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Does higher phenol content really confer resistance to anthracnose disease in chilli?

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ABSTRACT: *Chilli (Capsicum annuum L.) is an important crop in many parts of the world. The major constraint to chilli production includes various biotic stresses among which anthracnose or fruit rot caused by Colletotrichum spp., is the major disease mainly affecting fruit quality. Anthracnose is mainly a problem on mature fruits, causing severe losses via both pre and post-harvest fruit decay (Hadden and Black, 1989; Bosland and Votava, 2003). Most chilli producing areas of the world experience heavy reduction in produce quality on infestation with this disease (Than et al., 2008). Several species of Colletotrichum viz., C. capsici (Butler and Bisby), C. gloeosporioides (Penz.), C. acutatum (Simmonds), C. atramentarium (Berk and Broome), C. dematium (Pers.) and C. coccodes (Wallr.), Glomerella cingulata (Stoneman) along with A. alternata (Keissler) are involved in spread of this disease worldwide (Than et al., 2008). Typical anthracnose symptoms on chilli fruit include sunken necrotic tissues, with concentric rings while in some cases the lesions are brown and then turn black. Fruit rot reduces dry weight, capsaicin and oleoresin content of affected fruits (Mistry et al., 2008), leading to reduction in the medicinal properties of chilli.*

Key words: *Phenol content, lesion diameter, anthracnose resistance, correlation coefficients*

Plants display a great diversity in the array of secondary compounds synthesized by them when compared to animals as they face constraint for physical mobility to escape their predators and therefore have evolved a chemical defence against such predators. Plant phenolics are one category of secondary metabolites that are needed for resistance to pathogens and for many other functions. Generally, the role of phenolic compounds in defence is related to their antibiotic, antinutritional or unpalatable properties. Plant phenolics may be divided in two classes: (i) preformed phenolics that are synthesized during the normal development of plant tissues and (ii) induced phenolics that are synthesized by plants in response to physical injury, infection or when stressed by suitable elicitors. Induced phenolics may also be constitutively synthesized but, additionally, their synthesis is often enhanced under biotic or abiotic stress.

Role of phenolics in providing resistance to fungal diseases was first reported in onion where sufficient quantities of catechol (I) and protocatechuic acid (II) (phenolic compounds) was found to prevent onion smudge disease caused by *Colletotrichum circinans*. These two phenols were present in sufficient quantity in onion scales to reduce spore germination of *C. circinans* to below 2%, while susceptible varieties lack these compounds and the germination rate is over 90% (Walker and Stahmann, 1995). Resistance of potato tubers against *Streptomyces scabies*, *Verticillium alboatrum* and *Phytophthora infestans* (Johnson and Schaal, 1952) have been associated to adequate levels of chlorogenic acid (III) while lower concentrations stimulates the growth of *P. infestans* and *Fusarium solani* var. *coeruleum*. Benzaldehyde (IV) in lower concentrations strongly inhibits spore germination of *Botrytis cinerea* and germination of *Monilia fructicola* (Wilson and Wisniewski, 1989). *In vitro* studies reveal that phenolic compounds extracted from olive plants (*Olea europaea* L.) tyrosol, catechin, and oleuropein, showed antifungal activity, thus affecting plant resistance against *Phytophtora* spp. (Del Rio, 2003). Naringenin (flavanone), dihydroquercetin (dihydroflavonol), kaempferol, and quercetin (flavonols) have been tested for their biological activity against two fungal rice pathogens, *Pyricularia oryzae* and *Rhizoctonia solani* (Amborabé, 200). In summary, preexisting antifungal phenolics are simple

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phenols, phenolic acids, flavonols and dihydrochalcones. This study focuses to understand the role of phenolics in the resistance mechanisms of chilli against *Colletotrichum spp*.

There are some reports trying to relate the phenol content with anthracnose resistance in chilli. Prasath and Ponnuswamy (2008) reported that resistant and moderately resistant genotypes to anthracnose disease had significantly higher amount of total and orthodihydroxy phenols as compared to susceptible ones. Similar reports have also been given by Kaur *et al*. 2011 stating that orthodihydroxy phenols were higher in resistant genotypes. Study conducted by Pratibha *et al*. (2013) revealed that there is substantial reduction in phenol content on infection by anthracnose fungus.

The experiment was conducted at the Department of Genetics and Plant Breeding, GKVK, UAS Bangalore during the year 2010-2011. The experiment consisted of two sets of material (i) A set of seventy chilli lines (including varietal lines from various parts of the country) and (ii) a set 240 F_2 individuals developed from the cross of *Capsicum baccatum* lines i.e PBC-81 and SB-1. There are many reports stating PBC-81 to record complete resistance to anthracnose and SB-1 showing extreme susceptibility to the disease among the *baccatum* complex. Because of crossing incompatibities between *C. annuum* and *C. baccatum*, the F_2 population was developed from within *C. baccatum* species cross.

Both the sets of two material included in the study were screened for resistance to anthracnose disease by artificial inoculation method. The Cc (*Colletotrichum capsici*.) 38 cultures maintained at the HPI unit, Department of GPB, CoA, UAS, GKVK, Bengaluru was used for microinjection method of screening for anthracnose reaction. Cc 38 culture of *Colletrotrichum* spp. was found to be most virulent strain among 72 *Colletotrichum* spp collected and screened for their virulence by Nanda (2011). The size (diameter) of the lesion was recorded nine days after by using a specialized scale and expressed in milli meters.

The 70 chilli lines were grown in two replication and 10 fruits from each replication were screened for lesion diameter upon inoculation. Similarly, each individual F_2 plant was numbered and the observations were recorded on response to anthracnose disease infection in each of the F_2 plant, their F_1 and the parents (PBC 81 and SB 1). Ten random fruits of each F_2 were picked at red ripe stage. The fruits were surface sterilised and inoculated with virulent strain of *Colletotrichum capsici i.e.*, '*Cc* 38'. Disease reaction was recorded in terms of lesion diameter.

Both sets of experimental material were also phenotyped for total phenol content. Fruits for phenol estimation were collected from the same plant from which they were collected for anthracnose screening. Total phenol content was estimated using the Folin-ciocalteau reagent technique (Singelton *et al*., 1999). In this method 500 mg of dry chilli powder was added with ten times the volume of 80 per cent ethanol. The homogenate was centrifuged at 10,000 rpm for 20 minutes, the supernatant was saved and the residue re-extracted with five times the volume of 80 per cent ethanol, centrifuged and supernatant was dissolved. The supernatant was evaporated to dryness and the residue was dissolved in a known volume of distilled water. Different aliquots were pipetted out into test tubes and volume was made up to 3 ml in each tube with water. 0.5 ml of folin-ciocalteau reagent was added and after 3 min, 2ml of 20 per cent Na_2CO_3 solution was added to each tube. The contents were mixed thoroughly, and the tubes were placed in boiling water for one minute. After cooling, absorbance was measured at 650 nm against a blank reagent. A standard curve was plotted using different concentrations of catechol. From this standard curve, the concentration of phenols in the test sample was determined.

Relation between lesion diameter (on artificial inoculation with *Colletotrichum capsici* and *Colletotrichum gleosporoides*) and total phenol content was determined using Pearsons correlation coefficients. Estimates of correlation coefficients between response to anthracnose recorded in lesion diameter and total phenol content was found to be significant and negative among the set of 70 chilli lines (Table 1). In contrast to this, correlation coefficient estimated among the F_2 individuals was also negative but non significant (Table 2).

Phenols are known to have a role in defense against biotic stresses. Association of total phenol content with response to anthracnose disease would provide clues to devise selection strategies to develop anthracnose resistant/tolerant cultivars. Significant and negative correlation was observed in the first of set of experimental material comprising of 70 *C. annuum* lines. This provides evidence of possible role of higher phenol content in conferring resistance to anthracnose disease. Contrary to this non significant correlation among the F_2 individuals suggested possible independent control of the genes controlling

Table 1
Estimates of correlation coefficients of lesion diameter on infection with Colletotrichum spp. with their total phenol content in 70 genotypes of chilli

	Lesion diameter on infection with Cg isolates	*Lesion diameter on infection with Cc isolates*	*Phenol content*
Lesion diameter on infection with *Cg* isolates	1		
Lesion diameter on infection with *Cc* isolates	0.707**	1	
Phenol content	-0.390**	-0.287**	1

Table 2
Estimates of correlation coefficients of lesion diameter on infection with Colletotrichum spp. with their total phenol contentamong the F2 individuals of the cross PBC-81×SB-1

	Lesion diameter on infection with Cg isolates	*Lesion diameter on infection with Cc isolates*	*Phenol content*
Lesion diameter on infection with *Cg* isolates	1		
Lesion diameter on infection with *Cc* isolates	0.927429**	1	
Phenol content	-0.043	-0.073	1

these two traits under study. Two contrasting results obtained from two different sets of experimental material in our study raises a question: Results from which of these two material are more reliable, collection of varietal lines or F_2 individuals? As argued in association mapping studies, reliability of results largely depends on the structure of the population under study. The population structure (existences of distinctly clustered subdivisions in a population) and population admixture are the main factors to create spurious association between unlinked loci. Theoretically, relatedness generates association between linked loci, yet it might also generate association between unlinked loci pairs when predominant parents exist in germplasm groups. This primarily happens due to the occurrence of distinct allele frequencies with different ancestry in an admixed or structured population. There are several evidences that relatedness has resulted in correlation between linked and unlinked loci in an equal proportion (Stich *et al*, 2005, 2006, 2007). Several reports of spurious association found between markers and traits under study in association genetics is good indicative to draw conclusion about the role of selection or population stratification (cryptic relatedness) in generating false correlation between two loci (Huttley *et al*., 1999, Remington *et al*., 2001, Gupta *et al*., 2005). The other factors such as genetic drift or bottlenecks might have also generated linkage disequlibrium (LD) among different loci in a genome (Pritchard, 2000), which is evidenced by nonuniform distribution of LD in chromosomes (Maccaferri, 2005). One of those factors is selection that generate LD between unlinked loci through "a hitchhiking" effect (high-frequency sweeping and fixation of alleles flanking a favored variant) and epistatic selection or the so-called coadapted genes (Cannon, 1963).

With the above discussions, it can be said that F_2 population forms a better population to study correlation statistics in comparison to the set of chilli varietal lines. This varietal collection includes lines primarily bred and developed in India. So it clearly supports the possibilty of common ancestry and relatedness among the varietal collection. Apart from this, these varieties have been developed upon strong selection pressure for similar kind of plant architecture as well as other preferred economic traits. This definitely would result in co-selection of loci during breeding for multiple traits (Caldwell, 2006), common in traditional crop breeding programs worldwide. F_2 population negates most of the possibilities of co-adaptation of genes due to common ancestry or high selection pressure. Hence, non significant associations precludes the possibility of total phenol content in conferring resistance to anthracnose in chilli as obtained from the results. Non significant correlation indicates independent genetic control of two categories of traits. Independent segregation is possible when the loci controlling these traits are present on different chromosomes or if present on the same chromosome, they are present farther apart to cause independent segregation. Such similar results were reported by Naveen (2009). Jabeen *et al.*, (2009) reported weaker role of phenols in conferring fusarium wilt resistance in *Capsicum annuum*.

SUMMARY

Pre-formed antibiotic compounds such as phenolic and polyphenolic compounds are ubiquitous in plants and have been found to play an important role in resistance to fungi. Fruit rot in chilli caused by the fungus *Colletotrichum spp.* causes heavy losses in chilli fruit quality. The presence of this disease in India has a great impact on chilli production and export. The present study was undertaken to find the possible role of phenols in conferring resistance to anthracnose in chilli. For this a set of 70 *Capsicum annuum* L. varietal lines and F_2 population developed from the cross of resistant and susceptible parents from *Capsicum baccatum* complex was screened for reaction to anthracnose and phenol content. Pearsons correlation (r) statistic was calculated between lesion diameter on artificial inoculation (with *C. capscici* and *C. gleosporoides*) and phenol content separately for the set of 70 chilli lines and F_2 individuals. Significant and negative correlation was found between lesion diameter and phenol content among the set of chilli lines while it was non-significant and negative among the F_2 individuals. F_2 populations are the best material to study correlation statistics as estimates of correlation coefficients from germplasm collection may be spurious in nature basically due to its population structure. This study therefore fails to support the hypothesized fact that phenols have a role in conferring resistance to anthracnose in chilli.

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