

# First International Symposium on Chili Anthracnose



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Seoul National University, Seoul, Korea

# First International Symposium on Chili Anthracnose

국제고추탄저병심포지엄

September 17-19, 2007



Hoam Faculty House, Seoul National University, Seoul, Korea

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# **First International Symposium on Chili Anthracnose**

Hoam Faculty House, Seoul National University, Korea, September 17–19, 2007

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- National Horticultural Research Institute, Rural Development Administration, Korea
- National Institute of Agricultural Science and Technology, Rural Development Administration, Korea
- Seoul National University, Korea
- AVRDC–The World Vegetable Center, Taiwan

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## WELCOME MESSAGE

Il-Gin Mok, Ph.D.

Director General

National Horticultural Research Institute

Distinguished guests, galaxy of scientists from Korea and abroad, students, members of media, ladies and gentlemen. It is my proud privilege to extend heartiest welcome to you to this opening session of the First International Symposium on "Chili Anthracnose".

Chili pepper has ranked first among vegetables in terms of both planted area and production value in Korea. It ranks second in value among all crops only after rice. The most devastating diseases of chili pepper include anthracnose and Phytophthora blight. Phytophthora could be managed with resistant varieties and grafting. Recently Korean seed companies released varieties resistant to Phytophthora and they are performing quite well in the farmer's field. However, anthracnose resistant variety is not available yet, and the augment of loss due to this disease could be substantial, especially under long rainy environment like this year. Neither grafting nor spraying could solve the problem.

This disease problem is not only restricted to the Korean farmers. On a worldwide basis, anthracnose is the single greatest economic constraint to chili and sweet pepper production in tropics and subtropics.

I understand that is the reason why we chose to face this subject here in Seoul. In the presence of the most qualified researches in the world, I am certain that the works of this meeting will greatly contribute to broaden our horizons and bring wisdom in developing anthracnose resistant varieties and improving management strategies.

I therefore renew my welcome, particularly to those who have had to travel far to join us, and wish you all a pleasant and fruitful stay. Lastly, I would like to recognize the organizers for their hard work to make this symposium such a successful one.



## CONGRATULATORY REMARKS

Sok-Dong Kim, Ph.D.

Director General

National Institute of Agricultural Science and Technology

I am very pleased and honored to offer my congratulations to the organizers of the first International Symposium on Chili Anthracnose. I would like to thank all participants for their keen interest and enormous efforts in helping us make this symposium possible in Seoul, especially more than 30 distinguished scientists from abroad.

Anthracnose is one of the most serious diseases of chili pepper in Korea as well as other countries. Pathogens associated with occurrence of the disease have been reported to be several species of a genus or some strains of a species. It has been known that the disease is very difficult to control in the field because of building up of resistance in the pathogen to some fungicides. In addition, none of the cultivating varieties shows satisfied level of resistance in the farmer's field.

I found from the program that the participants have diverse background and expertise in the area of chili anthracnose research. I hope that the presentations and discussions at this symposium will lead to a better understanding of chili anthracnose, and this symposium will provide the opportunity to exchange new scientific findings on pathogens and control of the disease.

I wish that each participant strengthens personal friendship and coalition among the participants, and all the distinguished scientists from abroad have a pleasant stay in Korea.



# First International Symposium on Chili Anthracnose

17-19 September 2007

Hoam Faculty House, Seoul National University, Seoul, Korea

## PROGRAM

### DAY 1, Monday, September 17

Registration and Opening Session

Master of Ceremony: Oh, Dae-Geun

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09:00–09:40 Registration

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09:40–10:00 Opening ceremony

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Opening remarks by the Chair of the Symposium Executive Committee  
and Head of AVRDC–The World Vegetable Center Pepper Breeding *Gniffke, Paul*

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Welcome address by the Director General of the National Horticulture  
Research Institute *Mok, Il-Gin*

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Congratulatory remarks by the Director General of the National Institute  
of Agricultural Science and Technology *Kim, Sok-Dong*

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10:00–10:20 Coffee break

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10:20–10:30 Introduction / Housekeeping *Oh, Dae-Geun*

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10:30–11:10 Introduction of speaker *Black, Lowell*

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Preamble: Problems of anthracnose in pepper and prospects for its  
management *Park, Hyo Guen*

---

11:10–11:50 Introduction of speaker *Han, Seong-Sook*

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Keynote address: Overview of the phylogenetics species concept in  
*Colletotrichum* as it relates to chili anthracnose *Correll, James*

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11:50–12:10 Introduction of speaker *Oh, Dae-Geun*

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Background information: Chili anthracnose research in AVRDC–The  
World Vegetable Center, 1993–2002 *Black, Lowell*

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12:10–13:20 Lunch break

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## DAY 1, Monday, September 17

### Country Reports

Chairs: Gniffke, Paul / Park, Hyo Guen

13:20–13:40	Country report: Korea	<i>Kim, Byung–Soo</i>
13:40–14:00	Country report: Thailand	<i>Poonpolgul, Srisuk</i>
14:00–14:20	Country report: Vietnam	<i>Le, Dinh Don</i>
14:20–14:40	Country report: India	<i>Ramachandran, N.</i>
14:40–15:00	Country report: China	<i>Zhang, Deyong</i>
15:00–15:20	Country report: Indonesia	<i>Widodo, Ir.</i>
15:20–15:40	Country report: Brazil	<i>Henz, Gilmar</i>
15:40–16:00	Coffee break	

### Pathogen Species Identification and Diagnosis

Chairs: Correll, James / Kim, Wan Gyu

16:00–16:20	Pathotypes of <i>Colletotrichum</i> spp. infecting chili peppers and mechanisms of resistance	<i>Taylor, Paul</i>
16:20–16:40	Characterization of <i>Colletotrichum</i> spp. associated with pepper anthracnose in China, India, Indonesia, Taiwan, and Thailand	<i>Wang, Tien–chen</i>
16:40–17:00	Taxonomic characteristics of <i>Colletotrichum</i> spp. and their teleomorphs causing anthracnose of chili pepper in Korea	<i>Hong, Sung Kee</i>
17:00–17:20	Application of ITS–RFLP analysis for identifying <i>Colletotrichum</i> spp. associated with pepper anthracnose in Taiwan	<i>Sheu, Zong–ming</i>
17:20–17:40	<i>Colletotrichum acutatum</i> associated with pepper anthracnose in Thailand ( <i>poster</i> )	<i>Poonpolgul, Srisuk</i>
17:20–17:40	The occurrence of chili anthracnose in Taiwan and its control ( <i>poster</i> )	<i>Sheu, Zong–ming</i>
17:20–17:40	Variability among <i>Colletotrichum capsici</i> causing chilli anthracnose in North Eastern India ( <i>poster</i> )	<i>Selvakumar, R.</i>
17:40–18:00	Discussion on international collaboration	<i>Black, Lowell</i> <i>Gniffke, Paul</i> <i>Oh, Dae–Geun</i>

## DAY 1, Monday, September 17

### Entertainment and Dinner

18:30–19:15 Korean folk music (percussions)

*Gongsaemi*

19:15– 20:30 Dinner

## DAY 2, Tuesday, September 18

### Pathology

Chairs: Kim, Young Ho / Wang, Tien-chen

08:30–08:50 *Colletotrichum acutatum* associated with anthracnose disease on chilli  
(*Capsicum* spp.) in Thailand

*Than, Po Po*

08:50–09:10 Occurrence of pepper anthracnose and the morphological  
characterization of its pathogen *Colletotrichum* spp. in Guangxi, China

*Liao, Yong-Mei*

09:10–09:30 Morphological and molecular diversity and distribution of hot pepper  
anthracnose causing *Colletotrichum* spp. isolates of Southern India

*Rao, Mohan*

09:30–09:50 The development of fungicide application system for the control of  
pepper anthracnose

*Kim, Heung Tae*

09:50–10:10 Development of forecasting system for anthracnose in pepper

*Yun, Sung-Chul*

10:10–10:30 Effect of different media, pH, and temperature on growth and  
sporulation of *Colletotrichum capsici* (*poster*)

*Garg, Ruchi*

10:30–10:50 Coffee break

## DAY 2, Tuesday, September 18

### Resistance Sources and Mechanisms

Chairs: Park, Kyung Seok / Taylor, Paul

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10:50–11:10	Evaluation of diverse chili pepper sources for resistance to anthracnose	<i>Gniffke, Paul</i>
11:10–11:30	Resistant sources of chili ( <i>Capsicum annuum</i> L.) anthracnose fruit rot disease [ <i>Colletotrichum capsici</i> (Syd.) Butler and Bisby] against different isolates collected from commercial chili growing areas of India	<i>Deshpande, Arvind</i>
11:30–11:50	Structural resistance and defense mechanisms of chili pepper against pepper anthracnose disease	<i>Kim, Young Ho</i>
11:50–12:10	Diagrammatic scale to evaluate anthracnose severity on sweet pepper fruits for disease progress studies	<i>Henz, Gilmar</i>
12:10–12:30	An early stage differentiation of pepper anthracnose pathogen, <i>Colletotrichum acutatum</i> JC24, on the fruit with or without wound (poster)	<i>Cho, In Joon</i>
12:30–13:40	Lunch break	

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### Breeding and Inheritance of Resistance

Chairs: Gniffke, Paul / Kim, Byung-Soo

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13:40–14:00	Backcross introgression lines between <i>Capsicum annuum</i> and <i>C. baccatum</i> for breeding chili pepper resistant to anthracnose caused by <i>Colletotrichum</i> spp.	<i>Yoon, Jae Bok</i>
14:00–14:20	Study on the characteristics of interspecific hybrids and their progenies for the breeding of anthracnose-resistant pepper	<i>Chae, Young</i>
14:20–14:40	Inheritance of resistance to anthracnose caused by isolates of <i>Colletotrichum acutatum</i> and <i>C. capsici</i> in <i>Capsicum</i>	<i>Kim, Sang Hoon</i>
14:40–15:00	Development of anthracnose-resistant chili pepper varieties at AVRDC–The World Vegetable Center	<i>Gniffke, Paul</i>
15:00–15:20	Breeding for anthracnose resistance in hot pepper ( <i>Capsicum annuum</i> ) (poster)	<i>Kim, Yong Kwon</i>
15:00–15:20	Evaluation of horticultural characteristics of anthracnose-resistant breeding lines in pepper (poster)	<i>Pae, Do-Ham</i>
15:20–15:50	Coffee break	

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## DAY 2, Tuesday, September 18

### Molecular Marker and Biotechnology

Chairs: Kang, Byoung-Cheorl / de la Peña, Robert

15:50–16:10	Construction of linkage map and mapping QTLs for anthracnose resistance in <i>Capsicum baccatum</i> population	<i>Kim, Ki-Taek</i>
16:10–16:30	Introgressed QTL mapping and developing molecular markers associated with anthracnose resistance in <i>Capsicum</i>	<i>Yoon, Jae Bok</i>
16:30–16:50	Transgenic peppers transformed with anthracnose resistance related genes	<i>Harn, Chee Hark</i>
16:50–17:10	Development and optimization of a microsatellite marker library for <i>Colletotrichum capsici</i> , a major causal agent of anthracnose disease in chilli pepper	<i>Ranathunge, Nalika</i>
17:10–17:30	Identification of hot pepper mapping population putative parents for molecular tagging of genes conferring resistance to anthracnose caused by <i>Colletotrichum</i> spp. isolates of Southern India	<i>Chinnaswamy, Nanda</i>
17:30–17:50	Expression profiling of genes involved in preinfection-related development stages of pepper anthracnose fungus, <i>Colletotrichum acutatum</i> (poster)	<i>Kim, Yoonhee</i>
17:30–17:50	Characterization of an ABC transporter gene, CaABC1, from the pepper anthracnose fungus, <i>Colletotrichum acutatum</i> (poster)	<i>Kim, Hyejeong</i>
17:50–18:30	Discussion	<i>Black, Lowell</i> <i>Gniffke, Paul</i> <i>Oh, Dae-Geun</i>
18:30	Dinner	

## DAY 3, Wednesday, September 19

### Day Tour

9:00	Departure from Hoam Guest House
13:30	Arrival at chili powder processing factory
15:00	Arrival at farmer's field
20:30	Expected arrival at Hoam Guest House

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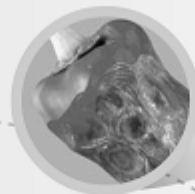
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**Symposium topics:**

Disease losses, mycology and pathology,  
breeding and molecular advances,  
and country reports on chili anthracnose

# First International Symposium on Chili Anthracnose



# Problems of Anthracnose in Pepper and Prospects for its Management

Hyo Guen Park

Pepper & Breeding Institute, Business Incubator, College of Agriculture and  
Life Science, Seoul National University, Suwon 441-853, Korea

The anthracnose has been well recognized as one of the most devastating diseases wherever peppers (*Capsicum* spp.) are grown under hot and rainy season. But it is somewhat strange that this disease had not received the deserved attention academically domestically as well as internationally until AVRDC started the well-organized approaches on this disease in the middle of '80's and NHRI and SNU in Korea started their breeding program in the middle of '90's. All of us who attending this symposium should agree that there is not a single resistant pepper variety available commercially in any where in the world.

It is very appropriate and timely to have the 1<sup>st</sup> International Symposium on Pepper Anthracnose in Korea, since this country has produced about annually \$1.5 billion worth of *Capsicum annuum*, both green and mature, definitely being the most important vegetable, and this crop has been seriously damaged by the anthracnose with about \$100 million annually for many years. The main objectives of this symposium, I think, are to find out why we could not develop resistant varieties to anthracnose and to set up strategies how we will work together to solve this problem caused by anthracnose. In Orient, we have an old proverb, which says that we will win every war out of hundreds if you know well your enemy and yourself. In this case, since we have a war against anthracnose, we should know about our enemy, which is anthracnose, as much as possible, and also we should know the resistant mechanism of host, pepper. It is getting very clear that our goal, minimizing the damage of anthracnose, can not be achieved without very close international co-works. Through this symposium, I hope we will be able to organize a good international working group consisted of pathologists, molecular biologists and breeders.

## Overview of the Phylogenetics Species Concept in *Colletotrichum* As It Relates to Chili Anthracnose

James C. Correll<sup>1</sup>, K. Cornelius<sup>1</sup>, Chunda Feng<sup>1</sup>, Sarah B. Ware<sup>1</sup>,  
Brad Gabor<sup>2</sup>, and Tyler L. Harp<sup>3</sup>.

<sup>1</sup>Department of Plant Pathology, University of Arkansas, AR, USA,

<sup>2</sup>Seminis, Woodland, CA, USA, <sup>3</sup>Syngenta, Vero Beach, FL, USA.

*Colletotrichum* is an important pathogen that causes anthracnose diseases on many diverse plant hosts. The current systematic arrangement within *Colletotrichum* remains problematic for many reasons. The morphological criteria often overlap and a number of "species" actually represent species "complexes" composed of genetically diverse but morphologically similar isolates. Many molecular studies have helped to resolve relationships within the genus *Colletotrichum*. Much of the initial molecular work on *Colletotrichum* was based on the ITS region; although ITS sequence data helped resolve some taxonomic boundaries within *Colletotrichum*, the use of ITS sequence data for phylogenetic analysis can be problematic. A more current and robust approach involves a phylogenetic species concept, whereby species boundaries are delineated based on sequence analysis of multiple unlinked loci (Taylor et al, 2000). Therefore, a more contemporary approach to infer the phylogenetic relationships among *Colletotrichum* species is the use of multiple nuclear loci (Crouch et al, 2006; Du et al, 2005; Guerber et al, 2003; Liu et al, 2007). One approach our laboratory has been using to examine genetic variation among populations of the chili anthracnose pathogen (*C. acutatum*, *C. gloeosporioides*, *C. capsici*, and *C. coccodes*) is the comparison of RFLP and sequence diversity of a 1 kb intron of glutamine synthetase (GS). This target DNA is valuable diagnostically (RFLPs) for identifying species of *Colletotrichum* on pepper and for examining inter- and intra-specific phylogenetic (sequence) relationships. Pepper anthracnose populations from a worldwide collection were also evaluated for mtDNA RFLPs, vegetative compatibility, and pathogenicity on foliage and detached red and green pepper fruit. *C. acutatum* has recently emerged as an important pathogen of green pepper fruit in a number of countries. Examination of *C. acutatum* from a limited collection of isolates from pepper

anthracnose epidemics in Taiwan, Thailand, Korea, Brazil and the U.S. indicated that they belong to a single mtDNA-RFLP and GS-intron haplotype and most isolates (36 out of 43) examined from the various countries belong to a single vegetative compatibility group (VCG). This VCG may represent a globally distributed clone of the pepper anthracnose pathogen, *C. acutatum*.

## Chili Anthracnose Research at AVRDC 1993-2002

Lowell L. Black<sup>1</sup> and Tien-chen Wang<sup>2</sup>

<sup>1</sup>Seminis Vegetable Seeds,  
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<sup>2</sup>AVRDC The World Vegetable Center, PO Box 42,  
Shanhua, Tainan, Taiwan 74199

Anthracnose of *Capsicum* spp. is especially damaging to crops grown during the warm, wet season in the tropics and subtropics. AVRDC initiated a pepper/chili program in 1986, identified anthracnose as a top priority, and began searching for resistance sources. For the first few years screening emphasis was on *C. annuum*; it was conducted in spray-inoculated fields and with detached fruit in the lab by pin prick, spray, or drop inoculation. Progress was limited. There was little correlation between field and lab results, i.e. some field resistance was identified but could not be confirmed by lab inoculation. Field resistance correlated fairly closely with fruit size suggesting that in many cases lower incidence of infected fruit in the field was due to escapes. During this period *C. capsici* and *C. gloeosporioides* were considered the major anthracnose pathogens in Taiwan and elsewhere. It was realized in 1997 that several isolates at AVRDC identified as *C. gloeosporioides* were in fact *C. acutatum*, and that some of these isolates were collected in the early 1990s. A subsequent survey showed *C. acutatum* to be responsible for 60% of the anthracnose lesions in Taiwan and to be the primary agent in green fruit lesions. In 1997, a microinjection inoculation method was developed to evaluate anthracnose reactions of detached fruit, and the search for resistance shifted to other *Capsicum* spp. Green fruit resistance to *C. acutatum* was identified in 14 *C. baccatum*, *chinense*, and *frutescens* lines and ripe fruit resistance in some of the *baccatum* and *chinense* lines. These lines were also shown to be resistant to Taiwan isolates of *C. gloeosporioides* and *C. capsici*. AVRDC immediately began to make interspecific crosses between *C. annuum* and two of the more promising lines PBC 932 (*chinense*) and PBC 81 (*baccatum*). F<sub>1</sub> seed were not viable, but interspecific progeny were recovered from both crosses by in vitro culture of immature F<sub>1</sub> seed. Emphasis was placed on a susceptible *C. annuum* x PBC 932 cross from which three BC<sub>3</sub>F<sub>5</sub> families expressing resistance in green and ripe fruit were identified in 2002.

# Country Report of Anthracnose Research in Korea

Byung-Soo Kim

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Anthracnose is known as one of the three major diseases with Phytophthora blight and viral diseases causing more than 10% of annual economic loss in Korea. Causal fungi of anthracnose in Korea have been changing with time. *Colletotrichum dematium* and *Glomerella cingulata* have caused mainly ripe rot and damage on immature fruits was rare or not observed up until 1970's but immature fruit rot caused by *C. gloeosporioides* began to emerge as major anthracnose on pepper from early 1980's. Meanwhile, fruit rot caused by *C. dematium* was getting rare. Leaf spot caused by *C. coccodes* was also found in 1980's. There also has been a controversy on identification of the causal fungus of anthracnose causing economically important immature fruit rot. *C. gloeosporioides* has been known as major pathogen of the immature fruit rot but an argument that *C. acutatum* is the cause of the immature fruit rot was present. As entering into early 2000's, *C. gloeosporioides* and *C. acutatum* could be more clearly distinguished by sensitivity to Benomyl and molecular technology. *C. acutatum* is currently known as a dominant species of *Colletotrichum* causing major fruit rot of pepper in Korea.

Resistance to anthracnose was sought in wide range of genetic resources with various ways of inoculation but reliable level of resistance to *C. gloeosporioides* was found only in *Capsicum baccatum*, a cultivated species but that is not readily crossed with *C. annuum*. Research efforts were taken to overcome the interspecific genetic barrier by bridge cross and embryo rescue method. Breeding efforts to introduce resistance in *C. baccatum* into *C. annuum* are in progress.

# Chili Pepper Anthracnose in Thailand

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Chili pepper including *Capsicum annuum* and *C. frutescens* one of Thai spices that is important in Thai food recipe. The production area is approximate 84,900 ha produced average 6 tons/ha. Thailand exports chili pepper produces worth 40 million USD to 27 countries. Anthracnose disease caused by *Colletotrichum* spp. has been reported since 1951. Local cultivars are susceptible to the disease. The disease severity and yield losses varied from 10 to 80 % due to season, rain precipitation and culture practices. Lines 83-168 and KKU-Cluster, have been reported as resistant and high yield varieties, respectively, were used in breeding program and inheritance study of resistance. The single dominant gene controlled the resistance was reported from the evaluation. To confirm fungi species identification, species specific primers were used to identify the causal agents along with the analysis of protease activity. There are three species of *Colletotrichum* associated with chili pepper anthracnose, *C. acutatum*, *C. capsici*, and *C. gloeosporioides* were reported. Good agriculture practice (GAP) for chili production and disease management has been used to control the disease. *Bacillus subtilis* as a biocontrol organism is investigated.

Key word: anthracnose, chili pepper, *Colletotrichum* spp.

## *Colletotrichum* spp.

### Attacking on Chili Pepper Growing in Vietnam

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Currently, area for chili cultivation in Vietnam is increasing due to the market demands. Private companies have intensively invested in processing the chili sauces, which are for domestic and export use. A total area for chili growing is approximately 5,000 ha per one crop in 18 provinces from north to south and from highland to lowland area. Even though it is different in cultivation conditions, the farmers have agreed that anthracnose is one of serious diseases on chili to cause the yield loss and to reduce the quantity of marketable fruits. Disease incidence is recorded from 20 to 80% on fruits of *Capsicum annum* and 5 to 20% on fruits of *C. frutescens* infected in the field conditions. Disease epidemiology and control strategy are not studied well in current. A severe damage to chili fields is recorded in rainy season from August to October annually, but less damage to that is recorded in dry season from January to April. The number of chili varieties is provided from four seed companies. However, it is lacking the information on anthracnose resistant genotype of chili varieties planted and on virulence of *Colletotrichum* spp. isolates responding to their hosts. Our studies indicated that there were at least three *Colletotrichum* species found on the immature and mature fruits of chili. They are *Colletotrichum acutatum*, *C. gloeosporioides*, and *C. capsici* identified based on morphological and biological characteristics, and pathogenicity tests. However, it may have other species such as *Colletotrichum nigrum* and *C. cocodes* as recognized formerly. We also found *Colletotrichum* isolates to attack only on the leaves of chili seedlings leading to defoliation and collapse. Difference in weather and soil conditions of chili cultivation areas throughout the country is suggesting that there are several *Colletotrichum* strains present and that it is needed to study on distribution of *Colletotrichum* species (strains) in chili cultivation areas.

## Current Status of Chili Anthracnose in India

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Anthracnose incited by *Colletotrichum* spp is one of the most damaging diseases of chilli in India. The severity of the disease varies depending on cultivars grown and the weather conditions prevailing in a particular region. In severe instances, the pre harvest and post harvest infections together account for more than 50% of the crop losses. Two species, namely *C. capsici* and *C. gloeosporioides* were known earlier to cause Anthracnose in chilli. Surveys conducted recently revealed the presence of *C. acutatum* also. However *C. capsici* was the most predominant species in the major chilli growing states namely Karnataka and Andhra Pradesh. Among 92 isolates of *Colletotrichum* collected from different chilli growing areas, 53 were identified as *C. capsici* 38 as *C. gloeosporioides* and one was found to be *C. acutatum*.

The fungus is both internally and externally seed-borne. Sowing such contaminated seeds results in pre emergent and post emergent damping-off of seedlings in nursery and field. These infected seedlings form the primary sources of inoculum. The fungus survives in an active form on the stems and branches causing die-back symptoms. The wet conditions caused due to monsoon rains that occur during the June-October period help in the outbreak and spread of the disease. Fungicides are mainly used to manage the disease but the control achieved is not satisfactory. Different cultivars are known to vary in their susceptibility to fruit rot. In order to locate host resistance, both indigenous and exotic sources of chilli germplasm were evaluated. Some of the chilli genotypes obtained from The Asian Vegetable Research and Development Centre (AVRDC), Taiwan were found resistant to Indian isolates of *Colletotrichum*. Breeding efforts are underway to incorporate Anthracnose resistance into local cultivars of chilli. The paper details the disease scenario, losses caused due to chilli Anthracnose, performance of various chilli cultivars and the disease management strategies adapted in major chilli growing regions of India.

# Chili Anthracnose Research in China: an Overview

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Chili, *Capsicum* spp., is one of the most important horticultural crops in China grown as cash crop. The production acreage of chili is increased successively at the average rate of 10% since 2000. However, many diseases are contributing to low yields and low quality of fruits. Among those, anthracnose, which causes 15-60% yield loss, is one of the important diseases. Recent research of chili anthracnose in China include strain isolation and identification, biology characteristics of different strains, the development of inoculation technique for resistant varieties screening, resistant screening of chili germplasm for different breeding goals and controlling of anthracnose through bio-fungicides and chemicals. This paper will give a brief overview of anthracnose study in favor of chili production in China during the past 10 years.

# Status of Chili Anthracnose in Indonesia

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In Indonesia, chili pepper is one of the important crops that has become priority of the government program and given great attention. So far, anthracnose is still to be key factor threatening in chili production among the most four important pests and diseases in the field, and rank first among fungal diseases. Yield losses due to the disease varied from approximately 10 to 80% and 2 to 35% in wet and dry season, respectively. Farmers more emphasize to fungicides application in overcoming this disease than other control measures, which contribute approximately 20% over the total cost of chili production. Rotation, intercropping, mix cropping, weeding, and plastic mulch use are common cultural practices performed by farmers with main purpose for the efficiency and economic reasons rather than reducing anthracnose disease. Some research has been adressed to this disease, includes pathogen identification, development of resistant cultivars, and biological and integrated control. Recently, research concerning the development of resistant cultivars has been accelerated by either national funding (Directorate of Higher Education, Ministry of National Education and Ministry of Research and Technology) or international collaboration (AVRDC). The causal agents of chili anthracnose; species, virulence, composition, and distribution over area in Indonesia should be clarified as complementary informations in the development of resistant cultivars. Studies subjected on the use biological control agent with the mechanisms of induce resistant and growth promoting, has being performed and will be one of the promising control measures for anthracnose disease on chili. Development of this economically and environmental friendly control measures of chili anthracnose will be appropriate choice in supporting sustainability of chili production, food safety, and better health of farmers and consumers. Through participatory approach in research and knowledges sharing with farmer groups, learning process and technology dissemination go smoothly.

# Present Situation of the Anthracnose Disease in Sweet and Hot Pepper in Brazil and Search for Sources of Resistance

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Anthracnose of hot and sweet peppers (*Capsicum* spp.) is one of the most destructive diseases, which might cause significant yield losses especially during the hot/rainy areas/seasons in Brazil. The most economically important damage results from the fruit symptoms with anthracnose. In *Capsicum* plantlets, leaves and stem, the disease is regarded as a minor problem. In Brazil, losses of up to 100% have been reported in highly susceptible *C. annuum* L. cultivars (Lopes & ?vila, 2003). It is common to observe many fruits with high levels of latent infection, which symptoms will manifest only after harvesting (Lopes & ?vila, 2003). Anthracnose of *Capsicum* in Brazil is caused by a complex of *Colletotrichum* species with *C. gloeosporioides* and *C. acutatum* being the major causal agents. *Capsicum* germplasm has been evaluated for anthracnose resistance under different conditions with different *Colletotrichum* isolates. Screening assays for resistance have been conducted using mainly detached fruit evaluation with wounding and also under natural field epidemics. Germplasm evaluation for resistance to isolates *C. gloeosporioides* in fruits indicated good sources of resistance in *C. chinense* (Henz et al., 1993a; 1993b; 1994; Lobo J?nior et al., 2001; Pereira, 2005), *C. annuum* (Henz et al., 1993b; Pereira, 2005) and *C. baccatum* (Pereira, 2005). Good levels of field resistance to natural epidemics of *C. gloeosporioides* were also found in *Capsicum* lines carrying the upright (up) gene (Boiteux & Lopes, 1993). A source of immune-like resistance has been identified in the line *C. chinense* 'PI 159236' under field conditions. Preliminary results indicated that resistance of 'PI 159236' to *C. gloeosporioides* is under polygenic control. The development of segregating populations aiming to map the genetic factors associated with the resistance of *C. chinense* 'PI 159236' is underway.

## Pathotypes of *Colletotrichum* spp. Infecting Chili Peppers and Mechanisms of Resistance

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Anthraco­nose disease is the most important biological constraint to chilli pepper (*Capsicum annuum* L) production in Southeast Asia. In Thailand, three *Colletotrichum* species: *C. capsici*, *C. acutatum*, *C. gloeosporioides*, species were isolated from chilli fruit that showed typical anthracnose symptoms. These pathogens were isolated separately or together from infected fruit however, *C. acutatum* and *C. capsici* appeared to be the most severe being able to infect a range of *Capsicum* species and resistant genotypes. Each species can be distinguished by distinct spore shape, growth rate in culture on potato dextrose agar and by phylogenetic analyses from DNA sequence data of the ITS rDNA and -tubulin (tub2) gene regions. At least two pathotypes were identified for both *C. capsici* and *C. acutatum* isolates which were able to infect resistant genotypes of *Capsicum chinense* PBC932 and *Capsicum baccatum*-PBC 1422. This result will have profound effect on Thai chilli breeding programs where novel sources of resistant genes from these related species are being incorporated into the commercial *Capsicum annuum* varieties to enhance resistance to anthracnose. Inheritance of resistance in fruit from a cross between *C. annuum* Bangchang and the resistant line *C. chinense* PBC932 indicated that resistance was controlled by a single recessive gene (*co1*). Resistance was assessed by measuring lesion area per fruit area on detached chilli fruits, using a laboratory-based injection inoculation method. Infection of leaves of resistant genotype PBC932 by *C. capsici* resulted in a

hypersensitive response (HR) between 40 to 72 hours after inoculation. Preliminary results have identified four gene coding sequences (PR-1 protein, PR-4 protein, NBS-LRR resistance protein RGH1 and leucine-rich repeat protein) to be significantly up-regulated at 12 and 36 hours after inoculation. These sequences were likely encoding chilli candidate defense response genes related to HR response to anthracnose.

# Characterization of *Colletotrichum* Species Associated with Pepper Anthracnose in China, India, Indonesia, Taiwan and Thailand

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Pepper anthracnose is one of the major constraints of pepper production in the hot, humid tropics and subtropics. Several *Colletotrichum* species have been reported as causal agents of the disease. Their identification was almost exclusively based on morphological and cultural characteristics. However, morphological plasticity of *Colletotrichum* is well known and can lead to misidentification of the species. Understanding the pathogen profile is a prerequisite for managing the disease, including breeding for resistance. The objective of this study was to evaluate several phenotypic traits and specific primers for rapid and accurate differentiation of *Colletotrichum* species associated with pepper anthracnose in China, India, Indonesia, Taiwan and Thailand. AVRDC mycologist contributed the technique of application of phenotypic and molecular criteria for the identification of *Colletotrichum* spp. Isolates used were originated from single conidial cultures and were maintained on silica gel at 4°C. They were then transferred from stock cultures to potato dextrose agar (PDA) to determine conidial morphology, colony morphology and growth rate, casein hydrolysis medium (CHM) for protease activity determination and to potato dextrose broth (PDB) for PCR reactions with species-specific primers. Several specific PCR primers derived from the sequence of the internal transcribed spacer (ITS) region of the rDNA gene, such as *Ca/INT*<sup>2</sup> (5'-GGGGAAGCCTCTCGCGG-3'), *Cg/INT* (5'-GGCCTCCCGCCTCCGGGCGG-3') and *Cc/INT* (5'-TCTCCCCGTCCGCGGGTGG-3') were used for the detection of *C. acutatum* (Ca.), *C. gloeosporioides* (Cg) and *C. capsici* (Cc) respectively. Three species Ca, Cg and Cc were identified in China, India, Indonesia, Taiwan and Thailand based on the phenotypic and molecular criteria. This is

the first report of the association of *Ca* species with pepper anthracnose in all countries except Taiwan. At AVRDC in Taiwan we have found diverse morphological characteristics and poor specificity in the case of *C. gloeosporioides* isolates from Taiwan-using the *Cg* specific PCR primer. This implies that previously identified *Cg* isolates may be a heterogeneous complex species. *C. boninense* was recently separated from *C. gloeosporioides* as a distinct species based on morphological and molecular evidence.

# Taxonomic Characteristics of *Colletotrichum* spp. and Their Teleomorphs Causing Anthracnose of Chili Pepper in Korea

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Seventy-seven isolates of *Colletotrichum* spp. from chili pepper fruits in Korea were identified by morphological characteristics and molecular analyses. Based on morphological characteristics, 62 isolates were identified as *C. acutatum* and the others as *C. gloeosporioides* and *Glomerella* spp. *C. acutatum* isolates were divided into three groups through analysis of random amplified polymorphic DNA (RAPD) patterns. One of the three groups was chromogenic in PDA culture, and the others were non-chromogenic. By phylogenetic analysis of the complete internal transcribed spacer (ITS1-5.8S-ITS2) sequences, the Korean isolates of *C. acutatum* were grouped as A2 and A3 out of global molecular groups A1-A8. However, analysis of sequences in partial beta-tubulin 2 (exons 3-6) showed that all the Korean isolates were located separately from A1 to A8. By comparative analysis of ITS sequences, *C. gloeosporioides* isolates from chili pepper were grouped with those from other hosts apple, grape and persimmon, which was supported by analysis of the partial beta-tubulin gene. Three isolates of *Glomerella* spp. from chili pepper were distinguished from the isolates of *C. gloeosporioides* and other *Colletotrichum* species in morphological features and sequences of ITS and beta-tubulin 2 genes. One of them was identified as *Glomerella septospora*, and the other two *Glomerella* spp. were assumed to be new species.

# Application of ITS–RFLP Analysis for Identifying *Colletotrichum* Species Associated with Pepper Anthracnose in Taiwan

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Anthracnose is one of the major constraints of pepper production in the hot and wet tropics and subtropics. Four causal agents responsible for the disease: *Colletotrichum acutatum* (Ca), *C. boninense* (Cb), *C. gloeosporioides* (Cg) and *C. capsici* (Cc), have been identified in Taiwan by AVRDC. Our previous studies indicated that morphotaxonomic criteria alone is not enough to effectively and accurately differentiate these *Colletotrichum* species, due to the morphological plasticity. This study is aimed to develop a rapid and accurate diagnostic tool for species identification. Sequencing of the internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) in a total of eighteen phenotypic-characterized *Colletotrichum* isolates were accomplished for analyzing nucleotide divergence among four *Colletotrichum* species. Few restriction polymorphisms were presumed to offer a simple way for species differentiation. In this study, restriction fragment length polymorphisms (RFLP) of ITS region resulting from AluI, RsaI, & BamHI digestion were employed to differentiate four *Colletotrichum* species. After PCR amplification with universal primers ITS4/ITS5, the generated products (approximately 600 bp) were respectively digested by AluI, RsaI, & BamHI, and analyzed through 2% agarose gel electrophoresis. Among these 18 isolates, three RFLP patterns were generated by AluI digestion; however, the PCR product of Ca isolates remained un-cut. Two RFLP patterns were generated by RsaI, and those of Cg isolates were cut into two fragments (200 & 400 bp). Two RFLP patterns were generated by BamHI, and only those of Cc isolates remained un-cut. All Ca, Cb and Cc isolates had their own respectively distinguishable RFLP patterns, while Cg isolates separated into two groups. The results suggested ITS-RFLP technique is an efficient method for rapid diagnosis of *Colletotrichum* species from pepper. Recently, a total of 412 Taiwan isolates collected from pepper production areas were analyzed through ITS-RFLP fingerprinting. Among them, 245 Ca, 34 Cb, 52 Cc and 69 Cg were identified. Other *Colletotrichum* isolates (3%) were not distinguishable, which inferred to

the various inter- and intra-species variations in *Colletotrichum* members. The results reflected the complex pathogen diversity of pepper anthracnose, and Ca is the predominate species in Taiwan.

# *Colletotrichum acutatum* associated with pepper anthracnose in Thailand

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Pepper anthracnose is one of the major constraints of pepper production in Thailand. Based on conidial morphological characteristics, *Colletotrichum capsici* and *C. gloeosporioides* were identified as the major causal agents of pepper anthracnose in Thailand in the past years. Recently a total of 24 *Colletotrichum* isolates were collected from typical anthracnose infected fruit samples and identified as 13 isolates of *C. capsici* and 11 isolates of *C. gloeosporioides*. However, different culture characters of *C. gloeosporioides* were found on media. Thereafter, we run PCR analysis with species-specific primers and analyze protease activity in casein hydrolysis medium, we have found that the primer CgINT (5'-GGCCTCCCGCCTCCGGGCGG-3') (Mills et al. 1992) amplified (450 bp) 5 isolates of previous 11 isolates as *C. gloeosporioides*, and the primer CaINT2 (5'-GGGGAAGCCTCTCGCGG-3') (Sreenivasaprasad et al. 1996) amplified (490 bp) 4 isolates of the remained *C. acutatum* isolates. Four *C. acutatum* isolates showed protease activity strongly than *C. gloeosporioides*. Based on the morphological, biochemical and molecular characteristics, *C. acutatum* was firstly confirmed as the causal agent of pepper anthracnose in Thailand.

Key words : anthracnose, chili pepper, *Colletotrichum* spp.

# The Occurrence of Chili Anthracnose in Taiwan and its Control

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Chili pepper is an important vegetable used for multiple purposes in Taiwan. During 2003-5, the annual chili crop was estimated around 9,000 tons produced on 1,200 ha. Most commercial production is for the fresh market, with relatively little for processing. In Taiwan, high precipitation brought by Plum rain during May-June, or typhoons (June-September) always leads to severe damage from anthracnose on immature as well as ripe fruit, and even causes post-harvest losses. Anthracnose greatly reduced the yield and marketable fruit percentage. This disease was the major constraint for chili pepper production in summer season in Taiwan. Little or no anthracnose infection is observed during November to April. A 2006 summer yield trial conducted by AVRDC demonstrated the close correlation between precipitation and anthracnose damage.

In Taiwan, four *Colletotrichum* species have been identified by AVRDC as causal agents for pepper anthracnose, using morphological and molecular indicators: *Colletotrichum acutatum*, *C. boninense*, *C. capsici* and *C. gloeosporioides*. In order to identify anthracnose pathogens accurately and rapidly, an ITS-RFLP fingerprinting was developed. During 1992-2006, a total of 412 Taiwan isolates collected from diseased fruits island-wide were characterized. 65% of these isolates were *C. acutatum*, and is broadly distributed throughout Taiwan production areas.

AVRDC has pursued breeding for resistance to anthracnose for many years. Currently, some lines genetically resistant to *C. acutatum* have been developed and released by AVRDC, but no resistant variety is yet commercialized. Therefore, chemical control and effective crop management practices are recommended for the management of anthracnose in peppers.

# Variability among *Colletotrichum capsici* Causing Chili Anthracnose in North Eastern India

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The research work was carried out in Arunachal Pradesh, the largest state in northeastern India. The plant parts showing symptoms either die back, anthracnose or fruit rots depending on expression were collected during crop seasons through surveys conducted from year 2002 to 2006 and evaluated for the variability. In total, 12 isolates were evaluated for their variability on morphology and pathogenicity. The Koch postulates were proved for the pathogenicity of the test isolates. Mainly *Colletotrichum capsici* was found to be associated in causing anthracnose. In addition *C. dematium*, *C. gloeosporioides*, *C. graminicola* and *C. atramentarium* have also been isolated in few cases. The diseased samples were used for isolation of pure culture of the fungus on Czapeck's Dox agar. The colony characters, morphology and margin architecture were recorded. Shape of conidia and morphology of isolates were also observed. The germination of conidia and formation of appresoria were also observed.

The colonies were circular, smooth, white having thick texture. The colour of the colony varies from gray, greenish to white. The growth rate of colony varies from media to media depending on the composition. The size of the conidia range between 25-26 x 3.2-3.72um and the variation is not significant among isolates. The conidia were formed at the tip of conidiophore, which is unbranched. The conidium is hyaline, single celled and fusoid. Conidiophore is unbranched and aseptate.

## *Colletotrichum acutatum* Associated with Anthracnose Disease on Chili (*Capsicum* spp.) in Thailand

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Anthracnose disease of chilli pepper (*Capsicum annuum*) in Thailand is to be caused by three *Colletotrichum* species: *C. capsici*, *C. acutatum*, and *C. gloeosporioides*. Validation of the taxonomy of these species was based on colony and spore morphology; rDNA sequence analysis and pathogenicity testing. *C. acutatum* and *C. capsici* were found to be equally more aggressive than *C. gloeosporioides* when fruit of *Capsicum annuum* cultivar Bangchang were inoculated with a mixed spore inoculation of the three pathogens. Isolates of *C. acutatum* from chilli were able to infect the resistant genotype of *C. chinense* PBC932 but only after wounding of the epidermis at inoculation. At least two pathotypes of *C. acutatum* were identified from fruit bioassays were detached fruits of six *Capsicum* spp. genotypes including two of *C. annuum*, three of *C. baccatum* and a F<sub>1</sub> hybrid of *C. annuum* and *C. chinense* were inoculated with ten aggressive isolates of *C. acutatum*. Three isolates of *C. acutatum* collected from north Thailand were able to severely infect *Capsicum baccatum* genotype PBC1422, whereas six other isolates could not infect this genotype. In contrast, *C. baccatum* PBC80 was very resistant to infection of all isolates of *C. acutatum*. All genotypes of *C. annuum* and the F<sub>1</sub> hybrid were susceptible to all isolates. *Colletotrichum acutatum* was also shown to be a pre- and post-emergent pathogen of *C. annuum*. Germination of seeds of Bangchang inoculated with conidia of *C. acutatum* was severely affected and the few seedlings that developed wilted and died within two weeks of germination.

# Occurrence of Pepper Anthracnose and the Morphological Characterization of its Pathogen *Colletotrichum* spp. in Guangxi, China

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Pepper is an economically important crop in Guangxi province of China with about 2 million tons of fresh chili produced in 2006. Guangxi locates in the subtropical region with high temperature and high moisture climate that is favorable for the epidemic of chili pepper anthracnose. In 2003, the incidence was 100% in the variety Man-tian-xing in Tian-deng County that resulted in a severe loss. In 2006, the incidence was 15% to 60% in the major chili producing regions in Guangxi.

The causal agent responsible for the chili pepper anthracnose in Guangxi was identified to be *Colletotrichum* spp. At least three morphological types of *Colletotrichum* were founded from diseased samples collected. The first type was with a colony of white to dark grey, reverse dark brown. Setae were abundant and the conidia were single cell, colorless, falcate, and 23.0-26.9×3.9-4.5 μm in size. The second type was with a colony of white to grey, reverse light pink. Acervuli were deficient. The conidia formed on the top of hyphae directly or inside the acervuli. Setae were absent and conidia were single cell, colorless, straight, cylindrical, apex obtuse, and 12.8-16.7 × 5.1-5.5 μm in size. Perithecia were flask like, dark brown that were formed after 20 days culturing on potato sucrose agar media under 28°. Asci were clavate, 41.0-48.6×9.0-13.4 μm in size, containing 8 ascospores. Ascospores were single cell, colorless, oval, and slightly bended in the middle. The third type was with colony of white to dark grey, reverse white grey to dark grey. Acervuli were abundant and setae were absent. The conidia were single cell, colorless, straight, cylindrical, apex obtuse, and 12.6-15.5 × 5.0-5.8 μm in size.

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## Diversity and Distribution of Hot Pepper *Colletotrichum* spp. Isolates in Southern India

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Hot pepper (*Capsicum annuum*) "Ripe Fruit Disease" caused by *Colletotrichum* species causes substantial losses in fruit quality and quantity in the major hot pepper growing regions of Southern India. Assessment of location-specific variability in and distribution of the pathogen will support the breeding of anthracnose resistant cultivars by improving the design of large-scale field screening techniques. We have studied the distribution and diversity of 72 *Colletotrichum* spp. strains isolated from diseased fruits collected at the fruit ripening stage from the major hot pepper growing regions of Southern India (Andhra Pradesh, Karnataka, Tamilnadu and Maharashtra provinces). Forty of these isolates were *C. capsici* (Cc) and 32 were *C. gloeosporioides* (Cg). Cc was predominant in Karnataka, Tamilnadu and Maharashtra, and Cg in Andhra Pradesh. The morphological diversity of the pathogen was assessed by scoring the colour of the mycelia, the overall colony type, the colony margin type, conidial size and radial growth. Thirty six isolates produced slightly fluffy type colonies, and the rest either fluffy or flat colonies. The latter type was generally produced by Cc isolates, while Cg ones produced either fluffy or slightly fluffy colonies. The majority of isolates produced white or whitish-grey mycelia when cultured on PDA. The isolates which produced either pink or orange mycelia were Cg. In general, Cg isolates grew faster than Cc ones. Four of the five slow growing isolates were Cc. Conidial size was more variable among Cg than among Cc isolates. AFLP fingerprinting showed the isolates to be highly diverse.

# The Development of Fungicide Application System for the Control of Pepper Anthracnose

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It was made the effort to develop the fungicide application system for the control of pepper anthracnose. In a greenhouse, it was performed to assess the controlling activity of 5 selected fungicides (chlorothalonil, carbendazim, trifloxystrobin, tebuconazole, BTH-mancozeb). Trifloxystrobin and BTH-mancozeb showed a good effect on pepper anthracnose by 64.8 and 70.3% of control value, respectively. In the field test, trifloxystrobin, tebuconazole and propineb applied by 10 day interval showed 87.8, 89.3 and 96.4% of control value, respectively at early season. However, the control activity of trifloxystrobin decreased dramatically to 41.3% in 20 day interval application, while tebuconazole activity continued to be high in 20 day interval application as well as 10 day interval. At the later season, propineb showed a good activity in the 10 day interval treatment only as 80.6% of control value, but activities of all the tested fungicides fell below 30% of control value. When another field experiment was conducted to determine the proper application timing, it was concluded that it was very important in the control system of pepper anthracnose not only the application of BTH-Mancozeb and/or a protective fungicide at a transplanting stage or a flowering stage but also that of strobilurins just prior to outbreak pepper anthracnose. At last it would be confirmed whether control system of fungicide application developed in our laboratory was effective on pepper anthracnose or not.

# Development of Forecasting System for Anthracnose in Pepper

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Anthracnose (*Colletotrichum acutatum*) is the major fungal disease affecting hot pepper fruit in Korea. With the goal of achieving better integrated pest management for hot pepper production, a disease-forecasting model was developed based on conidial germination and appressorium formation. And the model was compared to a conventional disease-control method. Experimental field plots were established in Asan, Chungnam, in 2005 to 2006, and hourly temperature and wetness-period data were measured and used as model inputs. Only 3 times of fungicide spray were advised with the model and as effective as 8~10 times of calendar-based spray. A web-based forecasting information system for anthracnose was developed and operated at <http://redpepper.epinet.co.kr>. This system consists of four subsystems weather data collection, job processing, data storage, and web service systems. The raw weather data from the nation-wide automated weather station network (Korean Meteorological Administration, 528 locations) were collected and created nation-wide mesh weather data by spatial interpolation methods. The job processing system produced nation-wide mesh forecasting information from the nation-wide mesh weather data by running the anthracnose forecasting model. Nowadays, the forecasting system is testing for the better application in commercial fields.

# Effect of Different Media, pH, and Temperature on Growth and Sporulation of *Colletotricum capsici*

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*Colletotrichum capsici* (Sydow) E. Butler and Bisby is most important pathogen causing severe damage in chilli crop due to anthracnose disease. Anthracnose of chilli is not only a problem of India but it occurs through out the world where chillies are growing. It causes heavy loss in chilli production on both pre and post harvest specially on ripe fruits.

The fifteen isolates i.e. VC-1, VC-2, VC-3, VC-4, VC-5, S1, S2, S3, S4, S5, S6, S7, S8, Ccc-2, and Ccf were collected from different agroclimatic conditions and studied the effect of different nutrient media, pH and temperature on growth and sporulation of the pathogen. The colonies of the pathogen on different nutrient media varies from grey to dark brown, on pH light grey to dark grey and on temperature only greyish. A significant cultural variability where recorded on different nutrient media, pH and temperature after six days of inoculation. The maximum radial growth were recorded in isolate S1 (74.80mm) with conidial size 22.5 X 3.3 um the most favorable temperature was observed between 20°C and 30°C for maximum radial growth and optimum pH found was 8 for the same. Among Ten different nutrient media Richard's Synthetic agar was best for maximum radial growth and Rose Bengal agar gave the minimum radial growth, while potato dextrose agar was best for sporulation after 6<sup>th</sup> DAI .

## Evaluation of Diverse Chili Pepper Sources for Resistance to Anthracnose

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Forty accessions of pepper, including representatives of *Capsicum annuum*, *C. baccatum*, *C. chinense*, and interspecific progenies, were evaluated at AVRDC during the spring and summer of 2007. Evaluation methods included field growouts, high pressure spray and microinjection assays on green and red-ripe fruit, and evaluation of foliar DNA with an AFLP-derived marker. Two isolates of *Colletotrichum accutatum* collected in Taiwan were used in the evaluations. Field performance, lab assays, and molecular marker characterization are broadly correlated, but frequent discrepancies arise. Marker appears to be valid only in accessions tracing their resistance to PBC932. Numerous accessions display good resistance under field conditions, but assay as susceptible under lab tests. Independent genetic factors may be found in *C. baccatum* as compared to *C. chinense*, and additional fruit features, such as cuticle and epidermis structure, may confer pre-infection protection.

Resistant Sources of Chili (*Capsicum annuum* L.)  
Anthracnose Fruit Rot Disease [(*Colletotrichum capsici* (Syd.)  
Butler and Bisby] against Different Isolates Collected from  
Commercial Chili Growing Areas of India

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Chilli (*Capsicum annuum* L.) is an important commercial crop of India, being grown in an area of 0.9 million hectares. Anthracnose fruit rot (*Colletotrichum capsici* (Syd.) Butler and Bisby) is an important fungal disease of dry chilli crop causing upto 75 per cent fruit infection. Though many fungicidal control measures are recommended, their efficacy under Indian farmer's commercial fields is reduced because of the prevailing weather conditions and extensive continuous areas.

In order to locate resistant sources of chilli against *C. capsici* 89 genotypes drawn from five species of *Capsicum* and inter-specific derivatives of *C. annuum* X *C. baccatum* var. *pendulum*, were subjected to artificial screening against six isolates of *C. capsici* collected from major chilli growing areas. Single spore isolates at 20-25 spores/10X microscopic field suspension in distilled water was used to dip the pin pricked fruits to cause infection and evaluated on disease scale of one to six scale. Besides, these genotypes were also field screened and evaluated under local conditions at Indian Institute of Horticultural Research, Bangalore, India during the rainy months from June to December for three consecutive years. Field screening was augmented by spraying Bangalore isolate fungal inoculum (20-25 spore /10X microscopic field) twice during the crop period coinciding with high humidity conditions.

*C. baccatum* accessions as a group were found to be resistant to all the isolates except Rahuri isolate. Two accessions of *C. baccatum* IHR 951 and 1263 were found to be resistant against all six isolates under laboratory screening and to local isolate under field screening. Thirteen genotypes were resistant to Bangalore isolate, 18 to

Bhubaneswar, 16 each to Dharwad and Rahuri, 33 to Thrissur and 10 to Sattur isolate. None of the released/ notified varieties were resistant to any of the isolates.

Field screening against Bangalore isolate gave similar results as in case laboratory screening against Bangalore isolate. Based on chilli genotypic differential resistant reaction against six isolates of *C. capsici* an attempt is made to identify host differentials.

# Structural Resistance and Defense Mechanisms of Chili Pepper against Pepper Anthracnose Disease

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Cytohistological responses of chili pepper fruit were examined in compatible and incompatible interactions with the anthracnose pathogen, *Colletotrichum* spp. using susceptible (*Capsicum annuum* cv. Jejujaerae) and resistant pepper (*Capsicum baccatum* PBC80). Compatible responses in the susceptible pepper were destruction of cell wall and cellular contents followed by intracellular colonization of the pathogen between plasmalemma and cell wall. Three conspicuous responses found as incompatible responses in the resistant pepper were as follows. 1) Cuticle layers of fruit pericarp were thicker than those of the susceptible pepper and thickened remarkably after the pathogen inoculation, which were not in the susceptible pepper. 2) Infected or affected fruit cells of the resistant pepper showed programmed cell death (PCD)-related and hypersensitive response (HR)-related cytological features, which were not found in the susceptible pepper. These cytological features were terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL)-positive reaction in fluorescent microscopy and subcellular changes in electron microscopy, respectively. 3) At the later stage of infection, especially by in planta inoculation, wound periderm-like boundary layers were formed around the inoculation site, following cell enlargement and profound cell division, which may have blocked disease progression. Wound periderm could be formed by wounding alone in susceptible peppers. In another experiment on disease development affected by delayed inoculation after wounding with a different temperature regime of 13°C, 18°C, 23°C, and 28°C, occurrence and severity of pepper anthracnose decreased proportionally with increased delayed time of inoculation after wounding and with decreased temperature. Wound periderm was formed on pepper pericarp tissues after wounding more extensively at 18°C than 28°C. Most of the anthracnose pathogens, including pepper anthracnose pathogen are facultative biotrophs, showing initial biotrophic infection strategy and subsequent necrotrophic infection mode. Cuticle thickening, PCD and HR

may be effective to the biotrophic phase of disease progression because these histopathological changes occur during pathogen penetration and early infection stages. Wound periderm formation, which is probably stimulated by PCD and HR, may be a defense mechanism against the necrotrophic infection stage of the anthracnose pathogen. Therefore, all of these defense mechanisms should be needed for fulfilling the complete resistance of chili pepper fruit against the anthracnose disease.

# Diagrammatic Scale to Evaluate Anthracnose Severity on Sweet-Pepper Fruits for Disease Progress Studies

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Anthracnose is one of main pepper diseases in Brazil, causing severe losses in fruits in the field and during the postharvest period. This paper describes a diagrammatic scale to evaluate anthracnose severity on sweet-pepper fruits under field conditions. An experiment was carried out in Bras?lia, DF, Brazil, during the dry season, from May to July 2005, with sweet-pepper hybrid 'Maximos F<sub>1</sub>' cultivated in plots of 20 plants (three replicates) in a randomized block design, with treatments varying in soil cover (plastic or straw mulch) and chemical control. Eighty days after transplant, the field was infested with five halves of *Colletotrichum gloeosporioides*-infected pepper fruits per plot. The field was sprinkler irrigated. Seven days after inoculation, 100 fruits of different sizes and growth stages and distinct degrees of anthracnose severity were evaluated with a 1cm-squared plastic sheet to estimate the diseased area. Anthracnose severity on fruit varied greatly, ranging from apparently sound, symptomless fruits, to fruits with more than 90% diseased area. Individual lesions ranged from one to 12 per fruit, in several sizes and shapes. The descriptive scale of anthracnose fruit severity was made up according to the number and size of lesions and the percentage of the diseased area. Fruits were separated into three groups by the diseased area: (a) fruits apparently sound, symptomless (0% diseased area); (b) fruits with 1 to 5 lesions, with diseased area ranging from 1% to 20%, and lesion diameter = 0.5cm; and (c) fruits with more than 20% of area diseased, with one or more lesions =0.5cm of diameter. Diseased fruits were further divided into nine classes accordingly to the percentage of diseased area, ranging from class 0 (0% of diseased area, soundfruits) to class 8 (81% or more of diseased area). The proposed scale proved easy to use, reliable and accurate to measure anthracnose severity of sweet-pepper fruits in field conditions, allowing repeated evaluations in the same fruits and plots, as needed in epidemiological studies.

# An Early Stage Differentiation of Pepper Anthracnose Pathogen, *Colletotrichum acutatum* JC24, on the Fruit with or without an Wound

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Appressoria of *C. acutatum* JC24 were produced at the tip of germ tubes on the epidermis without a wound between 1 or 2 days after inoculation with conidial suspension adjusted to  $1 \times 10^6$  conidia/ml. Observing conidia having appressoria even at 7 days after inoculation, any change was not found almost compared with conidia 1 or 2 days after inoculation. However, mycelial growth on the epidermis of pepper fruit with wounds was done plentifully 3 days after inoculation by using SEM. From 3 days after inoculation it was also found that a number of conidia were formed on conidiophores saprophytically. Investigating the population of *C. acutatum* JC24 on the epidermis through the semi-selective medium method, the number of colonies on the semi-selective media was increased from 3 days after inoculation. On the cellophane membrane, it was tested whether nutrient was able to incite the production of conidia of *C. acutatum* JC24 or not. By adding crude extract of pepper and Chinese juniper leaves, the number of conidia was increased in the proportion of diluted concentration of leaf extract. The increase of conidia was showed dramatically by the addition of potato dextrose broth. The increase of conidia could be also observed on the epidermis of pepper fruits by adding PD broth to inoculation point with conidia suspension 1 day after inoculation. Based by results, it was suggested that nutrients incited the conidial production of *C. acutatum* JC24 saprophytically, which play an important role of the second inoculum in the field. Especially it was suggested that exudates from the wound of pepper fruit be enough to incite conidial production.

# Backcross Introgression Lines between *Capsicum annuum* and *C. baccatum* for Breeding Chili Pepper Resistant to Anthracnose Caused by *Colletotrichum* spp.

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Our breeding program for resistance to anthracnose caused by *Colletotrichum* spp. was started from the interspecific BC<sub>1</sub>F<sub>1</sub> population derived from the cross between *Capsicum annuum* and *C. baccatum*. Among the 88 BC<sub>1</sub>F<sub>1</sub> progenies, four plants highly resistant to the anthracnose pathogen, KSCa-1 isolate of *C. acutatum* were selfed and backcrossed for developing advanced generations. In early generations such as BC<sub>1</sub>F<sub>2</sub> and BC<sub>2</sub>F<sub>1</sub>, hundreds of progenies were screened to select diverse resistant plants. On the other hand, dozen of plants were screened in somewhat fixed generations such as BC<sub>1</sub>F<sub>5</sub> and BC<sub>2</sub>F<sub>4</sub>. We could select several plants resistant to the disease from every generation and we found that the resistance was quite fixed after BC<sub>1</sub>F<sub>4</sub> and BC<sub>2</sub>F<sub>4</sub> generations. Consequently, several fixed lines, named backcross introgression lines (BILs), resistant to anthracnose were selected from the BC<sub>1</sub>F<sub>6</sub>, BC<sub>2</sub>F<sub>4</sub> and BC<sub>2</sub>F<sub>5</sub> generations, respectively. Their resistance was confirmed under the natural infection conditions of open fields but they had some problem in other horticultural traits including fruit size and shape, yield and susceptibility to some viral diseases. Therefore, we needed backcrossings between commercial inbred lines and the resistant BILs. In some combinations, the resistance of F<sub>1</sub> plants was not uniform, that is, the resistance level was somewhat fluctuated among plants. The result indicated that the resistance of some BILs was not fixed yet. In addition, even in the F<sub>1</sub> plants, many horticultural traits were not good yet. It suggested that more backcrossings would be necessary for the commercialization of the BILs. On the contrary, some F<sub>1</sub> combinations were considerably great in uniform resistance as well as other horticultural traits. This year, the F<sub>1</sub> varieties were planted in an open field with three replication and we are going to check their resistance.

# Study on the Characteristics of Interspecific Hybrids and Their Progenies for the Breeding of Anthracnose-Resistant Pepper

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Anthracnose disease, one of the limiting factors together with late blight disease in pepper production in Korea, has increased its level of damage every year. To introduce the anthracnose-resistant gene(s) into cultivated pepper, 881045, one of the *C. baccatum* var. *pendulum* selected for resistance against anthracnose, was crossed to five cultivars of *C. annuum*. As a result, 9 interspecific fertile plants were obtained, and 3 resistant plants were selected in F<sub>2</sub> generation. A backcross, BC<sub>1</sub>F<sub>1</sub>, was made between a selected resistant plant in F<sub>2</sub> and 881045. Four lines with comparable resistance as 881045 or a little less resistance to anthracnose were selected subsequent generations to BC<sub>1</sub>F<sub>5</sub> generation were developed.

The interspecific hybrid had yellow-green spots on petals. This characteristic is a distinctive morphological trait of *C. baccatum*. Twenty-four F<sub>2</sub> plants were obtained from the interspecific hybrid, but only three plants were fertile. The fertile F<sub>2</sub> plants were all anthracnose resistant. DNA composition of anthracnose-resistant plants, as characterized by the amplified fragment length polymorphism (AFLP), was more similar to that of *C. baccatum* var. *pendulum* 881045 than that of *C. annuum* L.F.. The lesion diameter, after artificial inoculation, varied widely, despite similar DNA composition of the individual plants. The anthracnose-resistant lines from interspecific hybrid showed high glucose content in fruits, but susceptible cultivars showed higher sucrose content than glucose.

To obtain basic information in introducing anthracnose resistance into cultivated pepper, cross compatibility and cross efficiency were evaluated in the developed

anthracnose-resistant lines from interspecific hybrid. The cross compatibility was compared in F<sub>1</sub>s as expressed by the number of seed setting. This seed count in F<sub>1</sub>s was significantly higher than the number of selfed-seeds of interspecific hybrids. Therefore, the four breeding lines developed in this study could be efficiently used for introgression of anthracnose-resistant gene(s) into various types of cultivars belonging to *C. annuum*.

# Inheritance of Resistance to Anthracnose Caused by Isolates of *Colletotrichum acutatum* and *C. capsici* in *Capsicum*

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Several genetic resources resistant to anthracnose were recently identified in *Capsicum* germplasm. To carry out inheritance analysis of the anthracnose resistance, we made three intraspecific cross combinations between *C. annuum* Yeoju x Daepoong for resistance to *C. capsici* isolate, HN11 x AR for *C. acutatum* isolate and Daepoong x AR for resistance to both isolates. In addition, an intraspecific cross between *C. baccatum*, Golden Aji x PI594137 was used for resistance to the *C. acutatum* isolate. Mature green fruits were inoculated with microinjection method and the inoculum density was adjusted to  $5 \times 10^5$  spore/ml. In Yeoju x Daepoong and HN11 x AR combinations, the segregation ratio of their F<sub>2</sub> populations were fitted to 1:3 (resistant:susceptible) as like the Mendelian manner. These results suggested that the resistance of Daepoong to *C. capsici* and AR to *C. acutatum* were qualitatively inherited by single recessive gene, respectively. The F<sub>2</sub> segregation ratio of Daepoong x AR was fitted to 1:0 and 1:3 for resistance to *C. capsici* and *C. acutatum*, respectively. The results indicated that the resistance of Daepoong and AR to *C. capsici* was controlled by the same recessive gene while the resistance of AR to *C. acutatum* was governed by different single recessive gene. On the other hand, the resistance of PI594137 (*C. baccatum*) seemed to be controlled by a dominant gene since the F<sub>2</sub> segregation ratio of Golden Aji x PI594137 was likely fitted to 3:1 Mendelian manner. Therefore, we can say that the inheritance mode of anthracnose resistance might be specified with different genetic resources and pathogen isolates used. Consequently, our results would be useful for developing molecular markers associated with the anthracnose resistance and it would be possible for breeding more durable resistant variety by means of pyramiding those different resistant genes.

## Development of Anthracnose Resistant Chili Pepper Varieties at AVRDC-The World Vegetable Center

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With the identification of good sources of resistance to Anthracnose, and the development of repeatable screening methodologies, AVRDC has pursued a program to develop and release chilli pepper varieties carrying resistance to Anthracnose. Interspecific cross between *Capsicum annuum* and *C. chinense* (PBC932), was followed by three backcrosses to *C. annuum*, producing a small number of selections displaying lab-based resistance equivalent to the *C. chinense* parent. One of these lines was used in inheritance studies, which have documented genetic control by at least one major gene, and evidence of independent control of resistance at immature green and mature red fruit stages. That mapping population generated AFLP-based molecular markers associated with green fruit resistance in progenies derived from PBC932. Resistance derived from *C. chinense* PBC932 has tended to be associated with irregular fruit shape, which is improving with continued backcrossing to elite *C. annuum* parents. Five lines have been released for public evaluation and use, mainly through AVRDC's International Chili Pepper Nursery program. Lines carrying resistance to other diseases, including CMV, CVMV, PVY, Bacterial Wilt, and *Phytophthora capsici*, in backgrounds popular in India and Thailand are being advanced.

# Breeding for Anthracnose Resistance in Hot Pepper

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Pepper anthracnose is the most destructive disease resulting in yield loss over 10% every year in Korea. Therefore, the farmers have sprayed with agricultural pesticide several times and have paid much cost for disease control. One goal of breeding is to develop hot pepper varieties with multiple disease resistance including virus, Phytophthora and anthracnose. For breeding resistance variety, *C. baccatum* variety PBC 81, resistant to anthracnose, was used. The availability of genetic tools such as cytoplasmic male sterile, maintainer and restorer lines which are essential to develop hybrid cultivar of pepper in Korea. To breed male sterile lines, elite maintainer and restorer lines with virus and Phytophthora resistance of N.A.C.F. were backcrossed to resistant lines selected in green house and field test respectively. The anthracnose resistance must be quantitatively inherited, backcross was carried out after resistance test of  $F_2$ ,  $BC_1F_2$ ,  $BC_2F_2$ ,  $BC_3F_2$  and  $BC_4F_2$ .

Anther culture breeding program is an efficient technique which can reduce the breeding period. Through anther culture of  $BC_3F_1$ , 193 plants have been developed. Among them, 2 plants showed resistance to anthracnose. These plants can be used genetic materials for resistance. The time period to develop a new variety of pepper by conventional method required long time under the Korean climatic weather conditions. Shuttle breeding scheme was effectively employed for the selection of anthracnose resistance.  $BC_2F_4$ ,  $BC_4F_2$  and  $BC_4F_3$  plants were grown and selected in Korea during monsoon season and at Hinan island of China during winter season. Pathogenicity of isolates is varied greatly by years and regions. Also resistance gene is not simple. Therefore it is quite difficult to transfer resistance gene of *C. baccatum* into cultivar of *C. annuum*. The success of breeding for resistance depends on how to select the resistance individuals in progeny generation. Since 2002 started breeding, backcross and selection have been practiced continuously every year. 65 plants in the  $BC_4F_3$  selected from the cross of resistance line and elite lines of N.A.C.F. showed resistance to anthracnose and phenotype similar to recurrent parents. We are making  $F_1$  cross combinations that can be evaluated next year.

# Evaluation of Horticultural Characteristics of Anthracnose Resistant Breeding Line in Pepper

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The  $F_1$ ,  $F_2$  and BC population of inbred lines derived from a cross between A8 (pbc385) which is an anthracnose susceptible line, high yield, and good horticultural characteristics, and A17 ((*C. annuum* x *C. chinense*)BC<sub>3</sub>F<sub>5</sub>) which is an anthracnose resistant line derived from AVRDC, were used to evaluate the horticultural characteristics. The seeds were sown in plug flats in a glasshouse on 2 March and seedlings were transplanted into the rain shelter house on 10 May, 2007. The evaluation was taken from 10 May to 30 July. Two parents,  $F_1$ ,  $F_2$ , BC<sub>11</sub>F<sub>1</sub>, and BC<sub>21</sub>F<sub>1</sub> populations were used. Fruit characteristics were investigated from samples which were taken at green full developed fruit stage. The characteristics of parent lines, P<sub>1</sub> and P<sub>2</sub> were flowering after sowing 116 and 109 days, fruit weight 11.6 g, 7.0 g, and fruit length 10.5 cm, 7.8 cm respectively. Flowering after sowing was 105 days, 100 to 125, 100 to 115 and 106 to 119 days in  $F_1$ ,  $F_2$ , BC<sub>11</sub>F<sub>1</sub> and BC<sub>21</sub>F<sub>1</sub> populations respectively. The fruit weight is 11.1 g, 1.6 to 23.1 g, 2.4 to 16.4 g and 4.7 to 23.9 g, fruit length 10.3 cm, 4.6 to 14.4 cm, 4.5 to 13.1 cm and 8.0 to 13.2 cm. Fruit position is pendent type in all lines. These samples would be used for developing the molecular marker of anthracnose resistance.

# Construction of Linkage Map and Mapping QTLs for Anthracnose Resistance in *Capsicum baccatum* Population

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Chili anthracnose caused by *Collectotrichum accutatum* is the most economically important disease in Korea and in other countries over the world. *Capsicum baccatum* var. *pendulum* (Cbp) was identified as a resistant source to anthracnose and actively used for inter-specific hybridization parents for introduction of resistance gene(s) into cultivated chili lines. To understand the genetic basis of anthracnose resistance on Cbp, a genetic mapping population consisting of 192 F<sub>2</sub> plants was developed from a cross between Cbp and *C. baccatum* 'Golden Aji', susceptible to anthracnose, and was used for linkage mapping with SSR and AFLP markers. A linkage map with fourteen linkage groups has been constructed with 57 SSRs and 150 AFLPs. Using this molecular linkage map, number, location, and effect of QTL for chili anthracnose resistance were compared between detached fruit artificial inoculation and field inoculation. Due to the inconsistent inoculation on detached fruits, four replications of inoculations were carried out and independently used for QTL mapping analysis. Several significant QTL were detected and were stable under different inoculation conditions, and now they are confirming with different breeding populations. Markers tightly linked to the QTL which are stable under environmental conditions are critical for the success of MAS in future anthracnose resistant chili breeding programs.

# Introgressed QTL Mapping and Developing Molecular Markers Associated with Anthracnose Resistance in *Capsicum*

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It has been reported that the anthracnose (*Colletotrichum* spp.) resistance was inherited qualitatively by single recessive or dominant gene or quantitatively depending on the genetic resources and the pathogen isolates used. We used PBC81 belonging to *Capsicum baccatum* as the resistant parent and made an interspecific cross with a *C. annuum* accession. Since the interspecific hybrid plants were completely male sterile, interspecific BC<sub>1</sub>F<sub>1</sub> progenies as the first segregating population were used for inheritance analysis on the resistance. Distribution pattern of disease response and comparatively lower heritability in the population suggested that the resistance might be controlled quantitatively. Therefore, we carried out QTL mapping in the two different BC<sub>1</sub>F<sub>2</sub> populations using AFLP and SSR markers. In the first population, total introgressed genome consisted of 7 linkage groups was about 250 cM and a major QTL explaining about 50% of genetic variation was identified on LG 5. In the second population, 13 linkage groups covering about 400 cM in genome size were mapped and a major QTL explaining more than 50% of genetic variation was detected on LG 8. And then, we integrated the both maps with common molecular markers to confirm whether the major QTLs of both maps are the same locus or not. As the result of that, the both major QTLs were same locus positioning on LG 2 of the integrated map. In addition, we found that a major QTL associated with the resistance to *C. capsici* isolate, located on LG 7 and explaining about 50% of variation in this integrated map. To develop molecular markers tightly linked to the major QTLs, extreme target BSA method was used. Consequently, we developed several new markers much tightly associated with the major QTLs. The selection efficiency of the closest marker around the major QTL associated with the resistance to *C. acutatum* and *C. capsici* isolate was about 80% and 77%, respectively. Now, we are going to convert the AFLP markers linked to the major QTLs to the simple PCR-based CAPS or SCAR marker using universal genome walking kit.

## Transgenic Peppers Transformed with Anthracnose Resistance Related Genes

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One of the most serious diseases that caused a drastic yield loss of pepper is Anthracnose. Although many laboratories in the world have identified the resistant sources and wanted to obtain a resistant pepper by classical breeding, no successful variety against Anthracnose has ever been bred. There are two major reasons why such dragging is present. First of all, no such a clear genetic source for Anthracnose resistance derived from *C. annuum* was available. Instead, several genetic sources from *C. baccatum* and *C. chinense* were selected and these were hardly crossed with *C. annuum* even though some successful resistant BCF lines obtained by crossing *C. baccatum* and *C. chinense* to *C. annuum* were reported from AVRDC and Seoul National University. However, a resistant line with *C. annuum* genetic background has not been established yet. Second, the Anthracnose resistance seems to be inherited by polygenes. Therefore, whenever the crossing continues for the breeding program, the resistant characteristics would be selected out and would not be stably fixed.

We have isolated genes related to Anthracnose resistance using differential screening from the resistant resources such as PI594137, and some of the full genes were transformed to pepper by callus induced transformation technique. Here, we present transgenic peppers transformed by Anthracnose resistant related genes. By doing transgenic research for many years from now, we hope we could be able to generate the Anthracnose resistance pepper.

# Development and Optimization of a Microsatellite Marker Library for *Colletotrichum capsici*, a Major Causal Agent of Anthracnose Disease in Chili Pepper

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Genomic variation within and among populations may directly impact on the ability of a pathogen to overcome control measures such as host resistance genes through recombination and adaptation. Therefore, to ensure that durable resistance is bred, the breadth of the available variation within the pathogen population must be examined. The amount and structure of genetic variation within populations of the fungal pathogen *Colletotrichum capsici*, the major causal agent of anthracnose disease in chilli (*Capsicum* spp.), has not been determined. However, morphological studies showed a wide variation among isolates collected from different regions of Thailand, compared to those collected from Australia. Molecular markers, such as sequence-tagged microsatellite sites (STMS) markers, enable a far greater depth of discrimination and potential to measure genetic variation among closely related genomes than traditional morphological or biochemical markers. Therefore the aim of the study was to develop and optimise a set of STMS markers to be used in the future to evaluate the population diversity and structure of *C. capsici* infecting chilli from Thailand and Australia. Since STMS markers are highly species specific, several insert clone libraries enriched for microsatellite repeats were developed based on inter simple sequence repeat (ISSR) amplification of the *C. capsici* genome. In this study, the repeat motifs of (TA)<sub>15</sub>, (AG)<sub>15</sub>, (TG)<sub>15</sub>, (GAA)<sub>14</sub>, (TAA)<sub>14</sub> and an admixture of the genomic DNA of five Thai isolates were used for library construction. To date, 31 robust and reliable STMS primer pairs were developed and optimised to amplify single locus alleles. Of these, 10 loci were polymorphic among 16 *C. capsici* isolates that were sourced from a wide geographic distribution within Thailand and Australia. At each of the 10 loci, 3-8 alleles were observed that ranged in size from ~100bp to 500bp. The loci *CCSSR1* appeared to be the most informative, at which eight alleles of

between 100bp and 300bp were amplified. Selected primer pairs will be labelled with fluorochromes and allele distribution within the isolates will be identified using capillary electrophoresis.

# Identification of Parents Suitable for Mapping and Tagging Genes Conferring Resistance to Anthracnose in Hot Pepper

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Field and laboratory investigations were carried out to identify contrasting lines of hot pepper (*Capsicum* spp.) to act as parents of a population for mapping and tagging genes conferring resistance to anthracnose (*Colletotrichum* spp.) in Southern India. The field disease screen involved 70 *C. annuum*, two *C. frutescens* and two *C. baccatum* lines. Seven lines (PBC 80, PBC 81 (*C. baccatum*), PBC 142, Kunchangi Local, Puri Local, male-sterile line CCA 7244 and its maintainer, 9907-9611 (*C. annuum*)) were free from anthracnose, while, fruits of Pusa Jwala, Utkal awa and CA 14 (all *C. annuum*) were severely infected. Fruits from each of these seven resistant lines and three highly susceptible lines were subjected to a lab-based screening for anthracnose resistance, using a pin prick method with five representative S. Indian isolates of both *Colletotrichum capsici* (Cc) and *C. gleosporioides* (Cg). The lines were categorized as immune, resistant, moderately resistant, moderately susceptible and highly susceptible, based on the number of infected fruits and lesion size 8 days after infection. PBC 80 was immune to all five isolates of Cc and Cg, and PBC 81 was immune to only two (one each of Cc and Cg) of the isolates and resistant to the other three. Five lines (PBC 142, Kunchangi Local, Puri Local, male-sterile line CCA 7244 and its maintainer 9907-9611) ranged from moderate resistance to complete susceptibility; and three lines (Utkal awa, Pusa Jwala and CA 14) were completely susceptible to all five isolates. The resistant (PBC-80 & PBC-81) and susceptible parents (Utkal awa, Pusa Jwala and CA 14) are currently being used to develop mapping populations to tag anthracnose resistance with molecular markers.

# Expression Profiling of Genes Involved in Preinfection-Related Development Stages of Pepper Anthracnose Fungus, *Colletotrichum acutatum*

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Species of *Colletotrichum* cause diseases on a wide range of crop plants. As *C. acutatum* especially is destructive on pepper fruits, yield losses due to pathogen is considerable. *C. acutatum* has been used as an important pathogen in the studies on development and gene expression of infection structure. *C. acutatum* has different preinfection-related development stages such as mycelium, spore, germinating spore and appressorium forming spore. This experiment describes the gene expression profiling through EST sequencing and microarray analysis on the preinfection-related development. EST sequencing was performed with germinating spore stage cDNA library to identify genes involved. ESTs of over 1,000 cDNA clones generated 197 contigs with more than two ESTs included. DNAs for microarray were available from amplifying the unique gene set of 192 cDNA clones. These clones have inserts with average size of 1.5kbp. DNAs for microarray were solubilized to 50uM (pmol/ul) in 50% DMSO and spotted on amine coated glass. Total RNAs were isolated from different development stages and labeled by Cy3 and Cy5 for cDNA probes. The cDNA microarrays were hybridized and analyzed for expression profiling study.

# Characterization of an ABC Transporter Gene, *CaABC1*, from the Pepper Anthracnose Fungus, *Colletotrichum acutatum*

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*Colletotrichum acutatum*, a causal agent of pepper anthracnose, is an important pathogen resulting major yield loss in pepper production. The disease has been mainly controlled by fungicides with selective or non-selective mode of action. Fungal plant pathogens use several mechanisms to circumvent the inhibitory actions of fungicidal agents. ATP-binding cassette (ABC) transporter is known to be involved in drug resistance in a number of pathogenic microorganisms. This research is to clone and analyze the function of putative ABC transporter gene, *CaABC1*, for understanding fungicide resistance in *C. acutatum*. Part of the gene fragment was obtained from the EST sequencing of *C. acutatum* cDNA library. *C. acutatum* genomic Fosmid library with average insert size of 35kb was constructed for gene cloning. To clone *CaABC1*, the library was screened by membrane hybridization and pooling PCR. The *CaABC1* genomic fragment was identified in three Fosmid clones from a pooling PCR screening. Partial sequence information of the gene shows homology against ABC transporter genes from other species. Genomic DNA Southern blot analysis shows that *CaABC1* exists as a single copy in the genome of *C. acutatum*. Expression pattern of *CaABC1* is being verified through Northern blot analysis.



## First International Symposium on Chili Anthracnose

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