Evaluation of downy mildew resistance in the cucumber inbred lines

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Abstract

Downy mildew, a foliar disease caused by the oomycete *Pseudoperonospora cubensis* (Berk. and Curt.) Rostov. is one of the most destructive diseases of cucumber (*Cucumis sativus* L.) in tropical and temperate areas of the world. Resistant cucumber cultivars in Thailand had the sufficient resistance to grow a successful crop without the use of fungicides. The objective of this study was to identify new sources of the resistance and the susceptible to downy mildew of inbred lines from the RMUTL and NSTDA cooperating project, elite cultivars, and breeding lines of cucumber. Eight inbred lines were evaluated in Lampang province, during 2012 to 2014 under the natural field and the nursery epidemics. To describe genetic homozygous, eight inbred lines were constructed using 70 pooled DNA and single plant DNA of simple sequence repeats (SSR) markers.

Keywords: cucumber, (*Cucumis sativus* L.), downy mildew, inbred lines, single sequence repeats (SSR)

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Introduction

Cucumber (*Cucumis sativus* L.; 2n = 2x = 14) is produced worldwide and is consumed as a fresh (fresh or slicing types) or a processed product in the several market classes (Staub *et al*., 2008). Thailand produced mostly fresh products only 25,000 hectares and 265,000 MT in 2012 (FAOSTAT, 2012). Cucumber has an extremely narrow genetic base with 3% to 8% polymorphisms among elite and exotic germplasm and 12% between botanical varieties (*C. sativus* var. *sativus* L. and var. *hardwickii* (R.) Alef.) (Dijkhuizen *et al*., 1996; Meglic and Staub, 1996; Horejsi and Staub, 1999). A reduction in vigor accompanying inbreeding has been found to occur in many cross-pollinated crop species. Cucumber is an annual outcrosses with monoecious (first staminate, then pistillate flowers) sex expression. Lack of severe inbreeding depression in cucumber indicates that the frequency of natural self-pollinations is high, or, as Allard (1960) suggests, that cucurbits evolved as small populations in nature, thus having a high level of inbreeding due to intercrossing of related individuals. Several workers have studied the effect of self-pollination on species of the Cucurbitaceae. Jenkins (1942) reported that several cucumber cultivars which were inbred for 5 generations were as vigorous as the commercial open-pollinated stocks. When a random sample of plants from an open-pollinated pickling cucumber population were self-pollinated for 6 generations, the yield of the resulting lines was unaffected in either of the seasons tested (Rubino and Wehner, 1986). Wehner and Jenkins (1985) measured a natural outcrossing rate of 50% (36% between row). Some have reported heterosis from the specific hybrid combinations (Ghaderi and Lower, 1979), especially when crossing diverse parents (Hays and Jones, 1916). Selection within adapted material would be expected to progress slowly. Because of this narrow genetic base, a source population with a broad genetic base should be more responsive to selection for quantitative traits. Where breeders have worked to develop elite hybrids, heterosis may average only a 5% advantage (Wehner, 1999).

Of the methods of traditional inbreeding available, self-pollination results in the most rapid approach towards homozygosity, whereas a method such as within family mating slows the loss of heterozygosity (Hallauer and miranda filho, 1988). Aim during the development of inbred lines is to achieve homozygosity while ensuring plant vigor and per se performance, which usually decreases with the level of homozygosity. The following many methods are used to develop the cucumber inbred lines such as inbred line extraction, pedigreed method,

back cross for increases qualitative traits. Quantitative traits work on the recurrent selection mostly S_1 selection method (Lower and Ewards, 1986). Before extracting inbred, it is important to ensure that the frequency of genes for other desirable traits is great enough to recover them at an early inbreeding stage. It would be pointless to advance lines to an inbred status and then be forced to abandon them due to insufficiency of necessary traits. This gene enrichment may take the form of population improvement with random mating or of strategic crossed.

One of the most successful breeding programs relies on selections of disease-resistant segregates from large segregating populations (Peterson, 1983). Selection that is resistant to several diseases is frequently recombined in early generations and then subject to additional screening for higher levels of resistance or multiple resistances. Inbreeding of resistant lines is practiced through F_6 to F_8 generations, at which time selection for other horticultural qualities: gynoecia's sex expression is undertaken. Fruit number and type are then assayed in both inbred and hybrids.

Cucurbitaceous is the hosts of this pathogen downy mildew which was first described on the cucumber by Berkeley and Curtis in 1868 and investigation into the genetic basis of resistance began in the early 20th century. Downy mildew of cucumber, caused by the oomycete *Pseudoperonospora cubensis*, is a devastating, worldwide disease of cucurbit crops both in the field and the protected culture. The oomycete pathogen *Pseudoperonospora cubensis* (Berk. And Curt.) Rostow is a major foliar disease of cucumber, especially in the humid regions of the world (Palti and Cohen, 1980). *P. cubensis* is an obligate oomycete pathogen with a host range limited to the family Cucurbitaceae (Savory *et al*., 2011). In addition to cucumber, more than 50 other species of the Cucurbitaceous are hosts of this pathogen, including other canonically important cucurbits such as melon, watermelon, and squash (Palti and Cohen, 1980; Lebeda, 1992). Control of downy mildew in cucumber production involves a combination of multiple fungicide applications and the use of moderately resistant cultivars. The infection and sporangia of this pathogen are affected by environmental conditions such as temperature and humidity (Inaba and Kajiwara, 1975; Cohen, 1977; Savory *et al*., 2011). Genetically conferred host resistance is the ideal means of overcoming the problems in current and future disease control of downy mildew throughout the world, and development of new cultivars with high levels resistance is highly desired.

The purpose of the work was to evaluate the downy mildew resistance of the eight cucumber inbred lines in the field and nursery by three seasons.

Materials and methods

1. Inbred line selection method

Field studies were conducted in the field at Technology Research Institute, Lampang with three seasons, during July 2012 to August 2013. The initial inbred lines were selected from S_4 and S_5 inbred lines in 2008 until 2011 from Cucurbits Germplasm Unit project (Khanobdee *et al*., 2011). There was only one nursery screening for downy mildew on October to November, 2012. In the field, plots were exposed to natural epidemics in the course of the growing season. Susceptible commercial cultivar was used in borders around the field to monitor and increase inoculum in the field. Epidemics were encouraged using overhead irrigation. Plots were planted when border rows displayed major symptoms of disease. Disease was evaluated as chlorosis, necrosis, and sporulation were rated on a 0 to 9 scale based on percentage of symptomatic leaf area (0 represents 0%, 1 represents 1–3%, 2 represents 3–6%, 3 represents 6–12%, 4 represents 12–25%, 5 represents 25–50%, 6 represents 50–75%, 7 represents 75–87%, 8 represents 87–99%, and 9 represents 100%) as described by Jenkins and Wehner (1983). For the greenhouse screening, cucumber leaves infected with *P. cubensis* were collected from the fields in Lampang, which had not been sprayed with fungicides. Leaves were collected in the morning, placed in plastic bags and stored in a cooler with ice, and transported to the laboratory where five heavily infected leaves were soaked in distilled water and rubbed gently with a glass rod to dislodge sporangia. The spore suspension was filtered through four layers of cheesecloth to remove dirt and debris and the concentration was determined with the use of a hemacytometer. The suspension was adjusted to a final concentration of 10,000 sporangia mL^{-1}. Immediately before inoculation, Tween 20 (0.06 g L^{-1}) was added to the inoculum suspension to keep the spores well dispersed in the solution. In the greenhouse, plants were inoculated at the one- to two-true leaf stage with a hand pump spray bottle. Inoculum was applied to upper and lower leaf surfaces of cotyledons and true leaves until run-off. Flats were placed in a dark growth chamber with humidifiers (100% RH, 20 $^{\circ}C$) for 48 h to maximize sporulation. Flats were then moved to a greenhouse (25 to 45 $^{\circ}C$) and plants were evaluated for disease 10 d after inoculation.

2. Purity data

The eight inbred lines were selected by downy mildew resistance and were self-

pollinated to increase the homozygosity. The percentage of the morphology traits were recorded by plant growth habit (determinate of indeterminate types), fruit color (green or white), and spine color (black, white or brown). The purity of eight inbred lines was investigated by using 70 primers (Simple Sequence Repeat markers; SSR) –in pooled DNA and single plant DNA. Genotypic data of two types of DNA was compared with morphology traits using T-test method.

Results and discussions

1. Downy mildew resistance of inbred lines

Eight elite inbred lines were evaluated for downy mildew for 45 days after transplantation. Five lines were highly resistance, namely Lanna 1, Lanna 2, Lanna 3, Lanna 6, and Lanna 7 (Table 1). The moderate resistant lines were Lanna 4 and Lanna 5. But, Lanna 8 was highly susceptibility line while the commercial check variety was moderate susceptibility. The serious damage by downy mildew on field screening was the plant grown during July to October, 2012, but the rest two seasons was intermediate damage. In Thailand, the downy mildew pathogen mostly outbreak of disease on the rainy season during July to October. The resistant cultivars are very rare and intermediate and highly susceptible expressed by commercial varieties. Farmer used fungicide and did not success in the serious times. For example, in the United States, the pathogen resurged as a major problem in 2004 and has continued to be a major disease of cucumber ever since, although available cultivars had sufficient resistance to be grown successfully without the use of fungicides from 1961 to 2003 (Call *et al*., 2012). In the second season (Oct – Nov, 2012), plants were tested downy mildew resistance in both nursery and field. The result showed that in nursery, the tested lines were highly resistance to downy mildew except Lanna 8. On the other hand, in field screening all tested lines were moderately resistant to downy mildew except Lanna 8. This result showed that there was some correlation between nursery and field screening but the degree of resistance was difference (Table 1).

Table 1 Downy mildew data of eight elite lines scored three seasons during July, 2012 to August, 2013.

lines	downy mildew score (45 DAT)[1/]			
	first crop (Jul – Oct, 2012) field	second crop (Oct – Nov, 2012) Nursery[2/]	filed	third crop (Apr to Aug, 2013) field
Lanna 1	5.8 inter	2.4 HR	4.8 inter	2.0 HR
Lanna 2	5.4 inter	0.0 HR	4.5 inter	1.5 HR
Lanna 3	6.8 MS	2.6 HR	4.8 inter	1.7 HR
Lanna 4	5.3 inter	3.3 MR	4.8 inter	3.1 MR
Lanna 5	5.6 inter	2.8 HR	4.9 inter	3.3 MR
Lanna 6	5.0 inter	2.8 HR	4.4 inter	2.1 HR
Lanna 7	5.8 inter	1.3 MR	4.9 inter	0.5 HR
Lanna 8	7.7 HS	5.4 inter	8.2 HS	dead
check	6.4 MS	4.2 inter	5.2 inter	5.3 inter

[1/] Downy mildew score (45 days after transplantation): highly resistant (HR) = 0 - 3, moderate resistant (MR) = 3.1 - 4.0, intermediate (inter) = 4.1 - 6.0, moderate susceptibility (MS) = 6.1 - 7.0 and highly susceptibility (HS) = 7.1 - 9.0, respectively.

[2/] Nursery scored 10 days after inoculation.

2. Purity data

Average percentage for horticultural traits of seven lines was 100% except Lanna 1, only 90.6%. Fruit color of Lanna 1 was 71.9% of green and 28.1% of white color. SSR markers showed nearly the same percentage of the homozygous from pooled and individual DNA. Using T-test analysis, there were not significant for all pairs, between morphology traits with SSR 1 and SSR 2, and between SSR ways (Table 2). All elite lines were highly homozygous genotypes and possible to improved combining ability and registered lines to next step.

Conclusion

Five inbred lines were highly downy mildew resistance and three inbred lines were moderately resistance but, Lanna 8 was susceptible line. However, these elite inbred lines had high homozygosity.

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Table 2 The percentage of morphology traits, pooled DNA and single plant DNA of simple sequence repeat markers (SSR).

lines	morphology traits (percentage)						SSR 1 [1/]	SSR 2 [2/]	all average
	indeterminate	fruit color		spine color		total	(%)	(%)	(%)
	type	green	white	brown	white				
Lanna 1	100	71.9	28.1	100	0	90.6	98.6	98.6	95.9
Lanna 2	100	0	100	100	0	100	100	100	100
Lanna 3	100	100	0	0	100	100	100	100	100
Lanna 4	100	100	0	0	100	100	78.3	94.3	90.8
Lanna 5	100	100	0	0	100	100	98.6	98.6	99.9
Lanna 6	100	100	0	0	100	100	98.6	100	99.5
Lanna 7	100	100	0	0	100	100	97.4	100	99.1
Lanna 8	100	100	0	0	100	100	98.6	100	99.5
T-test [3/]						ns	ns	ns	98.0

[1/] pooled DNA [2/] single plant DNA [3/] ns – not significant.

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