-1228-14-1) did not show any sign of infection. The results indicated that these crosses of the immune and resistant cultivars could possibly be considered to provide some splendid advantages against the disease. However, with the results derived from plants grown under field conditions of F_2 offspring, the results showed that two pairs of crosses of S x R (KKU25 x IT81D-1228-14-1) and R x S (IT81D-1228-14-1 x MSU1) gave disease infected ratio of 3:1 with Chi square values ranged from 0.167-3.05. The results indicated the effect of dominant genes against the disease where the F_2 offsprings have possessed a pair of dominant gene where it gave a complete dominant effect against the infection of the disease. The attained ratio of 3:1 could be of acceptable satisfaction and this outcome did not go against the principle of diallele crosses. However, with the results derived from crosses between I x S (KVC7 x MSU1) where they gave Chi square values of 6.44 and 4.46 for both field and detached leaf experiments, respectively and at the same time they were not able to provide a ratio of the disease infection of 3:1 as did with the rest. The reason for this could possibly be attributable to perhaps the small amount of the sampling populations. From the results attained with both field and detached leaf experiments of F_1 and F_2 offsprings, the results indicated that gene expression of the cowpea offsprings against the disease of *Pseudocercospora cruenta* being established was only a pair of complete dominant gene, whilst others performed susceptible characteristics of homozygote recessive gene. Fery *et al.* (1975) and Castro *et al.* (2003) reported a similar result when they carried out cowpea experiment with respect to *Cercospora cruenta* pathogenic disease. Bateman *et al.* (1989) also reported a similar result with the use of cowpea cultivars against *Phytophthora vignae* pathogenic disease and they stated that only a pair of complete dominant gene was found to

have its complete dominant effect against the disease. The results derived from the diallele crosses of F_1 and F_2 cowpea plants against the disease of the present work indicated an ultimate effect of complete dominant gene over recessive gene with respect to this particular disease and the attained cultivars of high resistant characteristics could undoubtedly be useful for further breeding purposes.

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