Proceedings of the Sixth Academic Sessions, University of Ruhuna 2009
Vol. 6 pgs 63 - 66

Screening of Tomato (*Lycopersicum esculentum* L.) Varieties Resistant to Anthracnose Caused by *Colletotrichum coccodes*

Rathnaweera, C[1], Abeysinghe, S[1]., Rajapakse, R.G.A.S[2]., and Sakalasooriya, S.M.I.S.K.[2]
[1]*Department of Botany, Faculty of Science, University of Ruhuna, Matara.*
[2]*Horticultural Crops Research and Development Institute, Gannoruwa, Peradeniya.*
✉ *saman@bot.ruh.ac.lk*

Abstract
Anthracnose caused by *Colletotrichum coccodes* is one of the major disease problems in tomato (*Lycopersicum esculentum*) cultivation in Sri Lanka. Currently fungicides are used to control this disease at an alarming scale. However, the use of resistant varieties is feasible, economical, and a reliable method to control the disease. There are about eighteen tomato cultivars available in Sri Lanka, which show differential disease response to this pathogen. Therefore, the current study was focused on developing a method *in vitro* to identify the resistant varieties of tomato to this disease. Studies were undertaken to find whether there was a relationship between varietal resistance/susceptibility and influence of conidia differentiation by leaf exudates of different tomato varieties. Stimulatory compounds which are responsible for conidia differentiation into appressoria were found in leaf exudates of all tested varieties of tomato. Percentage of conidia germination and appressoria formation in leaf exudates of varieties showed significant correlation ($P<0.05$) with lesion development of different tomato varieties. The highest stimulation of conidia differentiation of pathogen was observed in leaf exudates of the varieties Tharidhu and Rashmi which are highly susceptible to the disease and the lowest stimulation was found in variety Volcano which is highly resistant to the disease. Results revealed that the stimulatory effect of exudates on conidia differentiation could be used to compare degree of resistance to anthracnose to screen tomato germplasm as an *in vitro* test.

Keywords: Tomatoes, Anthracnose, *Colletotrichum coccodes*, resistant varieties

Introduction

The cultivated Tomato *Lycopersicum esculentum* Mill is an important food crop in the world. Its versatility in fresh or processed form and its adaptability have played a major role in its rapid and widespread use. It is cultivated in 5788 hectares (HORDI, 2003) in both wet and dry zones of Sri Lanka as a major commercial crop; mainly in the districts of Matale, Kandy and Anuradhapura. The Average tomato production in Sri Lanka from year 2002 to 2005 was 40,700 Mt/yr (HORDI, 2003). However one of the major constraints in tomato cultivation is the diseases caused by different pathogens. Anthracnose is a major disease of tomato. The causal agent of anthracnose disease is mainly *Colletotrichum* species (Rajapakse, 2007). The pathogen attacks flowers, leaves and fruits at any stage of the plant. Conidia bearing bodies i.e. acervuli are produced as small grey to brown depressions on mature fruits and leaves. Development of the fungal pathogen on the surface of the host is influenced by many factors. Of these, the role played by chemicals, normally exuded or leached by the host i.e. fruit peel exudates have been studied (Swinburne, 1976). Such substances can affect three distinct phases in infection process namely spore germination, germ-tube growth, and the formation of infection structure, the appressoria (Diehl, 1953).

It has been reported that several species of the genus *Colletotrichum* produce appressoria in response to specific chemical signals (Bailey and Jegar, 1992). Rajapakse (2007) showed that conidia germination and appresoria formation of *Colletotrichum gloeosporioides* were higher on fruit exudates on brinjal varieties which are susceptible to anthracnose than in resistant varieties. Therefore, the present study was carried out to determine whether there is any relationship between different Sri Lankan tomato verities in terms of conidia differentiation by leaf exudates.

Materials and Methods

Pathogen isolation and identification

Anthracnose affected tomato (*Lycopersicum esculentum*) leaves were collected from the field of Horticultural Crops Research and Development Institute, Gannoruwa. The causative agent of anthracnose was

isolated and cultured on Potato Dextrose Agar PDA). Isolates of pathogen was purified using single conidia grown on PDA.The pathogen was identified on the basis of size and morphology of sproulating acervuli, conidia, setae, and morphology of mycelium on culture medium by microscopic observations (Rajapakse, 1999). Pathogenicity of all isolates was tested by the direct inoculation with conidial suspensions and subsequent anthracnose development on tomato leaves (Rajapakse, 1999).

Collection of exudates from tomato leaves

Mature leaves of tomato varieties i.e. Rashmi, Volcano, Lank Savar, Ravi, Thilina, Bathiya, Maheshi, Tharidhu, and T-245 and Rajitha were collected from green house grown healthy plants. Leaves were carefully detached from plants, washed in sterile distilled water and wiped with cotton wool soaked in 90% alcohol to reduce microbes on leaf surfaces. Five sterile distilled water (SDW) drops (20 µl) were separately placed using a micropipette on the surface of each leaf of all test varieties and kept for 18 h in moist chamber. After 18 h exudates were collected into separate bottles using a micropipette and stored at -20 °C for further analysis (Rajapakse, 2007).

Fractionate of exudates

Compounds in exudates were separated into ether soluble and insoluble fractions. Two milliliters of the leaf exudates of each variety was shaken with 6ml of diethyl ether in a separating funnel. After stabilization, the ether fraction was removed from the water fraction using a micropipette. This was repeated 3 times and the pooled ether fraction was evaporated in a fume cupboard. The resulting residue was redissolved in 2 ml of SDW and it was used as the ether fraction. The same procedure was repeated for all 10 varieties and resulting water and ether fractions were stored in separate bottles at – 20°C in a deep freezer.

Effect of ether soluble and water soluble fractions of exudates on conidia germination and appressoria formation

Conidia germination, appressoria formation and anthracnose development in leaf exudates of ten tomato varieties were determined .*Colletotrichum coccodes* isolated from ten tomato varieties were used for all inoculation and bioassay experiments. Conidia for all experiments were obtained from cultures on Potato Dextrose Agar incubated for 14 days at room temperature (28-30 °C). Conidia were harvested by adding 20 ml of SDW to the culture dishes, which were gently shaken. The suspension was transferred into pre-sterilized centrifuge tubes, and spun at 3000 rpm for 3 minutes. The supernatant was discarded and the conidia containing pellets were resuspended in fresh SDW. This process was repeated thrice. Density of the conidia in the final suspension was measured using a haemacytometer and adjusted to $5x10^5$ conidia/ml with SDW (Rajapakse, 2007).

Then the bio assay was conducted on glass slides. Suspension of conidia in SDW was mixed with equal volumes of the exudates or ether fraction or water fraction and twenty microlitre drops were placed on clean glass slides which were then placed on sealed plastic moist chambers and incubated for 18 hrs at 30°C.At each time of observation, a drop of lactophenol containing trypan blue was added and percentage germination and percentage germinated conidia with appressoria were assessed by microscopic observations. The experimental design for the observation of germination of conidia in leaf exudates was a 3x6 factor factorial experiment in a Completely Randomized Design with three replicates. Data were analyzed statistically using ANOVA and mean separation was carried out using Least Significant Differences, using the SAS statistical software.

To determine the disease severity of tomato varieties, twenty microlitre drops of conidial suspensions were drop inoculated on the tomato leaves to observe anthracnose lesion development. Leaves were transferred to humid plastic boxes lined with moisture paper pre-soaked in SDW. The order of appearance of disease symptoms in each variety and severity of the disease was measured according to a disease rating scale developed (Table 1).

Table 1. Rating scale for Disease Severity Index (DSI) on anthracnose lesions on leaves.

Rating scale	Description
0	No disease
1	yellow spots- Few
3	yellow brown spots
5	Brown-Few
7	Brown more
9	Dark brown

The experiment was arranged as a completely randomized design (CRD) with three replicates. The disease severity index in each variety was calculated using following formula. (Rajapakse, 1999)

$$DSI\ (\%) = \frac{\text{Total sum of numerical ratings}}{\text{Maximum disease ratings x No. of observations}} \times 100$$

Results and Discussion

The isolated fungal species was identified as *Colletotrichum coccodes* by comparison of its culture characters on PDA (Table 2). Isolates produced a grey color mycelium on culture media (PDA) at early stages and which turned light brown after about 14 days. Conidial size varied among isolates but generally ranged from 16-22 x 3-4 µm in length. Conidia from all the isolates showed a similar shape.

Table 2. Characters of the fungal isolate collected from anthracnose affected tomato leaves.

Characters of the Fungal isolate	Morpholog ical and culture characters of the isolate
Colony color on PDA	Grey initially then turned light brown, colony margin wavy
Reverse colony color on PDA	Brown or dark brown
Acervuli	Brown color masses, conidia present inside
Setae	Present
Conidia -shape	straight, fusiform, attenuated at the ends
Conidia- size	16-22 x 3-4 µm.
Appressoria in leaf exudates	clavate, brown, 11-16.5 x 6-9.5 µm

When isolates were tested for their ability to induce lesions in mature tomato leaves using the direct application of a conidial suspension, the tested isolate of *Colletotrichum coccodes* developed anthracnose lesions on tomato leaves. The disease was identified by brown circular depressions and the fungus appeared as brown acervuli on the surface of inoculated leaves, the isolate was virulent.

Results of germination assay (Tables 3 and 4) showed that there was a significant difference ($P<0.05$) in conidia germination and appressoria formation of *Colletotrichum coccodes* in exudates of ten tomato varieties. However, numbers of conidia that germinated and formed appressoria in leaf exudates were significantly higher in the varieties Tharidhu, Rashmi, Thilina and Lanka Savar compared to Volcano and T-245. Conidia differentiation was very low in SDW on glass slides. Similar observations were made by Turkey (1970) with *Colletotrichum coccodes* on banana, which was attributed to the presence of solutes leaching into the inoculum drop from the host cell. Rajapakse (2007) showed that *Colletotrichum gloeosporioides* conidia germination and formation of appressoria varied in fruit exudates in brinjal varieties and also found that the highest germination of conidia was present in variety Padagoda, which was also highly susceptible to the disease. Lesion development was assessed by disease severity index (Table 5) after 10 days of inoculation and it showed a significant ($P<0.05$) difference between leaves of different tomato varieties. Higher disease severity was observed in Tharidhu compared to the other varieties.

Table 3. Effect of exudates of leaves of tomato varieties on C. coccodes conidia germination after 18 hours incubation.

Percentage conidia germination in exudates on glass slides											
	Ravi	Rashmi	Lanka	Thilina	Rajitha	Volcano	Bathiya	Maheshi	T-245	Tharidhu	SDW
LX	40.17^{b}	63.54^{ba}	50.28^{bc}	48.28^{bc}	37.53^{cd}	18.27^{d}	35.01^{cd}	31.02^{cd}	22.03^{d}	72.77^{a}	32.51^{e}
WF	42.90^{bc}	52.37^{ab}	49.49^{bc}	47.87^{bc}	31.14^{bcd}	14.51^{e}	30.43^{ecd}	27.22^{ed}	23.56^{e}	59.95^{a}	
EF	6.43^{b}	10.85^{b}	10.39^{b}	10.01^{b}	6.43^{b}	4.22^{b}	5.79^{b}	5.29^{b}	5.18^{b}	22.29^{a}	

LX-Leaf exudate, WF-Water fraction of leaf exudates, EF-Ether fraction of leaf exudate
Values followed by the same letters are not significantly different, by DMRT at $P < 0.05$

Table 4. Effect of exudates of leaves of tomato varieties on formation of appressoria by conidia of C.coccodes after 18 hours incubation.

Percentage appressoria Formation on glass slides											
	Ravi	Rashmi	Lanka	Thilina	Rajitha	Volcano	Bathiya	Maheshi	T-245	Tharidhu	SDW
LX	37.15^{ba}	37.67^{a}	36.48^{ba}	32.70^{ba}	34.59^{ba}	2.67^{c}	32.97^{ba}	31.32^{ba}	11.19^{c}	40.8^{a}	22.23^{d}
WF	17.75^{ba}	23.03^{a}	21.0^{ba}	19.10^{ba}	15.44^{ba}	1.23^{c}	15.22^{ba}	14.93^{ba}	10.75^{c}	32.22^{a}	
EF	2.06^{b}	3.02^{b}	2.39^{b}	2.64^{b}	2.14^{b}	0.34^{b}	0.76^{b}	0.70^{a}	1.01^{b}	13.55^{a}	

LX-Leaf exudate, WF-Water fraction of leaf exudates, EF-Ether fraction of leaf exudate
Values followed by the same letters are not significantly different, by DMRT at $P<0.05$

Percentage conidia germination and appressoria formation in leaf exudates have significant correlation with disease severity index of different tomato varieties (r^2 = 0.93 and 0.87 respectively) (Fig.1 and 2). Therefore, stimulatory effects of exudates of different varieties on conidia differentiation could be used to compare tomato varieties in terms of resistance as an *in vitro* test.

Water fraction and the original exudates significantly stimulated conidial germination relative to the water control. But in ether fraction, the conidial germination was lower than the water controls. Most of the chemicals responsible for conidia germination and appressoria formation are extracted by water, but ether fraction containing chemicals had an inhibitory action on conidial germination of the pathogen.

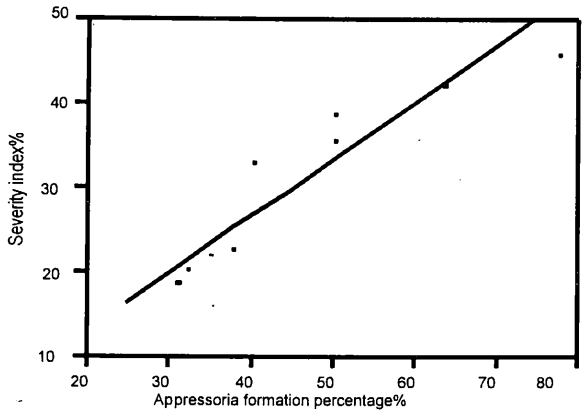

Figure 1. Relationship between appressoria percentage in leaf exudates and anthracnose disease severity index of tomato varieties.

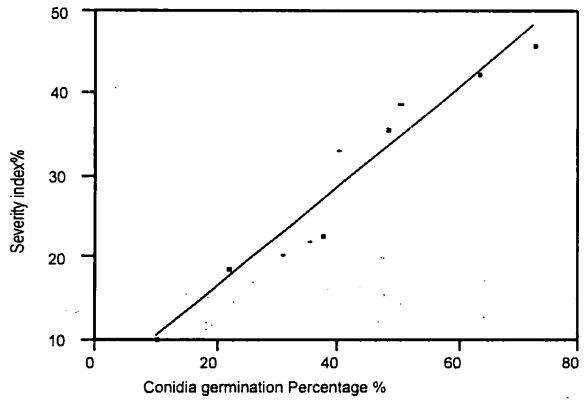

Figure 2. Relationship between appressoria percentage in leaf exudates and anthracnose disease severity index of tomato varieties.

Conclusion

The causal agent of tomato anthracnose was identified as *Colletotrichum coccodes*. Stimulatory compounds which are responsible for conidial differentiation into appressoria are present in leaf exudates of tomatoes. The highest stimulation for conidial differentiation was observed in leaf exudates of tomato variety Tharidhu which is highly susceptible to anthracnose disease. Rate of conidia differentiation by exudates could be used as an *in vitro* test to compare anthracnose susceptibility/resistance in tomato varieties.

Table 5. Disease severity of anthracnose on leaves of tomato varieties.

Variety	DSI
Ravi	33.34%
Rashmi	45.23%
Lanka Savar	38.95%
Thilina	35.78%
Rajitha	23.01%
Volcano	10.31%
Bathiya	22.23%
Maheshi	20.64%
T-245	19.04%
Tharidhu	46.35%

Referaneces

Anonymous 2003, Seasonal Reports, HoRDI

Bailey and Jegar .1992.Colletotrichum Biology, Pathology and Control. British Society of plant pathology.

Diehl, W.W.1953. Identifying a pathogenic Fungus in plant Diseases-The Year book of Agriculture

different brinjal varieties. Annals of Sri Lanka. Department of Agriculture.1:63-67

Rajapakse, R.G.A.S. 2007. Chemical factors stimulatory to anthracnose development in

Rajapakse, R.G.A.S.1999. Mode of anthracnose development in chilli (Capsicum annum L.) pods. Annals of Sri Lanka Department of Agriculture.1:247-266

Swinburne, T.R.1976. Stimulation of germination and appresoria formation by *Colletotrichum musae* (Berk. and Curt.) Arx.in banana leachete.Phytopathologische Zeitschrift.87:74-90

Turkey, H.B.1970. The leaching of substances from plants Annual. Review of plant Physiology.21: 305-324