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Screening for resistance to Anthracnose caused by *Colletotrichum acutatum* in chili pepper (*Capsicum annuum* L.) in Kediri, East Java

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Abstract. Anthracnose disease caused by Colletotrichum species is one of the most economically important diseases reducing marketable yield from 10 % to 80 % of the crop production in some developing countries, particularly in Indonesia. Disease control using pesticides is costly and has adverse impacts. Using resistant varieties is expected to be more economical and environmentally friendly. This study aims to evaluate 20 genotypes of chili pepper from AVRDC and Indonesia against anthracnose disease. Field and laboratory experiments were conducted in Kediri - East Java, Lembang - West Java respectively, during January to October 2012 using completely randomized block design with three replications. Twenty genotype developed by AVRDC, IVEGRI, private sector companies and farmers' local variety were evaluated for resistance to anthracnose in the field, an experiment unit consisted of 20 plants per plot. The observations were recorded for yield and some important economic characters. Fruit weight (g), length and width (cm) were average of 10 ripe fruits of the second harvest. Plant height (cm), canopy diameter (cm), pest and disease incidences (%) and fresh biomass (g) were average of five plants randomly taken from central rows. Data on fruit yield were recorded at plot basis. The impact of chili genotypes to another important of pest and diseases and financial analysis also observed. Financial analysis was conducted using partial budgeting approach. A further experiment was conducted in Laboratory and screen house of Indonesian Vegetables Research Institute (IVEGRI). Artificial inoculation was performed on green mature and ripe red fruits using AVRDC developed method and Yoon procedure with anthracnose isolates (AN 114). Symptoms were evaluated 3 - 7 days after inoculation. The results indicated that AVPP1102-B, AVPP0513, AVPP0719, AVPP0207, AVPP1004-B were found to be promising lines of chili pepper in terms of fruit yield and tolerance to anthracnose infection. There was no significant difference among Kencana, Flash 750, AVPP0207, AVPP0513 and AVPP0704. AVPP0712, AVPP0207 and AVPP0718 were showed consistent resistance in both in field and laboratory test. Four cultivars showed the highest profit. High resistance to both whitefly transmitted Gemini virus and anthracnose has been shown by AVPP0207, however fruit type does not match with consumer preferences. Thus, this line can be used as the source of resistance in breeding activity to develop resistance varieties for anthracnose and Gemini virus. The identification and development of new varieties of chili pepper with resistance to the disease would be most effective alternative for disease management. The results may provide useful information for stakeholder and breeding chili pepper anthracnose resistance.

Key Words: Gemini virus, environmental friendly, yield loss, crop production, susceptible, genotype.

Introduction. Chili pepper (*Capsicum annuum* L.) is one of the most important solanaceous vegetable crops in Indonesia with a total estimate production area of 239,770 ha in 2011 that accounted for 1,483,070 tons of fresh fruit with an average yield of 6.19 t ha⁻¹ (BPS 2012). Indonesia exports chili (0.60 % or US\$ 1,370,780) to Malaysia, Brunei Darussalam, Singapore, India, Republic of Korea, Vietnam, Hong Kong, Saudi Arabia, East Timor and Federal Republic of Germany (Dir. Hort. 2011). Several insect pests and diseases cause significant yield losses in chili pepper. One of the most important is anthracnose diseases. Anthracnose causes extensive pre- and post-harvest damage to chili fruits causing anthracnose lesions. Even small anthracnose lesions on chili fruits reduce their marketable value (Manandhar et al 1995). The disease has been observed to occur in three phases, they are: (a) seedling blight or damping off; (b) leaf spot and die back and (c) fruit rot (Mistry et al 2008). Typical anthracnose symptoms on chili fruit include sunken necrotic tissues, with concentric rings of acervuli. Fruits showing

blemishes have reduced marketability (Manandhar et al 1995), reduced fruit dry weight and quantities of capsaicin and oleoresin (Mistry et al 2008).

Chili pepper anthracnose caused by several *Colletotrichum species* including *C. acutatum*, *C. gloeosporioides*, *C. coccodes* and *C. capsici* leads to serious problems in pepper production in tropical and subtropical regions. Kim at al (2004) reported that different species of *Colletotrichum* cause diseases in different organs of chili plant. For example, *C. capsici* is widespread in red ripe fruits (Than et al 2008), whereas *C. acutatum* and *C. gloeosporioides* have been reported to be more prevalent on both young and mature green fruits (Park et al 1990; Kim et al 1999; Kang et al 2005). Harp et al (2008) reported the occurrence of anthracnose on immature chili pepper fruit caused by *C. acutatum*.

The damage caused by anthracnose is especially serious in many Asian counties including Indonesia. In these countries, *C. acutatum* is the most destructive of the several anthracnose pathogens present and is widely distributed (Syukur et al 2007). Thus, production in the tropics has serious diseases and quality problem (Kim et al 2010). It has been reported that apart from pre-harvest losses, fruit quality deterioration due to anthracnose range from 21 – 47 % in Sri Lanka (Anon 1993). Yield losses of 13 % have been recorded in Korea (Yoon et al 2004), about 50 % in Malaysia (Sariah 1994), 66 – 84 % in India (Thind & Jhooty 1985) and 75 % in Indonesia (Kusandriani & Permadi 1996). In Thailand, anthracnose can cause fruit yield losses greater than 50 up to 100 % (Pakdeevaraporn et al 2005).

In East Java (Indonesia), several fungicides have been used to reduce disease development (Setiawati et al 2011), but they are uneconomical and may cause environmental pollution. Among the various control methods, the use of host plant resistance is one of the most important components of the disease management strategies. The genotypes resistant to different Colletotrichum species and pathotypes (virulent isolates) within species have been identified (AVRDC 2003) and used to study inheritance of resistance (Kim & Park 1988). However, no commercial resistant varieties of C. annuum have been developed, due to the lack of resistance in the C. annuum gene pool (Temiyakul et al 2012). AVRDC (2003) and Pakdeevaraporn et al (2005) reported that current research is focusing on introgression of this resistance into susceptible commercial cultivars of C. annuum. At AVRDC, new crosses were made annually to combine superior sources of disease resistance with elite Indonesian OP varieties, mainly 'Jatilaba,' 'TitSuper' and 'KR-B' ('Keriting' from Bogor). Sources of resistance to anthracnose are progenies of inter specific crosses with PBC932, a Capsicum chinense germplasm selection. 12 advanced lines that had been identified as carrying anthracnose resistance in earlier generations (ACIAR 2011). Setiawati et al (2008) reported that Lembang – 1 dan Tanjung – 2 moderately resistant to anthracnose.

Several methods have been developed to assess anthracnose on chili pepper. They include the methods to be used in the field or in laboratory conditions. Excellent progress has been made in refining a laboratory assay method to evaluate anthracnose resistance in pepper fruit. In the meantime, however, less focus has been placed on field evaluations of candidate lines and selections. It is important to calibrate the laboratory assay results with real field disease reactions (AVRDC 2003).

The experiments were conducted to evaluate 20 genotypes of chili pepper from AVRDC and Indonesia against anthracnose diseases under field and controlled conditions.

Material and Method

Screening of chili pepper genotypes for anthracnose resistance under field conditions. Screening of varieties and breeding lines for anthracnose resistance was conducted at Menang village, Pagu Sub-district, Kediri (East Java) during the rainy season of January to May 2012. The location is low land which is about 100 m above the sea level. Randomized complete block design (RCBD) was used in this experiment with three replications and an experiment unit consisted of 20 plants per plot. Twenty genotypes developed by AVRDC, IVEGRI, private sector companies and farmers' local

variety (Tabel 1) were planted at a spacing of 70 cm between rows and 50 cm between plants within the row. Local variety bordered the experimental plants.

Table 1 20 genotype of chili pepper used in the experiment

No.	Genotype	Origin
1	AVPP1102-B	AVRDC, Taiwan
2	AVPP0719	AVRDC, Taiwan
3	AVPP0712	AVRDC, Taiwan
4	AVPP1103-B	AVRDC, Taiwan
5	AVPP0205	AVRDC, Taiwan
6	AVPP0708	AVRDC, Taiwan
7	AVPP0207	AVRDC, Taiwan
8	AVPP0514	AVRDC, Taiwan
9	AVPP1004-B	AVRDC, Taiwan
10	AVPP0513	AVRDC, Taiwan
11	AVPP1003-B	AVRDC, Taiwan
12	AVPP9813	AVRDC, Taiwan
13	AVPP0704	AVRDC, Taiwan
14	Kencana	IVEGRI, Indonesia
15	AVPP0718	AVRDC, Taiwan
16	AVPP0805	AVRDC, Taiwan
17	7 Flash 750 Private sector	
18	Elegans	Farmers local variety, Indonesia
19	Tanjung – 2	IVEGRI, Indonesia
20	Lembang – 1	IVEGRI, Indonesia

Chili seedling was prepared previously at nursery protected using bio-agent and nylon net to minimize attack of pests and diseases at very early stages. Transplanting was conducted when the seedling was 4 weeks old.

Land preparation started with ploughing and making beds. The size of bed is 1 m by 4.5 m. Organic materials consisting of mature compost and manure were applied as basal fertilizer along with NPK composite fertilizers. The dose of organic materials and NPK was 25 t/ha and 1 t/ha respectively. The beds were covered with silvery plastic mulch.

Pesticides were used adequately to maintain the plants. Pest control was conducted by spraying Alfamethrin 15 EC at 2 mL/L and Delthamethrin 2.5 EC at 2 mL/L. Spraying were based on field observation. Disease control was conducted by spraying Propineb and Mancozeb. After flowering not fungicides were applied to give chances for anthracnose diseases to attack. Weeding was conducted when necessary. Weeding was only applicable to the space of inter beds. The observations were recorded for yield and some important economic characters. Fruit weight (g), length and width (cm) were average of 10 ripe fruits of the second harvest. Plant height (cm), canopy diameter (cm) and fresh biomass (g) were average of five plants randomly taken from central rows. Data on fruit yield were recorded at plot basis. The intensity of anthracnose attack on chili fruit was measured using formula as follow:

$$\begin{array}{c} \Sigma \text{ infected fruits} \\ \text{Intensity} = ----- \times 100 \ \% \\ \Sigma \text{ observed fruits} \end{array}$$

Financial analysis was conducted using partial budgeting approach, where only different factors from control were. In this study, only cost of seeds and yield of each treatment were applicable to this financial analysis. Relative financial superiority was determined using formula as follow:

$$\Delta\pi = (R_T - R_C) - (C_T - C_C)$$

where:

 $\Delta\pi$ = additional profit comparable to control; R_T = revenue of treatment; R_C = revenue of control; C_T = cost of treatment; C_C = cost of control. The higher $\Delta\pi$ of treatments are comparable to $\Delta\pi$ of control ($\Delta\pi$ = 0), the more superior treatment.

Screening of chili pepper genotypes for anthracnose resistance under controlled conditions. A further experiment was conducted in Laboratory and screen house of Indonesian Vegetables Research Institute (IVEGRI), Lembang West Java at an elevation of 1250 m above sea level. All 20 *Capsicum* genotypes used in the field trial were tested under in vitro conditions using a completely randomized block design with three replications and five fruits per replications. Anthracnose (C. acutatum) isolated from infected of red chili pepper fruits from farmers' field at Kediri East Java (isolate AN 114) were used for all inoculation and bioassay experiments. Conidia of anthracnose pathogen for all experiments were obtained from cultures on PDA incubated for 14 days at room temperature (28 $^{\circ}$ C - 30 $^{\circ}$ C).

Chili fruits belonging green mature and ripe red fruits were harvested from screen house-grown plants. Fruits were carefully detached from plants and washed with sterile distilled water (SDW) and then wiped with cotton wood soaked in ethanol to reduce microbes on the surface. The concentration of inoculum was adjusted 5 x 10^5 conidia per mL. Artificial inoculation was performed on green mature and ripe fruit stages using microinjection method developed at the AVRDC (1999) and incubated for 3 days at a temperature of 25 °C and 95 % RH to observe anthracnose lesion development. The diameter of the lesions that developed on and around the puncture wound at 3 - 7 days after inoculation was recorded. Resistance score followed the modified procedure of Yoon (2003 in Syukur et al 2009) method. Disease incidence was evaluated using Yoon (2003 in Syukur et al 2009) method with slight modifications. The following rating system used was based on incidence of diseases. The formula as follow:

DI = diseases incidence;

n = infected fruits with lesio diameter > 4 mm;

N = total of fruits inoculated.

Note:

1. $0 \le X < 10$ highly resistant (HR);

2. $10 \le X < 20 \text{ Resistant (R)}$;

3. $20 \le X < 40$ moderately resistant (MR);

4. $40 \le X < 70$ Susceptible (S);

5. X > 70 highly susceptible (HS).

Statistical analysis. The collected data was analyzed with the software package SPSS 17.0 for Windows (SPSS Inc., Chicago, IL) using the analysis of variance to determine treatment significance. The analysis was continued with Least Significant Difference (LSD) at the 5 % probability level to determine comparison between treatment means. In graphs and figure, the original data and their standard errors are presented.

Results and Discussion

Growth parameter. The impact of *C. acutatum* on growth such as plant height, canopy diameter, and fresh biomass were found to be varied depending on the genotypes of chili. The result of measured average plant heights and canopy diameter in all the treatments indicated that AVPP102-B, AVPP0719, AVPP0708, AVPP0514, AVPP1004-B and AVPP1003-B shown the highest among the other lines and not significant different compare Tanjung-2, Elegance and Flash 750 at each sampling date. Comparatively low results was found on AVPP0207 has been observed (Table 1). Canopy diameter show that AVPP1102-B, AVPP1103-B, AVPP0513 and AVPP0805 resulted in significantly larger canopy diameter of plants and no significant different were found at Tanjung-2, Lembang-1, Elegance and Flash-750.

Kencana, AVPP0805, AVPP0514, AVPP0207, and AVPP9813 have a good performance in terms of fresh biomass compare to AVPP1004-B, AVPP0513, AVPP1103-B, AVPP0205 and Tanjung-2.

Anthracnose incidence. The incidence of anthracnose was observed every harvest period (4 times) and from total harvested (Table 2). Generally, anthracnose incidence at all genotypes tested was higher at third harvest period comparable to another harvest period. Significantly (P<0.05) different among 20 genotypes of chili pepper tested had observed. The incidence of anthracnose was varied from 1.05 % to 63.93 % depending on genotypes. The highest anthracnose incidence was found at Flash 750 (commercial variety) and Elegance (farmers' local variety) (70 - 80 %) followed by AVPP0513 and AVPP0719 (>50 %). AVPP0712, AVPP0207, AVPP9813 and AVPP0704 had the lowest anthracnose incidence (<10 %) at the periods harvest observed and total harvested, while the other lines (Kencana, Tanjung-2 and Lembang-1) had intermediate incidence. This results found here was similar with that reported by AVRDC (1999, 2003) and Mongkolporn et al (2010) which that in terms of anthracnose incidence in these lines. Sources of resistance to anthracnose in these lines are progenies of interspecific crosses with PBC932, a Capsicum chinense germplasm selection. Pedigree of AVPP0712 was (KBR/VC 246///KRB/// IR*4/PBC 932), AVPP0207 (IR*3/PBC 932), AVPP9813 (Kulin/HAD 295 (Berke's joy). Clearly some of these pedigrees are more promising than others. All of these lines that derived some parentage from the C. chinense accession PBC932. Wean while Tanjung-2 and Lembang-1 were lines that are moderately resistant to anthracnose (Setiawati et al 2008) and Kencana is the new variety that suitable in the extreme climate change (Setiawati et al 2013). This result has important information that commercial variety and farmers' local variety were susceptible to anthracnose. The result is in line with Temiyakul et al (2012) which reported that no commercial resistant varieties of C. annuum have been developed, due to the lack of resistance in the C. annuum gene pool.

Kim et al (2001) and Ko et al (2005) suggested that the resistant and moderately resistant genotypes under field conditions were found to be significantly superior with respect to total phenol as well as OD phenol content. Increased activity of polyphenol oxidase was reported in leaves of two resistant varieties of C. annuum. Upon infection with pathogen, the activity of the enzyme increased significantly in resistant varieties.

Plant growth and fresh biomass of various genotypes of chili pepper (Kediri, East Java, 2012)

Table 1

No Treatments	Treatments	Canopy width (cm) at WAP			Plant height (cm) at WAP				Fresh biomass	
		2	4	6	8	2	4	6	8	(5 plants)
1	AVPP1102-B	12.83 ^{bcd}	16.50 ^{fgh}	34.00 ^{ab}	39.33 ^{abc}	12.89 ^a	30.11 ^{abc}	42.11 ^{a-d}	56.89 ^{a-d}	2,208.33 ^{b-e}
2	AVPP0719	11.61 ^{cd}	18.33 ^{b-f}	30.11 ^{a-e}	38.67 ^{a-e}	8.78 ^{fg}	20.11 ^{d-i}	31.00 ^{d-g}	45.56 ^{e-h}	2,195.00 ^{b-e}
3	AVPP0712	9.67 ^e	18.44 ^{b-f}	24.11 ^{e-h}	29.78 ^{hi}	7.78 ^g	19.67 ^{f-i}	29.89 ^{efg}	46.44 ^{d-h}	1,966.67 ^{de}
4	AVPP1103-B	13.00 ^{bcd}	17.56 ^{d-h}	32.89 ^{abc}	38.78 ^{a-d}	11.89 ^{abc}	25.44 ^{a-h}	41.67 ^{a-d}	56.44 ^{a-d}	1,816.67 ^{ef}
5	AVPP0205	8.83 ^e	13.28 ⁱ	22.22 ^{fgh}	31.50^{e-i}	6.00 ^h	16.67 ^{hi}	25.56 ^{fg}	42.00 ^{fgh}	1,873.33 ^{ef}
6	AVPP0708	12.39 ^{bcd}	19.22 ^{a-e}	27.33 ^{c-f}	37.33 ^{a-g}	9.33 ^{ef}	21.22 ^{c-h}	31.33 ^{d-g}	44.89 ^{fgh}	1,986.67 ^{de}
7	AVPP0207	5.89 ^f	9.11 ^j	19.00 ^h	25.78 ⁱ	4.11 ⁱ	11.78 ⁱ	21.56 ^g	40.11 ^{gh}	2,716.67 ^{a-d}
8	AVPP0514	11.61 ^{cd}	17.92 ^{c-g}	29.00 ^{b-e}	37.22 ^{a-g}	11.00 ^{cd}	23.22 ^{b-h}	34.33 ^{b-f}	49.67 ^{b-g}	2,708.33 ^{a-d}
9	AVPP1004-B	12.17 ^{bcd}	13.67 ⁱ	30.56 ^{a-d}	38.22^{a-f}	5.56 ^h	18.56 ^{ghi}	29.56 ^{efg}	40.89 ^{gh}	1,831.67 ^{ef}
10	AVPP0513	12.94 ^{bcd}	15.22 ^{hi}	27.67 ^{c-f}	32.67 ^{c-i}	12.67 ^a	31.78 ^{ab}	45.67 ^{ab}	58.33 ^{abc}	1,861.67 ^{ef}
11	AVPP1003-B	12.72 ^{bcd}	21.44 ^a	34.44 ^{ab}	40.22 ^{ab}	8.22 ^{fg}	23.00 ^{b-h}	33.22 ^{c-f}	41.78 ^{fgh}	1,996.67 ^{cde}
12	AVPP9813	11.50 ^d	15.33 ^{ghi}	21.00 ^{gh}	29.67 ^{hi}	9.33 ^{ef}	17.67 ^{hi}	33.33 ^{c-f}	48.33 ^{c-h}	2,785.00 ^{abc}
13	AVPP0704	12.17 ^{bcd}	16.83 ^{e-h}	25.44 ^{d-g}	31.22 ^{f-i}	8.22 ^{fg}	19.22 ^{ghi}	28.78 ^{efg}	43.33 ^{fgh}	2,540.33 ^{b-e}
14	KENCANA	12.11 ^{bcd}	19.67 ^{a-d}	24.78 ^{d-h}	30.78 ^{ghi}	11.33 ^{bcd}	27.22 ^{a-g}	38.11 ^{a-e}	52.33 ^{b-f}	2,901.67 ^{ab}
15	AVPP0718	13.06 ^{bc}	16.33 ^{fgh}	31.89 ^{abc}	35.67 ^{b-h}	11.33 ^{bcd}	19.78 ^{e-i}	28.33 ^{efg}	38.56 ^h	2,175.00 ^{bcde}
16	AVPP0805	12.72 ^{bcd}	18.78 ^{b-f}	28.67 ^{b-e}	31.67 ^{d-i}	11.33 ^{bcd}	28.78 ^{a-e}	43.89 ^{abc}	58.33 ^{abc}	3,486.67 ^a
17	FLASH 750	13.28 ^{ab}	20.56 ^{ab}	34.22 ^{ab}	40.78 ^{ab}	12.56 ^{ab}	32.89^{a}	45.33 ^{ab}	63.78 ^a	2,265.00 ^{b-e}
18	ELEGANCE	14.72 ^a	19.28 ^{a-e}	35.44 ^a	41.00 ^{ab}	11.67 ^{abc}	28.67 ^{a-f}	42.33 ^{a-d}	56.11 ^{a-e}	2,031.67 ^{cde}
19	TANJUNG-2	13.56 ^{ab}	19.22 ^{a-e}	35.78^{a}	43.44 ^a	10.33 ^{de}	29.11 ^{a-d}	42.44 ^{a-d}	59.67 ^{ab}	1,153.33 ^f
20	LEMBANG-1	12.72 ^{bcd}	20.33 ^{abc}	30.67 ^{a-d}	37.56 ^{a-g}	10.22 ^{de}	31.11 ^{ab}	46.67 ^a	58.33 ^{abc}	2,150.00 ^{b-e}

WAP – weeks after planting.

Table 2 Anthracnose incidence of various genotypes of chili pepper (Kediri, East Java, 2012)

N/-	Tue of the state	Anthracnose (%) at (Harvest period)						
No.	Treatments -	1 st	2 nd	3 rd	4 th	Total		
1	AVPP1102-B	20.14 ^{bcd}	51.97 ^{ab}	68.82 ^{ab}	43.18 ^{bcd}	42.59 ^{cdef}		
2	AVPP0719	18.58 ^{bcd}	24.34 ^{abc}	75.77 ^a	44.84 ^{bcd}	53.49 ^{bcd}		
3	AVPP0712	9.11 ^{bcd}	27.44 ^{abc}	23.88 ^{cde}	16.46 ^{ef}	7.08 ^{hi}		
4	AVPP1103-B	21.37 ^{bcd}	42.03 ^{abc}	57.02 ^{abcd}	45.47 ^{bcd}	48.84 ^{bcde}		
5	AVPP0205	0.00^{d}	13.67 ^{abc}	11.67 ^e	33.56 ^{cde}	34.29 ^{defg}		
6	AVPP0708	6.60 ^{cd}	11.98 ^{abc}	55.94 ^{abcd}	27.62 ^{cdef}	26.20 ^{efgh}		
7	AVPP0207	0.00^{d}	0.00^{c}	27.78 ^{cde}	16.08 ^{ef}	1.05 ⁱ		
8	AVPP0514	9.81 ^{bcd}	11.43 ^{abc}	23.15 ^{cde}	16.17 ^{ef}	19.32 ^{fghi}		
9	AVPP1004-B	13.65 ^{bcd}	12.42 ^{abc}	32.23 ^{bcde}	40.20 ^{bcd}	20.01 ^{fghi}		
10	AVPP0513	20.18 ^{bcd}	33.13 ^{abc}	63.75 ^{abc}	58.60 ^b	63.93 ^{abc}		
11	AVPP1003-B	31.54 ^b	15.19 ^{abc}	33.31 ^{bcde}	29.29 ^{cde}	18.17 ^{ghi}		
12	AVPP9813	6.67 ^{cd}	0.00^{c}	3.46 ^e	5.64 ^f	5.99 ^{hi}		
13	AVPP0704	6.45 ^{cd}	9.09 ^{abc}	21.34 ^{de}	11.13 ^{ef}	2.37^{i}		
14	KENCANA	28.84 ^{bc}	38.40 ^{abc}	41.09 ^{abcde}	28.16 ^{cdef}	35.45 ^{defg}		
15	AVPP0718	13.57 ^{bcd}	5.03 ^{bc}	19.68 ^{de}	23.06 ^{def}	16.11 ^{ghi}		
16	AVPP0805	21.92 ^{bcd}	36.30 ^{abc}	34.73 ^{bcde}	25.81 ^{cdef}	26.81 ^{efgh}		
17	FLASH 750	13.73 ^{bcd}	40.15 ^{abc}	81.75 ^a	48.67 ^{bc}	71.92 ^{ab}		
18	ELEGANCE	84.84 ^a	57.41 ^a	54.40 ^{abcd}	87.29 ^a	83.42 ^a		
19	TANJUNG-2	4.39^d	41.16 ^{abc}	57.08 ^{abcd}	14.20 ^{ef}	46.56 ^{cde}		
20	LEMBANG-1	2.22 ^d	50.34 ^{abc}	24.82 ^{cde}	24.32 ^{def}	20.36 ^{fghi}		

Screening of chili pepper genotypes for anthracnose resistance under controlled conditions. The lesion development was assessed as diameter of lesion after 3 days of inoculation. Significant differences in anthracnose lesion development were found between the fruits of different maturity stages (Figure 1 & 2). The highest lesion development was observed on ripe red fruits. Disease incidence was different among 20 genotypes based on the number of days of fruits lesion development. Anthracnose symptoms on susceptible lines, started to develop at 3 DAI, and there was no further change in diseases incidence after 7 DAI. No lesion observed in AVPP0708, AVPP0207, AVPP0514, AVPP9813 and AVPP0704 at 3 DAI at green mature fruits. AVPP1102-B and Elegance had the highest diseases incidence of all the lines test at 4 – 6 DAI. After 7 DAI, test genotypes were grouped in three categories based on lesion diameter. High diseases incidence in AVPP0805, low diseases incidence in AVPP0205, AVPP0207, AVPP0514, AVPP1004-B, AVPP1003-B, AVPP0805 and Kencana and the rest of the genotypes tested had medium diseases incidence (Figure 1). AVPP0205, AVPP0207, AVPP0514 are lines had have anthracnose resistance from a Capsicum chinense accession (PBC932) into C. annuum. Two recurrent parents were used, Kulai and IR. These lines high levels of resistance at the green fruit stage under laboratory assay methodology (AVRDC 2003).

On ripe red fruits, AVPP0207 and AVPP0513 had the least diseases incidence and had lesions of less than 0.5 cm diameter followed by AVPP1102–B, AVPP0719 and AVPP103–B. From 20 genotypes tested, 8 lines had the highest diseases incidence (AVPP0514, AVPP1004–B, AVPP1003–B, AVPP9813, AVPP0704 and AVPP0805) and no significant different with Kencana and Elegance (Figure 2).

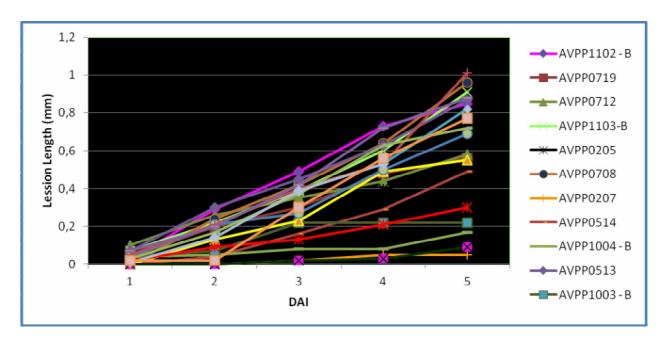


Figure 1. Lesion development of anthracnose on detached chili pepper at green mature stage after inoculation with *Colletotrichum acutatum* (Lembang, West Java, 2012).

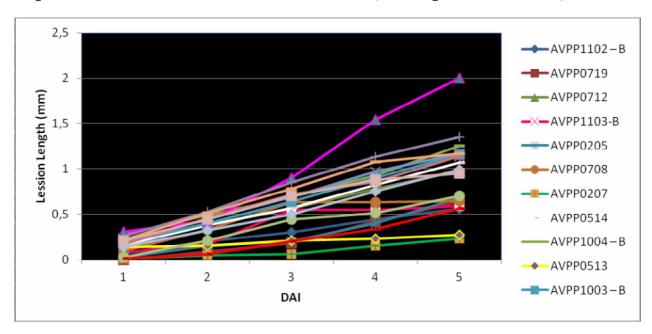


Figure 2. Lesion development of anthracnose on detached chili pepper at ripe red stage after inoculation with *Colletotrichum acutatum* (Lembang, West Java, 2012).

Chili pepper fruits at ripe red stage are more susceptible to *C. acutatum* than green fruits. Lesion diameter of anthracnose on green fruits range from 0.05 cm to 1.01 cm while lesion diameter on ripe red fruits from 0.23 cm to 2.0 cm at 7 days after inoculation. This quiescence of anthracnose pathogen on immature fruit can occur due to a number of preformed antifungal compounds detected in unripe fruits, which declined during ripening (Rajapakse & Ranasinghe 2002). The inheritance of chili anthracnose resistance in *C. baccatum* PBC80 by Mahasuk et al (2009) indicated that the resistance genes in mature green and ripe fruit stages were different and controlled by single dominant genes. Independent genetic studies have also identified resistance genes in *C. chinense* and *C. baccatum* that were differentially expressed at different fruit maturity stages. The resistance genes in mature green and ripe fruit stages were different and controlled by single dominant genes.

Breeding for anthracnose resistance has utilized *C. chinense* and *C. baccatum* as sources to introgress the resistance into *C. annuum*. Host resistance in these species has been shown to be differentially expressed at different fruit maturity stages (Montri et al 2009; Mongkolporn et al 2010). Ko et al (2005) reported on differential resistance in green and ripe fruit of the same chili variety as revealed by high expression of *PepEST* gene, while Manandhar *et al* (1995) reported that diseases incidence was correlated to cuticle and exocarp thickness and varied by fruit maturity.

Table 3 showed that on green stages fruits of 20 genotype chili pepper tested are infected with *C. acutatum*. No lesions developed on AVPP0207. Less than 20 % of the chili pepper genotypes were rated as highly or resistant and 80 % of the lines were rated as susceptible and highly susceptible. On ripe red fruits, AVPP0513 was highly resistant. 4 lines were moderately resistant and the other (75 %) of the lines on chili pepper tested was rated as susceptible and highly susceptible. Kim et al (2001) and Ko et al (2005) reported that expression of an esterase gene isolated from chili pepper is involved in anthracnose resistance by ripe fruits. Prasath & Ponnuswani (2008) reported that phenol and enzyme plays a role is resistance against fungal infection on chili pepper.

Table 3
Reaction of 20 genotypes of chili pepper against anthracnose under artificial inoculation
(Lembang, West Java, 2012)

No.	Genotype	Green mature fruits stage	Remarks	Red ripe fruits stage	Remarks
1	AVPP1102-B	85.00	HS	36.84	MR
2	AVPP0719	57.50	S	22.22	MR
3	AVPP0712	55.00	HS	100.00	HS
4	AVPP1103-B	85.00	HS	57.50	S
5	AVPP0205	52.50	S	22.22	MR
6	AVPP0708	87.50	HS	89.66	HS
7	AVPP0207	0.00	HR	30.00	MR
8	AVPP0514	45.00	S	97.50	HS
9	AVPP1004-B	12.50	R	100.00	HS
10	AVPP0513	80.00	HS	8.11	HR
11	AVPP1003-B	12.50	R	95.00	HS
12	AVPP9813	52.50	S	100.00	HS
13	AVPP0704	10.00	R	95.00	HS
14	KENCANA	35.00	MR	97.37	HS
15	AVPP0718	65.00	S	69.70	S
16	AVPP0805	61.25	S	100.00	HS
17	FLASH 750	40.68	S	60.00	S
18	ELEGANCE	70.00	HS	95.00	HS
19	TANJUNG-2	90.00	HS	92.50	HS
20	LEMBANG-1	90.00	HS	100.00	HS

HR=High resistant, R = Resistant, MR = moderately resistant, S = Susceptible, HS = High susceptible.

Table 4 present the data incidence of anthracnose from natural field and artificial laboratory at green mature and ripe red stage. Comparing natural field and artificial laboratory at green mature and ripe red stage, three lines (AVPP0712, AVPP0207 and AVPP0718) showed consistent resistance in field as well as in laboratory to the disease with incidence of anthracnose (>20 %) and average lesion diameters ranging from 0.05 – 0.98 cm. AVPP0514, AVPP9813, and AVPP0714 had have lower incidence of anthracnose only in field and in laboratory at green mature. Clearly some of these pedigrees are more promising than others.

Table 4
Reactions of 20 genotypes of chili pepper in natural field and artificial laboratory inoculation of anthracnose (*Colletotrichum acutatum*)

No.	Treatments	Field incidence (%) -	Laboratory lesion on fruits (cm)			
- NO.	Healments	Tield incluence (78)	Green mature	Ripe red		
1	AVPP1102-B	42.59 ^{cdef}	0.85 ^{abcde}	0.56 ^{ef}		
2	AVPP0719	53.49 ^{bcd}	0.56 ^{defg}	0.64 ^{de}		
3	AVPP0712	7.08 ^{hi}	0.58 ^{cdefg}	0.98 ^{cd}		
4	AVPP1103-B	48.84 ^{bcde}	0.91 ^{ab}	0.59 ^{ef}		
5	AVPP0205	34.29 ^{defg}	0.48 ^{fghi}	0.68 ^{de}		
6	AVPP0708	26.20 ^{efgh}	0.96 ^{ab}	0.65 ^{de}		
7	AVPP0207	1.05 ⁱ	0.05 ^j	0.23 ^f		
8	AVPP0514	19.32 ^{fghi}	0.49 ^{fgh}	1.07 ^{bc}		
9	AVPP1004-B	20.01 ^{fghi}	0.17 ^{ij}	1.25 ^{bc}		
10	AVPP0513	63.93 ^{abc}	0.87 ^{abcd}	0.27 ^f		
11	AVPP1003-B	18.17 ^{ghi}	0.18 ^{hij}	1.16 ^{bc}		
12	AVPP9813	5.99 ^{hi}	0.55 ^{efg}	2.00^{a}		
13	AVPP0704	2.37 ⁱ	0.09 ^j	1.17 ^{bc}		
14	KENCANA	35.45 ^{defg}	O.30 ^{ghij}	1.14 ^{bc}		
15	AVPP0718	16.11 ^{ghi}	0.69 ^{bcdef}	0.70 ^{de}		
16	AVPP0805	26.81 ^{efgh}	1.01 ^a	1.35 ^b		
17	FLASH 750	71.92 ^{ab}	0.72 ^{abcdef}	0.56 ^{ef}		
18	ELEGANCE	83.42 ^a	0.88 ^{abc}	1.17 ^{bc}		
19	TANJUNG-2	46.56 ^{cde}	0.82 ^{abcde}	0.99 ^{bcd}		
20	LEMBANG-1	20.36 ^{fghi}	0.77 ^{abcdef}	0.95 ^{ef}		

Yield parameter. Marked differences were observed among the various genotypes for yield and some economic characters (Table 5). As indicated in the table, statistically (P<0.05) the maximum fruit yield was obtained from lines AVPP1102–B, AVPP0719, AVPP1004-B, and AVPP1003-B and no significant different with Tanjung-2 and Elegance. AVPP0205 and AVPP0207 had the lowest fruit yield. Marketable yield was generally attributed to the combination of resistance to pest and diseases attacked such as anthracnose, Gemini virus and fruit flies. AVPP0712, AVPP0704 and Lembang-1 had significantly higher marketable yield compared to another genotypes.

The present study showed that very good performance in terms of fruit weight, length and width was also observed in AVPP1004-B, AVPP0513, AVPP1102-B, AVPP0719, AVPP0805, AVPP1003-B and AVPP9813. These lines were category as a large type ("Cabai Besar varieties"). However, the incidence of anthracnose was not correlated with types of fruit.

Financial analysis of field trials. Among chili lines tested in this trial, 8 lines were inferior and 11 lines were superior to Flash 750 (Figure 3). Four top superior lines were AVPP0719, AVPP0704, Tanjung-2, and AVPP1003-B. These lines, under trial conditions, can provide additional net income of more than US\$ 1000/ha. Note that this financial analysis is based on the experiment condition where all lines were not normally sprayed because of screening purpose. Result of financial analysis will be different if all lines are under normal cultivation practices.

Table 5
Fruit characters and fruit yield of various genotypes of chili pepper (Kediri, East Java, 2012)

No.	Treatments	Average fruit			Fruit	Fruit yield		
		Weight (g)	Length (cm)	Width (cm)	Туре	Marketable (%)	Fruit weight/Plot (g)	
1	AVPP1102-B	5.67 ^{abcd}	6.75 ^e	1.30 ^a	L	25.24 ^{de}	3,692.00 ^{abcd}	
2	AVPP0719	4.73 ^{cd}	9.87 ^{bc}	0.67 ^g	S	41.56 ^{cde}	4,155.33 ^{abc}	
3	AVPP0712	4.80 ^{cd}	9.66 ^{bc}	0.88 ^{c-g}	S	76.70 ^a	1,052.67 ^{efg}	
4	AVPP1103-B	5.67 ^{abcd}	8.00 ^{cde}	1.10 ^{a-d}	L	18.10 ^{de}	4,080.00 ^{abc}	
5	AVPP0205	6.33 ^{abc}	7.78 ^{cde}	1.15 ^{abc}	L	35.65 ^{de}	810.00 ^{fg}	
6	AVPP0708	7.00 ^{abc}	8.53 ^{bcde}	1.08 ^{a-e}	L	39.66 ^{cde}	2,889.00 ^{abcde}	
7	AVPP0207	4.67 ^{cd}	7.02 ^{de}	1.11 ^{a-d}	L	40.98 ^{cde}	649.00 ^g	
8	AVPP0514	7.50 ^{ab}	7.97 ^{cde}	1.22 ^{ab}	L	29.97 ^{de}	2,777.00 ^{bcdef}	
9	AVPP1004-B	8.00 ^a	8.31 ^{bcde}	1.31 ^a	L	39.01 ^{cde}	3,548.00 ^{abcd}	
10	AVPP0513	8.00 ^a	8.07 ^{bcde}	1.18 ^{ab}	L	27.43 ^{de}	3,371.67 ^{abcd}	
11	AVPP1003-B	7.67 ^{ab}	9.15 ^{bcde}	1.18 ^{ab}	L	40.78 ^{cde}	3,728.67 ^{abcd}	
12	AVPP9813	7.33 ^{ab}	7.47 ^{cde}	1.30 ^a	L	40.49 ^{cde}	2,266.00 ^{bcdefg}	
12	AVPP0704	7.00 ^{abc}	8.10 ^{bcde}	1.15 ^{abc}	L	78.53 ^a	2,105.33 ^{cdefg}	
14	KENCANA	4.67 ^{cd}	8.15 ^{bcde}	0.81 ^{defg}	S	40.09 ^{cde}	1,711.67 ^{defg}	
15	AVPP0718	5.33 ^{bcd}	7.87 ^{cde}	0.98 ^{bcdef}	S	54.00 ^{cde}	2,334.67 ^{bcdefg}	
16	AVPP0805	4.67 ^{cd}	10.45 ^{ab}	0.80 ^{efg}	S	36.57 ^{cde}	3,023.67 ^{abcde}	
17	FLASH 750	4.67 ^{cd}	12.33 ^a	0.76 ^{fg}	S	28.08 ^{de}	2,918.33 ^{abcde}	
18	ELEGANCE	5.67 ^{abcd}	9.49 ^{bc}	0.88 ^{cdefg}	S	14.96 ^e	4,174.67 ^{ab}	
19	TANJUNG-2	5.67 ^{abcd}	7.49 ^{cde}	1.33 ^a	L	31.54 ^{de}	4,869.00 ^a	
20	LEMBANG-1	3.67 ^d	9.27 ^{bcd}	0.61 ^g	S	73.45 ^{ab}	822.67 ^{fg}	

S = small ("Keriting variety"), L = Large ("Cabai Besar variety").

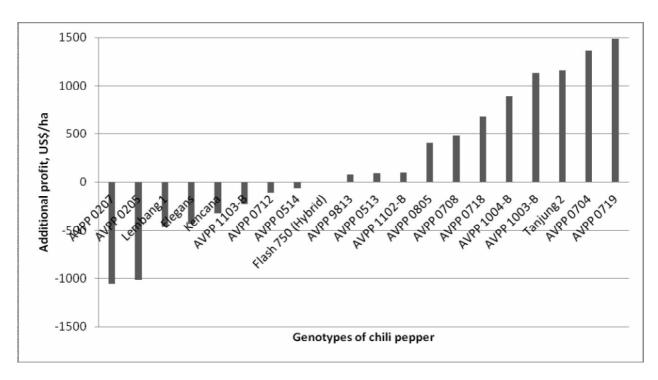


Figure 3. Result of financial analysis using partial budgeting approach.

Another pest and diseases incidence. Another pest and diseases was found during the experiment, namely Gemini virus and fruit flies. Percentage of Gemini virus incidence is presented at figure 4. AVPP1103–B, AVPP0207, AVPP1004–B, Kencana and Flash 750 had the lowest Gemini virus incidence (<20 %). AVPP0719, AVPP0718, Tanjung–2 and Lembang-1 had the highest Gemini virus incidence (>50 %), while the other lines had intermediate incidence.

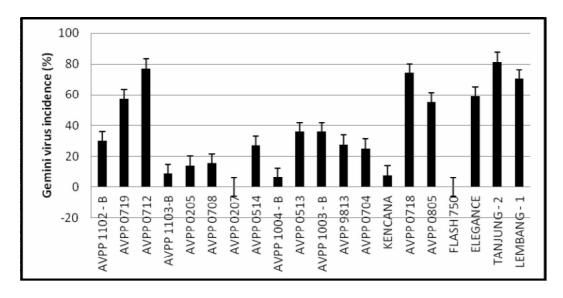


Figure 4. Gemini virus incidence at 20 genotypes of chili pepper (mean \pm SE).

Chili fruits harvested were also infected with fruit rot caused by *Bactrocera* spp. (Fruit flies) (Figure 5). Chili fruit infected with fruit flies become rotten and it will be unmarketable. Two lines of chili pepper (AVPP0719, AVPP0515) followed by Flash 750, Elegance and Lembang-1 had the lowest fruit flies incidence compare to another lines of chili pepper tested.

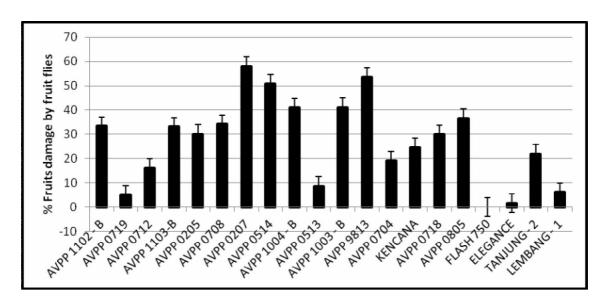


Figure 5. Percentage of fruits damage by fruits flies at 20 genotypes of chili pepper (mean \pm SE).

Conclusions. Based on analyses, we conclude that AVPP1102-B, AVPP0513, AVPP0719, AVPP0207, AVPP1004-B were found to be promising lines of chili pepper in terms of fruit yield and tolerance to anthracnose infection. There was no significant different from Kencana and Flash 750, AVPP0207, AVPP0513 and AVPP0704 and Kencana were more resistant to anthracnose than others, AVPP0205, AVPP0708, AVPP0514 and AVPP0718 were susceptible to anthracnose. Comparing field and in vitro evaluations, three lines (AVPP0712, AVPP0207 and AVPP0718) showed consistent resistance in field as well as in vitro to the disease. Three lines (AVPP1003-B, AVPP0704 and AVPP0719) and Tanjung-2 had the highest profit. High resistance to both whitefly transmitted Gemini virus and anthracnose has been found (AVPP0207), however fruit type does not match with consumer preferences. Thus, this line can be used as the source of resistance in breeding activity to develop resistance varieties for anthracnose and Gemini virus. In terms of financial aspect, AVPP0719, AVPP0704, Tanjung-2, AVPP1003B, and AVPP1004-B were superior to Flash 750 (commercial variety).

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