ENHANCED RESISTANCE AGAINST ANTHRACNOSE DISEASE IN CHILI PEPPER (*CAPSICUM ANNUUM* L.) BY SOIL APPLICATION OF POTASSIUM

K. S. Somapala[1]
[1]*Department of Botany, Faculty of Natural Sciences*
The Open University of Sri Lanka
Nawala, Sri Lanka
koshala9@gmail.com

H. L. D. Weerahewa[2*]
[2]*Department of Botany*
The Open University of Sri Lanka
Nawala, Sri Lanka
weerahewa@gmail.com

S. Thrikawala[3]
[3]*Department of Agricultural and Plantation Engineering*
Faculty of Natural Sciences,The Open University of
Sri Lanka, Nawala, Sri Lanka
sthri@ou.ac.lk

ABSTRACT

Resistance to anthracnose disease was investigated in Capsicum annuum L. 'Hungarian Yellow Wax' by soil application of potassium in a pot experiment. Three different concentrations of muriate of potash as the source of potassium: 2.7 (control), 5.4 and 7.1 g per plant were used in the experiment. Resistance to anthracnose in the fruit of plants treated with different levels of potassium was assessed by challenged inoculation with Colletotrichum gloeosporioides or C. capsici. Significant reductions in lesion areas were observed in fruit from plants treated with higher levels of potassium compared to the control. In fact, 71% and 62% reductions in disease against C. gloeosporioides in fruits of plants treated at 7.1 and 5.4 g per plant potassium and 63% and 59% disease reduction against C. capsici. Similarly a delay in anthracnose symptom development was observed in fruits obtained from plants treated at higher levels of potassium. The mechanisms underlying the potassium induced resistance was investigated by measuring cell wall thickness of the fruit exocarp, analysing total soluble phenolic (TSP) compounds. cell wall-bound phenolic (CWBP) compounds and by measuring the degree of formation of fungal appressoria on fruits. A significant increase in the thickness of the cell wall was observed of the fruits treated at higher levels of potassium than that of the control. The TSP content was found to increase significantly in the fruits treated at 7.1 g potassium inoculated with C. gloeosporioides. However CWBP was not significantly affected by increased level of potassium in either fruits inoculated with C. gloeosporioides or C. capsici. Although the percentage in appressoria formation was higher in C. gloeosporioides their germination and disease initiation was delayed in fruits harvested from 7.1 g potassium treatment. However, the delaying of appressoria germination was not observed in C. capsici. The germination of appressoria might have delayed by increased cell wall thickness of the fruits and/or biochemical resistance due to incresed levels of TSP.

Key words: : Capsicum, *Colletotrichum*, Potassium

Introduction

The genus *Capsicum* is one of the most important commercial grown spices and vegetable crop. It is a rich source of vitamin A and vitamin C among the vegetables. (Majumdar, 1994). The fruits with seeds are used commonly in spices and food due to their pungent flavor and has been known to possess several medicinal properties like anti-inflammatory, rubefacientm, carminative, analgesic (Sim and Sil, 2008). In addition, capsicum acquires antioxidant, hypoglycemic (Monsereenusorn, 1980), antifungal and antimicrobial (De Lucca et al,2006) activities.

Capsicum is susceptible for considerable postharvest loss due to the disease anthracnose which is caused by *Colletotrichum* species (Pereira et al., 2011; Park et al., 2012). In Sri Lanka, the post harvest loss of capsicum cultivation due to anthracnose disease is estimated around 21-47% (Rajapakse, 1999). The disease is caused by mainly five *Colletotrichum* species: *C. acutatum*, *C. capsici*, *C. gloeosporioides*, *C. nigrum (*Don et al., 2007) and *C. coccodes* (Johnston and Jones, 1997) in the world while *C. gloeosporioides and C. capsici* (Rajapakse and Ranasighe, 2002) are the commonly found species in Sri Lanka.

Various strategies are being practiced in controlling Capsicum anthracnose including chemical fungicide application. Fungicides have negative impact on the environment and human health and thus, alternative methods for disease management have an increasing concern. Numerous promising results have been achieved by application of potassium (K) for controlling several

fungal diseases in number of crops. In our previous studies it was revealed that potassium suppressed the anthracnose disease in capsicum (Somapala *et al*., 2015). Application of extra dose of potassium was effective in reducing the disease in tomato cv. 'Thilina' and 'Maheshi' (Weerahewa and David, 2015) and in banana cv.'Embul' (Weerakkon et al., 2005). However, the mechanism underlying in disease resistance mediated by potassium in plants is not yet fully understood. Therefore, the current research was conducted to investigate the effect of K on anthracnose disease in chili cv. ' Hungarian Yellow Wax' caused by either *C. gloeosporioides and C. capsici* and the possible mechanisms involved in disease resistance.

Research design

Plant material and nursery management

Capsicum cv. 'Hungarian Yellow Wax' seeds (Polo-seed international International Ltd, Thailand) were sawn in a 1:1 mixture of well decomposed farmyard manure and top soil and maintained according to the DOA recommendation until transplantation.

Treatments and experimental design

Healthy six weeks old seedlings were transplanted in pots of 0.004 m^3 containing top soil. The basal fertilizer application comprising 3.5 g of urea, 7.8 g of triple super phosphate (TSP) and 2.3g of murate of potash (MOP) was provided to each pot four days prior to transplanting. For the first and the second top dressing three potassium treatments were applied in fact, 2.3 g-control (K1), 4.6 g (K2) and 6.9 g (K3) along with 3.5 g of urea (Table 01). The treatments were arranged in a complete randomized design (CRD) with four replicates.

Table 1: Levels and time duration of application of urea, triple super phosphate and murate of potash for a chili pepper planted in 0.004 m^3 pots.

Treatments	Urea g/per plant	TSP g/per plant	MOP g/per plant
Basal	3.5	7.8	2.3
1st top dressing (4 weeks after transplanting)	3.5	-	2.3
	3.5	-	4.6
	3.5	-	6.9
2nd top dressing (6 weeks after transplanting)	3.5	-	2.3
	3.5	-	4.6
	3.5	-	6.9

Isolation and identification of *C. gloeosporioides and C. capsici*

Colletotrichum species from anthracnose lesions of diseased capsicum fruit were surface sterilized with 1% (v/v) NaOCl for 1 min followed by rinsing with sterile distilled water and cultured on potato dextrose agar (PDA). Ten culture plates were incubated at 27 – 30°C (Narayanasamy, 2010) and observed for mycelial growth, the morphology of the culture, and the shape of the conidia using a compound microscope (Daffodil MCX100, Vienna, Australia). *C. gloeosporioides* was identified by its orange cotton-like mycelium (Sutton, 1980) and ovoid-shape conidia (Du *et al*., 2005). *C. capsici* was identified by its sickle-shaped, aseptate conidia, the presence of prominent setae (Sutton, 1992), and its brown colour colony with concentric markings. (Rajapakse and Ranasighe, 2002). Koch's postulates were performed by inoculating the two pathogens to mature capsicum fruits with. The pathogen was re-isolated from the infected fruits on PDA. Seven to ten day old pure cultures of *C. gloeosporioides* and *C. capsici* were used for all inoculation experiments.

Assessing the degree of anthracnose disease development on capsicum fruits inoculated with either *C. gloeosporioides* or *C. capsici*

Conidial suspensions (10^5 conidia ml^{-1}) of *C. gloeosporioides* and *C. capsici* were prepared by scraping the mycelium from 7 d old pure cultures and suspending them in sterilised distilled water followed by filtering through glass wool. Fifteen capsicum fruits from each treatment were washed in 70% ethanol followed by dipping in 0.1% sodium hypochlorite solution and rinsed with SDW (Etebarian et al. 2005). Each fruit was challenge-inoculated by placing drops of 20µl conidial suspension at three different points on fruit surface. Inoculated fruits were incubated in moist chambers with 95-100% relative humidity at 28±3 °C. The number of days taken for disease initiation after inoculation and the lesion area was recorded daily for 10 days after inoculation (DAI) and the mean lesion area per treatment was calculated.

Measuring the cell wall thickness of capsicum fruits

The cell wall thickness of the fruits was measured in 0.1 mm thickness transverse sections. Twenty fruits from each potassium treated plant were used for obtaining measurements. Three cross-sections of each fresh fruit were mounted on a glass slide and the cell wall thickness was measured using a calibrated ocular micrometer at a magnification of 400x using a compound microscope (Daffodil MCX100, Vienna, Australia).

Evaluating the extent of formation of appressoria at inoculated zones

A thin layer thick fruit peels were taken from the *C. gloeosporioides* or *C. capsici* inoculated areas and were observed under the light microscope after 2, 3, 4, 5 and 6 DAI. The total number of spores and the number of appressoria formed spores were counted in five fields of vision under 400x using the light microscope. The percentage of appressoria formed spores per vision was calculated and averaged per treatment. The procedure was repeated four times per treatment for either organism.

Measuring the concentrations of cell wall bound phenolic compounds (CWBPC) and total soluble phenolic compounds (TSPC)

Fifteen fruits from each treatment were inoculated with *C. capsici* and samples of fruit peals were obtained from each inoculated spots at 2, 3, 4 and 5 d after inoculation. Twelve replicate measurements were carried out each day for each treatment. Fruit tissues were extracted separately with 80% (v/v) methanol and levels of TSPC in each fruit extract was determined using Folin-Ciocalteu reagent with ferulic acid as a standard (Ascensao and Dubery, 2003).

Each residue remained after the methanol extraction above was dried at 70°C for 24 h. The dried residue was suspended in 0.5 M NaOH (1 ml/10 mg) for 1 h at 96°C. The pH of the supernatant was adjusted to pH 2 using HCl, and centrifuged at 10,625 x g for 10 min. The mixture was then extracted with 99% (v/v) diethyl ether and was evaporated to dryness, and suspended in 10 ml of 80% (v/v) methanol. Contents of CWBPC were determined using Folin-Ciocalteu reagent (Ascensao and Dubery, 2003).

Statistical analysis

The collected data were analysed using one way ANOVA in SPSS 16.0 statistical package for determining whether there are any significant differences among the treatments at $P \leq 0.05$. The means were compared using the Duncan's multiple range test (DMRT)

Results and discussion

Assessing the degree of anthracnose disease development on capsicum fruits inoculated with either *C. gloeosporioides* or *C. capsici*

The lesion area observed at 10 days after inoculation on the fruits inoculated with *C. gloeosporioides* were 110, 41.8 and 31.9 mm^2 in K1, K2 and K3 treatments whereas that was 121.4, 49.3 and 45.1 mm^2 in *C. capsici inoculated fruits.* In both the fruits inoculated with either *Colletotrichum* species, the disease reduction was significant in the fruits treated at higher doses of potassium. In fact, *C. gloeosporioides* inoculated, K3 treated fruits showed 71% lesion development reduction compared to the control while in *C. capsici* inoculated fruits it was 63%. In K2 treated fruits, the disease reduction was 62% and 59% in fruits inoculated with *C. gloeosporioides* or *C. capsici* respectively. Total lesion area development for 10 days after inoculation was found to be grater in the fruits inoculated with C. capsici than that of C. *gloeosporioides* assuming that the K treated fruits are more vulnerable for *C. capsici* than C. *gloeosporioides.* Further, it was observed two or three days delaying of disease initiation in either K2 or K3 treated fruits than that of the control, inoculated with either of two *Colletotrichum species (Table 2).*

Cell wall thickness of the fruits

Fruit cell wall thickness was found to increase significantly with increased level of potassium (Table 2). I. e. 15.78% and 10.53% increase in cell wall thickness than that of the control.

Table 2: Cell wall thickness of the fruits,and anthracnose disease development on fruits inoculated with *C. gloeosporioides* or *C. capsici*

Treatment g MOP/ plant	Fruit cell wall thickness (µm)	Anthracnose disease development			
		C. gloeosporioides		*C. capsici*	
		Total lesion area (mm^2)	Day of disease initiation	Total lesion area (mm^2)	Day of disease initiation
2.3 (control)	0.19 ^{c}	110 ^{a}	2 ^{c}	121.4 ^{a}	3 ^{b}
4.6	0.21 ^{b} (10.53%)	41.8^{b} (62%)	3 ^{b}	49.3 ^{b} (59%)	3 ^{b}
6.9	0.22 ^{a}(15.78%)	31.9^{c} (71%)	6 ^{a}	45.1 ^{b} (63%)	6 ^{a}

Means in each column followed by different letters are significantly different at $P \leq 0.05$ according to the DMRT.

Total soluble phenolic compound (TSPC) and Cell wall bound phenolic compound (CWBPC) concentrations

The cell wall bound phenolic content of capsicum fruits obtained from either of potassium treated plants inoculated with either of the *Colletotrichum* species were fluctuated between 110 – 175 mg/ g during the period of testing. In general there was no significant effect of K treatment on CWBPC of chili pepper fruit inoculated with both the species. However, the TSPC was significantly greater in fruits (inoculated with *C. gloeosporioides*) from K-treated plants than that of the control at 4 and 5 DAI while such increase was not observed in the fruits inoculated with *C. capsici* (Table 3). *Colletotrichum* is a pathogen which directly penetrates the host at infection. Its penetration and further infection procedure can be hindered by induced or preformed chemical inhibitors in plant cells (Pruskey, 1998). In cell walls, phenolic polymers act as obstructions rendering cell walls highly resistant to mechanical and enzymatic disruption by pathogens (Kolattukudy, 1987). In grape, potassium fertilization has significantly influenced (at $p < 0.05$) the content of total polyphenols (Delgado, et al., 2004)

Table 3: Cell wall-bound and total phenolic compound concentrations in fruit from Ki-treated chili pepper plants inoculated with two *Colletotrichum* species.

DAI	Phenolic compound concentrations in capsicum fruits inoculated with *C. gloeosporioides* (mg/ g)					
	CWBP			TSP		
	K1	K2	K3	K1	K2	K3
2	123.1^{ab}	131.0^{a}	112.3^{b}	333.4^{a}	339.3^{a}	311.4^{a}
3	125.4^{a}	130.7^{a}	124.4^{a}	512.5^{a}	493.9^{a}	479.4^{a}
4	117.8^{b}	169.5^{a}	125.3^{b}	497.1^{b}	537.4^{a}	554.1^{a}
5	141.9^{a}	156.0^{a}	152.7^{a}	522.5^{b}	531.3^{b}	563.3^{a}
DAI	Phenolic compound concentrations in fruits inoculated with *C. capsici* (mg/ g)					
	CWBP			TSP		
	K1	K2	K3	K1	K2	K3
2	93.5^{b}	111.0^{a}	112.3^{b}	142.3^{b}	175.6^{a}	155.4^{b}
3	154.2^{a}	142.3^{a}	155.3^{a}	98.35^{b}	184.6^{a}	172.3^{a}
4	123.4^{b}	95.64^{b}	174.3^{a}	165.4^{a}	155.64^{a}	163.5^{a}
5	156.4^{a}	132.4^{b}	154.3^{a}	155.6^{a}	174.5^{a}	162.7^{a}

Mean values (n = 15) followed by the same lower-case letter for each category of phenolic compounds at each DAI are not significantly different at $P \leq 0.05$ as determined by one way ANOVA.

Changes in appressoria formation at inoculated zones

A significance difference was observed in the percentage of appressoria formation among the treatments in both the pathogens. The day of disease initiation and the percentage of appressoria formation was not found to be related. Though the disease initiation of the fruits inoculated with either of the *Colletotrichum* spp. found to be on 6 DAI, a larger proportion of increase of the appressoria formation could be observed from DAI in both the species. On the 5DAI the percentage of appressoria formation of *C. gloeosporioides* was 4.11% greater than *C. capsici.* While, an increase of appressoria formation was present in both the species, the germination was hindered on the fruits treated with 7.1 g K. similarly, an increase of the percentage appressoria formation was observed on higher dose of potassium treated tomato fruits inoculated with *C. gloeosporioides* (Weerahewa and David, 2015). However, the delaying of appressoria germination was not observed in *C. capsici.*

Table 3: Degree of formation of appressoria on capsicum fruits inoculated with *Colletotrichum gloeosporioides* and *C. capsici*

Colletotrichum spp.	Treatment (g KCl/ plant)	% Appressoria formation			
		2DAI	3DAI	4DAI	5DAI
C. gloeosporioides	2.3	0 ^{c}	3.21 ^{c*}	10.45 ^{b}	15.42 ^{c}
	4.6	1^{b}	4.41 ^{b*}	12.72 ^{a}	16.32 ^{c}
	6.9	2^{a}	5.33 ^{a}	12.81 ^{a}	23.34 ^{a}
C. capsici	2.3	1 ^{b*}	1.34 ^{a}	9.13 ^{a}	**
	4.6	0 ^{c}	3.51 ^{a}	10.98 ^{b*}	**
	6.9	1.2 ^{b}	3.75 ^{a}	11.23 ^{b}	19.23 ^{b}

Means of each column followed by different letters are significantly different at $P \leq 0.05$ according to the DMRT. (n = 20).
*The day of disease initiation
**It was difficult to obtain fruit peal samples due to lesion sporulation

Prevalence of a higher percentage of appressoria without germination in fruits from K3 treated fruits might be due to a barrier present in the fruits such as increased cell wall thickness.

Conclusion

It can be suggested that the inhibition of fungal appressoria germination and the reduction of disease development by increased level of potassium treatments might be due to formation of a physical barrier due to increased fruit cell wall thickness and/ or induced biochemical reactions with increased levels of TSP.

Acknowledgement

The Financial Assistance given by the National Science Foundation, Sri Lanka is gratefully acknowledged

References

Bailey, J. A., O'Connell, R. J., Pring, R. J. & Nash, C. (1992). Infection strategies of *Colletotrichum* species. In: *Colletotrichum*: Biology, Pathology and Control. (J. A. Bailey & M. J. Jeger, eds.) CAB Int., Wallingford, UK. 88-120.

Barksdale, T. H. (1972). Resistance in tomato to six anthracnose fungi. *Phytopathology*, 62(6), 660-663.

Byrne, J. M., Hausbeck, M. K. & Hammerschmidt, R. (1997). Conidial germination and appressorium formation of *Colletotrichum coccodes* on tomato foliage. *Plant Disease*, 81(7), 715-718.

Cano, J., Guarro, J. &Gené, J. (2004). Molecular and morphological identification of *Colletotrichum* species of clinical interest. *Journal of clinical microbiology*, 42(6), 2450-2454.

De Lucca AJ, Boue S, Palmgren MS, Masko K, Cleveland TE, Fungicidal properties of two saponins from *Capsicum frutescens* and the relationship of structure and fungicidal activity, Canadian Journal of Microbiology, April 1, (2006).

Du, M., Schardl, C. L., Nuckles, E. M. &Vaillancourt, L. J. (2005). Using mating-type gene sequences for improved phylogenetic resolution of *Colletotrichum* species complexes. *Mycologia*, 97, 641-658.

Etebarian, H. R., Sholberg, P. L., Eastwell, K. C. &Sayler, R. J. (2005). Biological control of apple blue mold with *Pseudomonas fluorescens*. *Canadian Journal of Microbiology*, 51, 591–598.

Freeman, S., Katan, T. &Shabi, E. (1998). Characterization of *Colletotrichum* species responsible for anthracnose diseases of various fruits. *Plant disease*, 82(6), 596-605.

Fuchs, W. H. & Grossmann, F. (1972) Nutrition and resistance of crop plants against pathogens and pests. In: Linser H (ed) Handbuch der pflanzenerna¨hrung und du¨ngung, Vol 1, Part 2. Springer-Verlag, Vienna, pp 1008–1107

International Plant Nutrition Institute. (2010, winter). The role of potassium in reducing the incidence of crop diseases.Retrievedfromhttp://www.ipni.net/ipniweb/pnt.nsf/5a4b8be72a35cd46852568d9001a18da/bc94c4a2c66bdca6852577ec0072616c!OpenDocument

Karunanayake, K. O. L. (2008). Natural defense mechanisms in mango fruit and their potential in management of postharvest diseases (Doctoral dissertation), University of Peradeniya, Sri Lanka, 240.

Martínez, E. P., Hío, J. C., Osorio, J. A. & Torres, M. F. (2009). Identification of *Colletotrichum* species causing anthracnose on Tahiti lime, tree tomato and mango. *AgronomíaColombiana*, 27(2), 211-218.

Monsereenusorn, Y Effect of *Capsicum annuum* L. on Blood Glucose Level. Pharmaceutical Biology, 1(18), pp: 1-7(1980).

Narayanasamy, P. (2010). *Microbial Plant Pathogens-Detection and Disease Diagnosis: Viral and Viroid Pathogens* (Vol. 3). Springer Science & Business Media.

Sim HK, Sil YH. Antioxidant activities of red pepper pericarp and seed extracts. International journal of food Science and Technology, 43. pp: 1813-1823(2008).

Smith, B. J. & Black L. L. (1990). Morphological, cultural, and pathogenic variation among *Colletotrichum* species from strawberry. *Plant Disease*, 74, 69-76.

Sutton, B. C. (1980). The coleomycetes. Fungi Imperfecti withPycnidia, Acervula and Stromata.Commonwealth Mycological Institute. Kew, Surrey, UK. 696 pp.

Sutton, B. C. (1992). The genus *Glomerella* and its anamorph*Colletotrichum*. In: J. A. Bailey and M. J. Jeger (Eds.), *Colletotrichum*: *Biology, pathology and control*.(pp. 1-26) CAB International, Wallingford, Oxon, UK.

Weerahewa, D. and David, D. (2015), Effect of silicon and potassium on tomato anthracnose and on the postharvest quality of tomato fruit (*Lycopersicon esculentum* Mill.). *J.Natn. Sci. Foundation Sri Lanka 2015 43 (3):271-278*.

Weerakoon, W. R. W. M. A. U., Abayasekara, C. L. and Adikaram, N. K. B. (2005). The effect of soil potassium on postharvest fungal diseases of banana. Proceedings and abstracts of University of Peradeniya Research sessions, 10, 94.

Weiss EA, Spice crops: Technology and Engineering, Published by CAB International Wallingford Oxon OX108DE, U.K. pp: 411. ISBN: 0-85199-605-1.