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A Varietal Screening of Cowpea Cultivars (*Vigna unguiculata*) for a High Resistance to *Pseudocercospora cruenta* (Sacc.) Deighton in Northeast Thailand

[1]N. Sinsiri, [2]S. Loahasiriwong, [2]S. Jogloy, [2]B. Toomsan and [3]W. Saksirirut
[1]Department of Agricultural Technology, Mahasarakham University, Mahasarakham 44000, Thailand
[2]Department of Agronomy, Khon Kaen University, Khon Kaen 40002, Thailand
[3]Department of Plant Pathology, Khon Kaen University, Khon Kaen 40002, Thailand

Abstract: This investigation consisted of four experiments, i.e., two pot experiments were carried out under glasshouse conditions and another two experiments were conducted under field conditions. The glasshouse pot experiments were carried out at Mahasarakham University, whilst the field experiments were carried out at Khon Kaen University. Twelve cowpea cultivars were used for both the first glasshouse and the first field experiments. The second glasshouse experiment had 14 cowpea cultivars and the same number of cultivars was used for the second field experiment. The four experiments aim to search for outstanding cowpea cultivars where they manifest high resistance to Pseudocercospora leaf spot disease for further breeding programme. A Completely Randomized Design (CRD) with four replications was used for both glasshouse experiments, whilst a Randomized Complete Block Design (RCBD) with four replications was used for both field experiments. The results showed that eight out of fourteen cowpea cultivars of the glasshouse and field experiments possessed a ranking score of Immunity (I). They were ranked accordingly as the best gene resources for future breeding programme. They include IT84E-1-108 as the best cultivar followed by KKU35, Ubonrachatanee1, KKU305, KKU264B, KKU264R, KVC#7 and IT86D-812 with seed yields of the field Experiment 2 of 1,480, 944, 864, 860, 738, 714 and 614 kg ha^{-1}, respectively. The spread out of the disease was encouraged most by high relative humidity of the environmental conditions.

Key words: Cowpea cultivars, disease infection, incidence%, severity, ranking score

INTRODUCTION

Cowpea (*Vigna unguiculata*), a kind of leguminous crop has a wide range of adaptability to environmental conditions, the crop could thrive on well at high North latitudes of 40-45° in Minnesota State, USA and in most of the tropical areas even at high elevations where it ranges from 1,300-1,600 m above sea level. This crop is known to have a high resistance to drought conditions (Davis *et al*., 1986). The land areas of the world being used for the cultivation of cowpea reach a figure of seven million hectares, particularly in most of the tropical and subtropical countries. Approximately three out of four portions of world supply of cowpea came from African countries, whilst a portion of the production comes from North and South America, Asia and Europe, particularly from Italy (Ehlers and Hall, 1997). Cowpea crop is widely known to consumers as an important source of proteins for human diets, animal feed and other uses, particularly in most of the Asian and African countries (Adejumo *et al*., 2001).

The cultivation of cowpea crop is also facing a lot of problems on diseases such as the problem on Pseudocercospora leaf spot disease causes by *Mycosphaerella cruenta* Latham and later this pathogenic disease was known as *Pseudocercospora cruenta* (Sacc.) Deighton where in many published works, it revealed that this pathogenic disease has its characteristics similar to *Cercospora cruenta* (Sacc.), which causes Cercospora leaf spot disease in cowpea plants. However, there are some distinctive differences found between these two diseases, i.e., Pseudocercospora leaf spot produces chlorotic or necrotic appearances on the upper surface of leaf while the underneath surface, it produces conidiophores and conidia with gray or dark gray appearances whereas Cercospora leaf spot produces a number of circular to irregular spots on both surfaces of leaf and it has cherry

Corresponding Author: Mr. N. Sinsiri, Department of Agricultural Technology, Faculty of Technology, Mahasarakham University, Mahasarakham 44000, Thailand

red to reddish brown appearances on both surfaces of leaf (Emechebe and Lagoke, 2003). It has been advocated that these two types of diseases could manifest its rapid growth on crop residues and in seeds of most leguminous crops throughout the year regardless of the seasons (Allen *et al*., 1998). It has also been reported that *P. cruenta* could be found in plant species of *Canavalia ensiformis* (Ram and Mallain, 1992). However, it is conceivable that Pseudocercospora leaf spot disease has a greater significant effect on cowpea production than Cercospora leaf spot disease (Emechebe and Lagoke, 2003). Another published work on cowpea revealed that pathogenic disease of *Mycosphaerella cruenta* Latham severely decreased cowpea seed yield up to 35.6% in the poor resistant cultivars and it decreases number of pods/plot, number of seeds/pod and a 100-seed weight of 25.4, 9.6 and 3.1%, respectively (Frey *et al*., 1977). The results on the effect of climatic conditions in India on the spread out of *P. cruenta* with Kala Jhamala cowpea cultivar revealed that environmental conditions of 81-86% relative humidity, 7.5-8.5 h photoperiod and with ages of 57-65 days after sowing were the most favourable conditions for growth of this pathogenic disease (Pandey and Pandey, 2002). In another work on the effect of sowing dates of cowpea, Awurum (2000) showed that the three planting dates (being used) (1st, 21st June and 12th July) gave a severely degree of infection more than the sowing dates in August(2nd and 23rd August). Therefore, it is of important value to carry out more research experiments on different cowpea cultivars in Northeast Thailand in order to search for the highest resistant cultivar for maximum seed yield in order to collect the promising cultivars as gene resources for further breeding programme.

MATERIALS AND METHODS

This study investigation was divided into two environmental conditions, i.e., glasshouse and field conditions. The glasshouse pot experiments were carried out at Mahasarakham University, with the use of a number of pots, whilst the field experiments were carried out at Khon Kaen University, Northeast Thailand. The glasshouse experiments consisted of two experiments, i.e., the first experiment was carried out in February-May 2003 with the use of 12 cowpea cultivars (KKU25, KKU305, KKU35, KKU264B, KKU264R, KVC#7, Suranaree1, KKSJ28-1-10-2-1-1, MSU1, Ubonrachataneel, IT81D-1228-14-1 and IT81D-1228-14-2) and the second experiment was done in August-October 2003 with the use of 14 cowpea cultivars, i.e., all cowpea cultivars being used in the first experiment were added with IT84E-1-108 and IT86D-812 cultivars. The field experiment at Khon Kaen University consisted of two experiments, i.e., the first experiment was carried out in February-May 2003 with 12 cowpea cultivars and the second experiment was conducted in August-November 2003 with 14 cowpea cultivars, i.e., the same number of cultivars being used in glasshouse experiments was used accordingly again in field experiments.

A Completely Randomized Design (CRD) with four replications was used in both glasshouse experiments. A number of plastic pots with diameter of 26 cm containing growing medium of 6 kg was used. The growing medium consisted of garden soil, burned rice husk, dry coconut husk particles and fermented compost at a ratio of 7:1:1:1 kg, respectively. Watering of the pots was carried out thoroughly then 3-4 seeds of each cowpea cultivar were sown by hand directly into the medium in each pot. Thinning of the seedlings was carried out at 14 days after sowing leaving only two seedlings per pot. An equal amount of water for each pot was given daily and regularly to maintain adequate amount of moisture content in the growing medium of the pots. After the thinning of the seedlings, an inoculation of spores of *Pseudocercospora cruenta* (Sacc.) Deighton was carried out. The *P. cruenta* (Sacc.) spores were mixed into distilled water at a spore concentration of 5.0×10^4 mL^{-1} and then sprayed to the plants at a rate 10 mL pot at 33 days after sowing. After the spraying process was completed then each pot was covered with a transparent plastic bag for three days and then the plastic bags were taken out. Thereafter the cowpea plants in the glasshouse were allowed to expose to radiant energy from the sun under the glasshouse conditions. Each plastic bag has a number of small holes to prevent the rising up of temperature and to ventilate the airflow in the pots. Examinations on disease infection of leaves were carried out three times at 10 day intervals starting from 40 days after sowing.

For both field experiments, Yasothon soil series (Oxic Paleustults) was used. Soil analysis data of initial mean values of soil pH (1:2.5 soil:water by volume), organic matter, nitrogen, available phosphorus and exchangeable potassium were 5.3, 0.79, 0.046%, 75 ppm and 89 ppm, respectively. A Randomized Complete Block Design (RCBD) with four replications was used for both experiments. The land areas were ploughed twice followed by harrowing once. The plot dimension used was a 2×4 m with 2.0 m in between the plots were used for infector row surrounded each plot. The sowing distance used was a 50×25 cm between rows and within

rows. Fifteen days before sowing seeds into the experimental plots, cowpea seeds of KKU25 (the most susceptible cultivar being used as control treatment) were sown by hand as infector rows surrounded the plots and then a few cowpea seeds/hole were sown again by hand directly into the experimental plots. One week after sowing, the cowpea seedlings were thinned out leaving only one seedling per hole. Weeding was carried out twice, one at 20 days after sowing and the other at 35 days after sowing. A complete chemical fertilizer 15-15-15 (NPK) was evenly applied to the plots by hand right after the thinning of the seedlings at a rate of 156.25 kg ha^{-1}. A spraying of Benlate fungicide was carried at 10 and 20 days after sowing to control the breaking out of other undesirable fungous diseases. At 20 days after sowing of the infector rows, the cowpea plant materials collected from the nearby area, which were severely infected by *Pseudocercospora cruenta* (Sacc.) Deighton were introduced to the seedlings of the infector rows. An examination for the infected plants was carried out for three times at 10 day intervals starting from 40 days after sowing for the first experiment and four times at 10 day intervals starting from 40 days after sowing for the second experiment.

The method in counting for the infected symptoms in primary and tri-foliage leaves of cowpea plants was carried out by the method of Adejumo *et al.* (2001). Incidence% = numbers of infected symptom compared with the weakest cultivar (control treatment) where the weakest cultivar is given 100%. Whilst that scores of severity of 1 = none infection; 2 = 1-3 spots on leaf surface with a diameter lesser than 5 mm; 3 = spot numbers greater than 3, each with a diameter lesser than 5 mm; 4 = spots of dark grayish spores with a diameter greater than 5 mm where at least one spot appears on primary leaf surface and three spots/leaf of tri-foliage leaves and 5 = more than three spots of dark grayish spores on leaf surface where it appears on primary and tri-foliage leaf numbers 1-4. Disease resistant degrees were also scoring as:1 = high immunity (I) = 0% incidence and severity = 1; 2 = resistance (R) = 1-25% incidence and severity = 1.1-2.0; 3 = susceptible (S) = 26-100% incidence and severity = 2.1-5.0. Yield components (number of pods/plant, seeds/pod, 100 seed weight) and seed yield data of the cowpea cultivars were also recorded. The collected data were statistically analysed using analysis of variance and Duncan's Multiple Range Test where appropriate (Nissen, 1989).

RESULTS

For Glasshouse Experiment 1 at 40 days after sowing, the results showed that there was a highly significant effect on incidence % found among 12 cultivars tested where the highest incidence % of infection was found with KKSJ28-1-10-2-1-1, whilst the rest were similar. At 50 days after sowing, there were no statistical differences found among 12 cowpea cultivars although KKSJ28-1-10-2-1-1 ranked the highest % followed by KKU25. However, at 60 days after sowing the results showed that incidence % were highest with KKSJ28-1-10-2-1-1 cultivar followed by KKU25 whilst the rest were similar. The differences were large and highly significant (Table 1). The results on severity scores indicated that severity scores for the three sampling intervals followed a similar trend to that of the incidence % where the most susceptible cultivars were found with KKSJ28-1-10-2-1-1 followed by KKU25 and MSU1. These three cultivars attained severity ranking standing of 'S', whilst resistant cultivars (R) were with KKU35 followed by KKU264B, IT81D-1228-14-1, Suranaree1 and IT81D-1228-14-2, respectively. For immunity, the immune cultivars (I) were KKU305, KKU264R, Ubonrachataneel and KVC7 and each of them had a ranking score of 'I', i.e., these four cultivars possessed a similar degree of immunity.

With Glasshouse Experiment 2, the spread out of the disease in primary and tri-foliage leaves of the fourteen cowpea cultivars at 40 days after sowing showed that incidence % of disease in primary leaves were highest with both MSU1 and KKU25 cultivars followed by KKSJ28-1-10-2-1-1 cultivar with percentages of 40, 40 and 15, respectively whilst the rest were not infected by the disease. The differences were large and highly significant. At 50 days after sowing, the results showed that MSU1 was the highest with 51.11% of infection followed by KKSJ28-1-10-2-1-1 and KKU25 where both attained 15%, whilst the rest were not infected by the disease (Table 2). With tri-foliage leaves at 40 days after sowing, the results indicated that there were only four cultivars infested by Pseudocercospora leaf spot disease, i.e., the highest intensity of the disease was found with the control treatment (KKU25) followed by MSU1, KKSJ28-1-10-2-1-1 and Suranaree1, respectively. The infected symptoms were severely greater in tri-foliage leaves than the primary leaves. At 50 days after sowing, the results showed that ranking positions of the infected cultivars were changed, i.e., MSU1 ranked the first followed by KKU25, KKSJ28-1-10-2-1-1 and Suranaree1 with values of 62.90, 52.42, 46.77 and 33.87%, respectively. At 60 days after sowing, the cowpea cultivars of IT81D-1228-14-1 and IT81D-1228-14-2 were infected by the disease, thus added to the four infected treatments, which were found at 50 days after sowing where the intensity of infection was relatively smaller than the rest.

Table 1: Incidence % of infected symptoms, severity and ranking score of Pseudocercospora leaf spot disease in leaves of 12 cowpea cultivars taken at 40, 50 and 60 days after sowing, grown in the dry season under glasshouse conditions (Glasshouse Experiment 1) at Mahasarakham University, Northeast Thailand

	Glasshouse Experiment 1						
Cultivars	Incidence (%) 40 DAS	Incidence (%) 50 DAS	Incidence (%) 60 DAS	Severity (1-5) 40 DAS	Severity (1-5) 50 DAS	Severity(1-5) 60 DAS	Ranking score
KKU25	18.55^{b}	31.73	84.47^{ab}	1.50^{b}	2.50^{ab}	4.50^{a}	S
KKU305	0.00^{b}	0.00	0.00^{c}	1.00^{b}	1.00^{b}	1.00^{c}	I
KKU35	0.00^{b}	7.88	10.85^{c}	1.00^{b}	1.25^{b}	2.00^{bc}	R
KKU264B	0.00^{b}	3.02	5.74^{c}	1.00^{b}	1.25^{b}	2.00^{bc}	R
KKU264R	0.00^{b}	0.00	0.00^{c}	1.00^{b}	1.00^{b}	1.00^{c}	I
KVC#7	0.00^{b}	0.00	0.00^{c}	1.00^{b}	1.00^{b}	1.00^{c}	I
Suranaree1	0.00^{b}	0.00	4.82^{c}	1.00^{b}	1.00^{b}	1.50^{c}	R
KKSJ28-1-10-2-1-1	98.06^{a}	95.10	98.14^{a}	3.50^{a}	4.50^{a}	4.00^{ab}	S
MSU1	0.00^{b}	16.10	32.74^{bc}	1.00^{b}	2.50^{ab}	3.00^{b}	S
Ubonrachatanee 1	0.00^{b}	0.00	0.00^{c}	1.00^{b}	1.00^{b}	1.00^{c}	I
IT81D-1228-14-1	0.00^{b}	23.90	15.76^{c}	1.00^{b}	1.50^{b}	2.00^{bc}	R
IT81D-1228-14-2	0.00^{b}	11.68	6.13^{c}	1.00^{b}	1.00^{b}	1.50^{c}	R
F-test	**	NS	**	**	**	**	
CV (%)	25.41	39.72	19.63	21.81	27.18	24.29	

Letter(s) within the same column indicate Duncan's Multiple Range Test; S = Susceptible; I = Immune; R = Resistance. **p = 0.01, NS = non significant, CV = Coefficient of Variations, DAS = Days After Sowing

Table 2: Incidence % of infected symptoms, severity and ranking score of Pseudocercospora leaf spot disease of 14 cowpea cultivars, taken at 40 and 50 days after sowing for primary leaves and at 40, 50, 60 and 70 days after sowing for tri-foliage leaves, grown under glasshouse conditions (Glasshouse Experiment 2) at Mahasarakham University, Northeast Thailand

	Primary leaves		Tri-foliage leaves					
Variety	Incidence (%) 40-DAS	Incidence (%) 50-DAS	Incidence (%) 40-DAS	Incidence (%) 50-DAS	Incidence (%) 60-DAS	Incidence (%) 70-DAS	Severity (1-5) 70 DAS	Ranking score
KKU25	40.00^{a}	15.00^{ab}	76.62^{a}	52.42^{ab}	54.04^{ab}	60.49^{ab}	4.50^{a}	S
KKU305	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}	1.00^{c}	I
KKU35	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}	1.00^{c}	I
KKU264B	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}	1.00^{c}	I
KKU264R	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}	1.00^{c}	I
KVC#7	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}	1.00^{c}	I
Suranaree1	0.00^{c}	0.00^{b}	26.19^{b}	33.87^{b}	37.94^{bc}	47.58^{bc}	2.75^{b}	S
KKSJ28-1-10-2-1-1	15.00^{ab}	15.00^{ab}	55.95^{a}	46.77^{ab}	61.29^{a}	70.97^{ab}	5.00^{a}	S
MSU1	40.00^{a}	51.11^{a}	70.24^{a}	62.90^{a}	75.00^{a}	75.00^{a}	4.25^{a}	S
Ubonrachatanee 1	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}	1.00^{c}	I
IT81D-1228-14-1	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}	20.16^{cd}	22.58^{c}	2.00^{b}	R
IT81D-1228-14-2	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}	8.06^{d}	12.09^{cd}	2.00^{b}	R
IT84E-1-108	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}	1.00^{c}	I
IT86D-812	0.00^{c}	0.00^{b}	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{d}	1.00^{c}	I
F-test	**	*	**	**	**	**	**	
CV (%)	29.05	24.33	21.33	24.28	18.81	16.99	21.64	

Letter(s) within the same column indicate Duncan's Multiple Range Test; S = Susceptible; I = Immune; R = Resistance; p** = 0.01, * = 0.05, CV = Coefficient of Variations, DAS = Days After Sowing

At 70 days after sowing, the results revealed that the six infected cultivars possessed a similar pattern of infection as found with the sampling at 60 days after sowing. For infection severity results, it revealed that the highest score on severity was found with KKSJ28-1-10-2-1-1 followed by KKU25, MSU1, Suranaree1, IT81D-1228-14-1 and IT81D-1228-14-2, respectively yet the last two cultivars possessed a similar degree of severity and both had a ranking score of 'R' whilst the four cultivars attained a ranking score of 'S', i.e., KKSJ28-1-10-2-1-1; KKU25 and MSU1 cultivars are the most susceptible cultivars to Pseudocercospora leaf spot disease. The cultivars those possess immunity score of 'I' include KKU305, KKU35, KKU264B, KKU264R, KVC #7, Ubonrachatanee1, IT84E-1-108 and IT86D-812. Leaves of these eight cultivars had no sign of infected symptom.

For Field Experiment 1 at 40 days after sowing, the results showed that the highest incidence % of infected symptoms was found with KKSJ28-1-10-2-1-1 followed by KKU25 (control treatment) with 77.60 and 35.56%, respectively. Other cultivars being used in the experiment had no signs of infection of the disease. Similarly, at 50 days after sowing, a similar result to that found at 40 days after sowing was shown, i.e., KKSJ28-1-10-2-1-1 had the highest incidence of 87.50% whilst KKU25 had 83.75%, both cultivars ranked at the same level of infection. The differences found among the cultivars were relatively large and highly significant (Table 3).

Table 3: Incidence % of infected symptoms, severity and ranking score of Pseudocercospora leaf spot disease in leaves of 12 cowpea cultivars, taken at 40, 50 and 60 days after sowing, grown under field conditions (Field Experiment 1) at Khon Kaen University Experimental Farm, Northeast Thailand

Cultivars	Incidence (%) 40 DAS	Incidence (%) 50 DAS	Incidence (%) 60 DAS	Severity (1-5) 60 DAS	Ranking score
KKU25	35.56^{a}	83.75^{a}	82.06^{a}	5.00^{a}	S
KKU305	0.00^{b}	0.00^{b}	0.00^{b}	1.00^{c}	I
KKU35	0.00^{b}	0.00^{b}	0.00^{b}	1.00^{c}	I
KKU264B	0.00^{b}	0.00^{b}	0.00^{b}	1.00^{c}	I
KKU264R	0.00^{b}	0.00^{b}	0.00^{b}	1.00^{c}	I
KVC#7	0.00^{b}	0.00^{b}	0.00^{b}	1.00^{c}	I
Suranaree1	0.00^{b}	0.00^{b}	0.00^{b}	1.00^{c}	I
KKSJ28-1-10-2-1-1	77.60^{a}	87.50^{a}	96.07^{a}	4.75^{a}	S
MSU1	0.00^{b}	0.00^{b}	4.64^{b}	2.50^{b}	S
Ubonrachatanee 1	0.00^{b}	0.00^{b}	0.00^{b}	1.00^{c}	I
IT81D-1228-14-1	0.00^{b}	0.00^{b}	0.00^{b}	1.00^{c}	I
IT81D-1228-14-2	0.00^{b}	0.00^{b}	0.00^{b}	1.00^{c}	I
F-test	**	**	**	**	
CV (%)	21.34	14.60	9.15	15.10	

Letter(s) within the same column indicate Duncan's Multiple Range Test; S = Susceptible; I = Immune; **p = 0.01, CV = Coefficient of Variations, DAS = Days After Sowing

Table 4 Incidence % of infected symptoms, severity and ranking score of Pseudocercospora leaf spot disease in leaves of 14 cowpea cultivars and seed yields, taken at 40, 50, 60 and 70 days after sowing, grown under field conditions (Field Experiment 2) at Khon Kaen University Experimental Farm, Northeast Thailand

Cultivars	Incidence (%) 40-DAS	Incidence (%) 50-DAS	Incidence (%) 60-DAS	Incidence (%) 70-DAS	Severity (1-5) 40 DAS	Severity (1-5) 50 DAS	Severity (1-5) 60 DAS	Severity (1-5)70 DAS	Ranking score	Yields Kg ha^{-1}
KKU25	100.00^{a}	88.89^{a}	71.67^{b}	77.28^{a}	3.00^{a}	4.75^{a}	4.75^{a}	5.00^{a}	S	339^{f}
KKU305	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{c}	1.00^{c}	1.00^{c}	1.00^{d}	1.00^{d}	I	860^{bc}
KKU35	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{c}	1.00^{c}	1.00^{c}	1.00^{d}	1.00^{d}	I	944^{b}
KKU264B	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{c}	1.00^{c}	1.00^{c}	1.00^{d}	1.00^{d}	I	738^{bcd}
KKU264R	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{c}	1.00^{c}	1.00^{c}	1.00^{d}	1.00^{d}	I	714^{bcd}
KVC#7	0.00^{c}	0.00^{c}	0.00^{d}	0.00	1.00^{c}	1.00^{c}	1.00^{d}	1.00^{d}	I	394^{ef}
Suranaree1	0.00^{c}	0.00^{c}	12.34^{c}	17.80^{b}	1.00^{c}	1.00^{c}	2.00^{c}	2.00^{c}	R	516^{def}
KKSJ28-1-10-2-1-1	37.50^{b}	58.34^{b}	88.34^{a}	82.78^{a}	2.00^{b}	4.00^{b}	5.00^{a}	5.00^{a}	S	378^{f}
MSU1	0.00^{c}	0.00^{c}	12.34^{c}	27.27^{b}	1.00^{c}	1.00^{c}	2.50^{bc}	3.75^{b}	S	513^{def}
Ubonrachatanee 1	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{c}	1.00^{c}	1.00^{c}	1.00^{d}	1.00^{d}	I	864^{bc}
IT81D-1228-14-1	0.00^{c}	0.00^{c}	0.00^{d}	6.64^{c}	1.00^{c}	1.00^{c}	1.00^{d}	1.75^{c}	R	690^{b-e}
IT81D-1228-14-2	0.00^{c}	0.00^{c}	0.00^{d}	9.96^{c}	1.00^{c}	1.00^{c}	1.00^{d}	1.75^{c}	R	877^{bc}
IT84E-1-108	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{c}	1.00^{c}	1.00^{c}	1.00^{d}	1.00^{d}	I	1480^{a}
IT86D-812	0.00^{c}	0.00^{c}	0.00^{d}	0.00^{c}	1.00^{c}	1.00^{c}	1.00^{d}	1.00^{d}	I	614^{c-f}
F-Test	**	**	**	**	**	**	**	**		**
CV (%)	28.03	12.44	28.29	19.96	17.97	17.74	31.64	31.92		30.23

Letter(s) within the same column indicate Duncan's Multiple Range Test; S = Susceptible; I = Immune; R = Resistance. **p = 0.01, CV = Coefficient of Variations, DAS = Days After Sowing

At 60 days after sowing, the results showed that both KKSJ28-1-10-2-1-1 and KKU25 possessed the same level of infected symptoms where both ranked the first followed by MSU1 with incidence of 96.07, 82.06 and 4.64%, respectively. The differences were large and highly significant. There were no signs of infected symptom found with other cultivars being used. Severity of infection at 60 days after sowing was highest with KKU25 followed by KKSJ28-1-10-2-1-1 and MSU1 with severity levels of 5, 4.75 and 2.5, respectively. For ranking score, the results showed that there were three cultivars attained 'S'. They include KKU25, KKSJ28-1-10-2-1-1 and MSU1 cultivars, whilst the rest attained an immunity of 'I'. Therefore, these three out of twelve cultivars being used in the experiment are the most susceptible cultivars to Pseudocercospora leaf spot disease.

With Field Experiment 2 at 40 days after sowing, the results showed that incidence % on infected symptom was highest with KKU25 followed by KKSJ28-1-10-2-1-1 with values of 100 and 37.50%, respectively whilst the rest were not infected. A similar result was found at 50 days after sowing, i.e., both infected cultivars possessed a similar trend on disease to the first sampling period but attained different incidence %, i.e., 88.89 and 58.34 for KKU25 and KKSJ28-1-10-2-1-1, respectively (Table 4). However, at 60 days after sowing, incidence % ranking was changed, i.e., the highest infected symptom was found with KKSJ28-1-10-2-1-1 followed by KKU25, Suranaree1 and MSU1, respectively where the last two cultivars attained a similar level of infected symptom with values of 88.34, 71.67, 12.34 and 12.34%, respectively. A similar trend on infected symptom was found at 70 days after sowing but two more cultivars were added to the list, i.e., there was a small degree of infected symptom found with IT81D-1228-14-2 and IT81D-1228-14-1 where both cultivars attained a similar intensity of infected symptom of 9.96 and 6.64%, respectively. The results on

Table 5: Correlation coefficients (r^2) on disease incidence and severity in leaves, number of pods/plant, number of seeds/pod, 100-seed weight and seed yields of 14 cowpea cultivars of Field Experiment 2 at 60 and 70 days after sowing, grown at Khon Kaen University Experimental Farm, Northeast Thailand

Items	Incidence 60 DAS	Severity 60 DAS	Incidence 70 DAS	Severity 70 DAS
Severity (60 DAS)	0.995**			
Incidence (70 DAS)	0.984**	0.992**		
Severity (70 DAS)	0.886**	0.897**	0.870**	
Number of pods/plant	-0.328	-0.354	-0.391	-0.352
Number of seeds/pod	-0.150	-0.143	-0.119	0.010
100-seed weight	-0.390	-0.411	-0.416	-0.448
Seed yields	-0.534*	-0.557*	-0.577*	-0.509

p** = 0.01, * = 0.05

severity level of infection (1-5) indicated that both sampling periods taken at 40 and 50 days after sowing had significantly different intensities of infected symptoms, i.e., KKU25 had the highest followed by KKSJ28-1-10-2-1-1 with severity intensities at 40 days after sowing of 3 and 2 and at 50 days after sowing of 4.75 and 4.0, respectively. There were three cultivars attained 'S' severity ranking score, i.e., KKU25, KKSJ28-1-10-2-1-1 and MSU1, whilst other three cultivars attained resistant score of 'R', i.e., Suranaree1, IT81D-1228-14-2 and IT81D-1228-14-1, whilst the rest attained an immune score of 'I', i.e., KKU305, KKU35, KKU264B, KKU264R, KVC#7, Ubonrachatanee1, IT84E-1-108 and IT86D-812.

With the results on correlation coefficients (r^2) of 14 cowpea cultivars being used in Field Experiment 2 on disease incidence and severity of infection at 60 and 70 days after sowing, the results showed that there were highly significant correlation coefficients due to both incidence and severity. Correlation coefficients (r^2) on incidence % in relation to seed yields at 60 and 70 days after sowing were -0.534* and -0.577*, respectively, whilst that of severity levels on seed yields at 60 and 70 days after sowing were -0.557* and -0.509, respectively. There were no significant correlation coefficients on number of pods/plant, number of seeds/pod and 100-seed weight (Table 5). Seed yields for the immune cultivars rank highest with IT84E-1-108 followed by KKU35, Ubonrachatanee1, KKU305, KKU264B, KKU264R and IT86D-812, with seed yields of 1,480, 944, 864, 860, 738, 714 and 614 kg ha^{-1}, respectively. The susceptible cultivars include KKU25, KKSJ28-1-10-2-1-1, MSU1 with seed yields of 339, 378 and 513 kg ha^{-1}, respectively.

DISCUSSION

The cowpea plants of Glasshouse Experiment 1 grown under glasshouse conditions were relatively normal although the inoculated plants of 12 cultivars showed different intensities of infection, particularly at 60 days after sowing. For incidence %, the results revealed that out of 12 cowpea cultivars only 4 cultivars showed no sign of incidence %, i.e., KKU305, KKU264R, KVC#7 and Ubonrachatanee1. Both KKSJ28-1-10-2-1-1 and KKU25 had the highest incidence % followed by MSU1, whist the rest were similar. The results indicated that these three cowpea cultivars possess somewhat susceptible gene to the disease. Similarly, the results on severity levels at 60 days after sowing indicated that both KKSJ28-1-10-2-1-1 and KKU25 (Control treatment) attained the highest level of severity followed by MSU1, whilst the rest attained a smaller severity level. The results on ranking score indicated that only four cultivars possessed immunity (I) to the disease, i.e., KKU305, KKU264R, KVC#7 and Ubonrachatanee1. Whilst five cultivars possess resistant ranking score, i.e., KKU35, KKU264B, Suranaree1, IT81D-1228-14-1 and IT81D-1228-14-2 and eventually the last three cultivars attained a severity score. They include KKU25, KKSJ28-1-10-2-1-1 and MSU1 cultivars. The results indicated the various disease resistances of the cowpea cultivars where it provides evidences and information for further selection (Adejumo *et al*., 2001).

With the second experiment carried out under glasshouse conditions at 60 and 70 days after sowing, the results showed that incidence % of infection followed a similar pattern as that of Glasshouse Experiment 1, i.e., incidence % of the disease was highest with MSU1, KKU25 and KKSJ28-1-10-2-1-1. The three cultivars attained a similar intensity of infected symptom, whilst Suranaree1 attained a smaller intensity of infection and the rest had much smaller intensity of infection. The results on severity levels indicated that only three cultivars attained the highest severity level, i.e., KKSJ28-1-10-2-1-1, MSU1 and KKU25, whilst Suranaree1, IT81D-1228-14-1 and IT81D-1228-14-2 attained a lesser severity intensity. There were eight cultivars possess immunity ranking score (I), i.e., KKU305, KKU35, KKU264B, KKU264R, KVC#7, Ubonrachatanee1, IT84E- 1-108 and IT86D-812, whilst other four cultivars rated at a susceptible ranking level. The results also indicated that only two cultivars attained resistant ranking score, i.e., IT81D-1228-14-1 and IT81D-1228-14-2. This could be attributable to genetic traits of individual cowpea cultivars (Emechebe *et al*., 2003). It is evidently shown that the results of Glasshouse Experiment 1 were, in some cases, not repeatable in Glasshouse Experiment 2. It was found in Glasshouse Experiment 1, which was carried out in the dry season that KKU35 and KKU264R attained a score of 'R', whilst that of the glasshouse Experiment 2 both cultivars attained a score of Immunity

(I). Similarly, Suranaree1 attained a score of 'R' in Glasshouse Experiment 1 and a score of 'S' in Glasshouse Experiment 2. This may be due to the differences in relative humidity where the cowpea plants grown in May, which was the beginning of the rainy, may have exposed to somewhat higher percentages of relative humidity than those in Glasshouse Experiment 2, thus the disease spread out severely. Nevertheless, the differences were not statistically significant. It has been advocated that the spread out of this type of disease in the rainy season is greater than in the dry season due to wet environmental conditions (Adejumo *et al*., 2001; Pandey and Pandey, 2002).

The results found with Field Experiment 1 indicated that there were three cultivars attained a ranking score of Susceptible (S), i.e., KKU25, KKSJ28-1-10-2-1-1 and MSU1, whilst the rest attained a ranking score of 'I' (immune). The results indicated that only three cultivars possessed its susceptibility to the disease, whilst the rest did not. The results seem to indicate somewhat dry conditions at Khon Kaen Experimental Farm when the disease was not severely found. With Field Experiment 2 where the crop plants were grown in the rainy season, the results showed that three cultivars attained 'R' ranking score whereas in the dry season they attained 'I', whilst the rest repeated the same ranking score as that of field Experiment 1. The results indicated that the rainy season did provide important evidences on severity of the disease where it indicates high relative humidity due to rainfalls (Pandey and Pandey, 2002). The differences in the response to the *Pseudocercospora cruenta* (Sacc.) Deighton disease of the cowpea plants of the four experiments could be partly attributable to the differences in gene combination of the cowpea cultivars (Emechebe and Lagoke, 2003; Allerd *et al*., 1992; Allard, 1999). The results of the four experiments showed that out of fourteen cowpea cultivars only eight cultivars gave a score of 'I' where no disease was infected. They include IT84E-1-108, KKU35, Ubonrachatanee1, KKU305, KKU264B, KKU264R, KVC#7 and IT86D-812 cultivars with seed yields of 1,480, 944, 864, 860, 738, 714, 394 and 614 kg ha^{-1}, respectively. The differences in responding to the disease of the cowpea cultivars may be attributable to the differences in both environmental conditions and the seasons where G×E interactions may cause differences in the response to the disease and it may be possible that the cultivars had more than a pair of resistant gene where they possess differences in disease resistance (Allerd *et al*., 1992; Allard, 1999). Another interesting point for the growth of cowpea plants under field conditions is soil conditions. If the crop plant is to be grown on Yasothon soil series, a great soil group of Oxic Paleustults (Pirmpoon, 1984) then it is necessary to improve soil conditions before sowing, particularly soil pH where the mean value should be at a range of 6-6.5 (1:2.5 soil:water by volume). This range of soil pH could facilitate better the release of soil nutrients (Miller and Donahue, 1990; Suksri, 1999). However, with this work it was assumed that all cultivars being used received, more or less, a similar environmental condition for growth in each experiment, thus the attained results could be of important information. It was found with the four experiments that eight cowpea cultivars possess immunity score of 'I'. Therefore, in order to justify which cultivar is of the highest value for future breeding purposes, hence their seed yields were used as an index for selection. It was found that IT84E-1-108 ranked the first followed by KKU35, Ubonrachatanee1, KKU305, KKU264B, KKU264R, KVC#7 and IT86D-812 with seed yields of 1,480, 944, 864, 860, 738, 714 and 614 kg ha^{-1}, respectively. These cowpea cultivars have possessed their high resistant to Pseudocercospora leaf spot disease under glasshouse and field conditions in Northeast Thailand.

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