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Influence of Capsaicin and Phenols on Resistance to *ChiLCV* and Anthracnose Diseases in Chilli (*Capsicum* spp.)

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Abstract

Chillies are integral and the most important ingredient in many different cuisines around the world as it adds pungency, taste, flavour and color to the dishes. Being rich in different secondary metabolites such as capsaicin and phenols, chilli also harbours various pests and pathogens leading to major economical loss in its production. Chilli Leaf Curl Virus (*ChiLCV*) and *Colletotrichum capsici* (anthracnose) are the two major diseases, whose resistance varies across the genotypes. As there is also a range of variation in capsaicin and phenolic content of different chilli genotypes, we decided to investigate for any association between these two secondary metabolites on development of *ChiLCV* and anthracnose diseases in a set of 144 chilli genotypes using F-test. Based on spectrophotometrically estimated amount of capsaicin and phenols present in chilli fruit, 144 genotypes were classified into three different classes *i.e.,* 33, 96 and 15 genotypes with High, medium and low capsaicin content as well as 14, 75 and 55 genotypes with high, medium and low total phenolic content, respectively. Significant difference between these distinct groups based on capsaicin and phenols for *ChiLCV* and anthracnose disease development, respectively suggested the influence of capsaicin on *ChiLCV* and phenols on anthracnose disease resistance. Further correlation analysis indicated, the investigated association from F-test to be significantly negative, implying that breeding for chilli genotypes with high capsaicin and phenolic contents may enhance their resistance to *ChiLCV* and anthracnose diseases.

Keywords : Chilli, Capsaicin, Phenols, *ChiLCV*, Anthracnose, F-test, Correlation

Chilli, also known as hot pepper/mirch, is an indispensable ingredient in every diet of almost all the households in tropical and subtropical countries. Chilli belongs to the genus *capsicum* that comprises of approximately 30 different species, out of which five (*C. annuum, C. chinense, C. baccatum, C. frutescens, C. pubenscens*) are cultivated worldwide. Among five cultivated species, *C. annuum* contributes majority of India's overall production followed by *C. chinense* and *C. baccatum*. India contributing to 43 per cent of the global chilli production, is the world's largest chilli producer as well as exporter. As per the advanced estimates of 2022-2023, India produces 4582.90 and 2060.00 Metric tonnes (Mt) of green and dry chillies in an area of 4.32 and 8.50 lakh hectares, respectively. In India, Andhra Pradesh produces the most chillies, followed by Telangana, Madhya Pradesh and Karnataka, accounting for 32, 28, 16 and 9 per cent of the nation's total production (Indiastat, 2023). Bellary, Gadag, Dharwad and Haveri are the principal chilli producing districts of Karnataka (Ganesh *et al.,* 2022).

Capsicum spp., enjoying the positions in both vegetable as well as spice groupings (Kumar *et al.* 2021), is one of the most consumer admired and economically important plants worldwide. Since chilli

fruit harbours most of the secondary metabolites, probably no other cultivated species is used in as many ways as chilli, like vegetable, spice, condiment, ornament and medicine. Capsaicin, one of the most abundant secondary metabolites present in chilli fruit can be credited for its therapeutic values such as antioxidant, anti-inflammatory, antitumoral and weight loss properties in addition to the hotness it offers. Generally, antioxidants present in plants such as chilli, facilitate to enhance the defence mechanisms against various diseases, including viral infections as well (Rajput *et al.,* 2021). Phenolic compounds are another class of secondary metabolites found in plants, and they have been shown to play various roles in plant biology including biotic stress resistance by acting as anti-nutritional repellent for most of pests and diseases (Florencio-Ortiz and Casas, 2021), thereby adding to the economic importance of chilli fruit. Hence, their antioxidant property makes capsaicin and phenols stand unique in maintaining plant defence mechanism by influencing plant metabolic pathways such as phytoalexin biosynthesis and reactive oxygen species generation (Pratyusha, 2022), which is the reason for concentrating on theses two secondary metabolites in the present study.

Alike any other crop species, chilli also experiences major production loss from different pest infestation and diseases infection. Numerous pathogens, including bacteria, viruses, fungi and insects are known to live and thrive in chilli, resulting in major economic losses. Among the viruses, Chilli leaf curl virus (*ChiLCV*) is one of the major pathogens infecting chilli resulting in worldwide economic losses. It is known to cause up to 100 per cent marketable yield losses (Kumar *et al*., 2015 and Rao *et al*., 2020). While, the virus causes symptoms in whole plant, fungus *Colletotrichum* sp. cause water-soaked sunken lesions on red and green fruits, leaving them unfit for marketing. The symptom caused by this fungal pathogen is widely known as chilli anthracnose. The pathogen can infect marketable fruits both in field and storage conditions. Anthracnose is known to cause up to 80 per cent losses (Cui *et al.,* 2023) as even a single anthracnose speck on the fruit reduces its marketability considerably. Thus, good fruits marketability requires that they should be free from *ChiLCV* and anthracnose diseases. If there exists positive strong genetic correlation between resistance to these diseases and higher levels of secondary metabolites, it is relatively easy for developing chilli cultivars with good level of resistance to these diseases. However, if there exists negative strong relationship between resistance to diseases and secondary metabolites, there is a need to strike a balance between levels of resistance to diseases and secondary metabolites.

On this backdrop, we hypothesised that secondary metabolites such as capsaicin and phenols present in chilli fruits might influence the development of these two major diseases, *ChiLCV* and anthracnose as the defensive role of plant's secondary metabolites have been confirmed in many of the previous studies (Zaynab *et al.,* 2018; Mes *et al*., 2000 and Mansfield 2000).

Material and Methods

The experimental material consisted of 144 genotypes, encompassing four different species of capsicum (Fig. 1). Out of 144 genotypes, 72 were procured from World Vegetable Centre (WVC), Taiwan and remaining were collected from chilli germplasm repository of Hot Pepper Improvement unit (HPI), Department of Genetics and Plant Breeding (GPB), University of Agricultural Sciences (UAS), Bangalore,

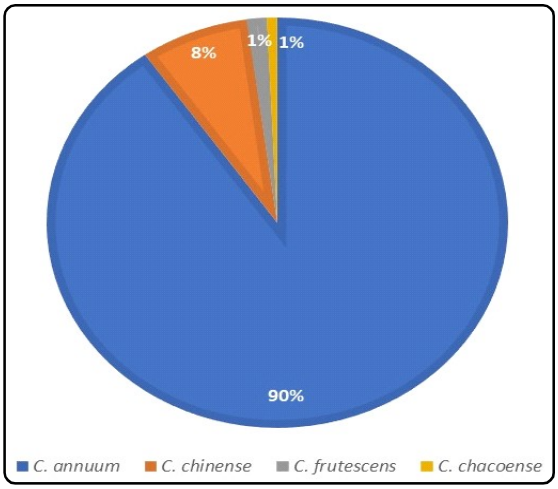


Fig. 1 : Different species of capsicum included in the present study

India. Three separate experiments were conducted to evaluate the genotypes for fruit capsaicin and phenolic content, responses to C. *truncatum (*previously known as *C. capsici)* infection and for challenge inoculation of *Chilli Leaf Curl Virus* (*ChiLCV*), respectively.

Fourty days old seedlings of the accessions were planted in alpha lattice design in single rows of 4.5m length with two replications at the experimental plots of Department of GPB, K-block, GKVK, UAS, Bangalore during summer 2021. Matured red fruits were collected from randomly selected plants from each genotype and in each replication. Further, collected fruits were shade dried. A fine red chilli powder was made from ten randomly selected sun-dried fruits. Dried red chilli powder of each genotype was used for the estimation of capsaicin and phenols present in the fruits.

Capsaicin Estimation

The capsaicin content of fruits was estimated by colorimetric method as described by Bajaj *et al*. (1980). Firstly, 0.5g of dry chilli powder was weighed into glass-stoppard test tube. Later, 10ml dry acetone (add 25g anhydrous sodium sulphate to 500ml of acetone at least one day before use) was added into the test tube and kept overnight for extraction. Next day samples were centrifuged at 10000 rpm for 10min to get clear supernatant. One ml of the supernatant was taken into a test tube and evaporated to dryness in a hot water bath. Then, the residue was dissolved in 5ml of 0.4 per cent of NaOH solution and 3ml of 3 per cent phosphomolybdic acid was added. The contents were shaken and left undisturbed for 1hr. After 1hr, the solution was quickly filtered into centrifuge tubes to remove any floating debris and then centrifuged at 5000rpm for 15min. The clear blue coloured solution was directly transferred into the cuvette and absorbance was read at 650nm along with a reagent blank. A standard graph was prepared using 0-200µg pure capsaicin. Simultaneously 0.2, 0.4, 0.6, 0.8 and 1ml of working standard solution (stock standard capsaicin solution was prepared by dissolving 50mg capsaicin in 50ml of 0.4 per cent NaOH solution (1000µg/ ml) and working standard solution prepared by diluting the 10ml of the stock standard to 50ml with 0.4 per cent NaOH solution (200µg/ ml)) was taken into new test tubes and proceeded as mentioned above (Tirupathamma *et al.,* 2018).

Phenol Estimation

Further, total phenolic content was measured from the dry red chilli powder spectrophotometrically following the procedure outlined by Siddhuraju (2007). The reaction mixture contained 50 per cent Folin-Ciocalteu reagent (0.5ml), 20 per cent (w/v) sodium carbonate solution (2.5ml) and methanolic extracts of sample (1.0ml). The mixture was placed in the dark for 40 minutes and the absorbance was recorded at 750nm against a blank with the spectrophotometer. Preparation of the calibration curve for total phenolic content determination was carried out using gallic acid (2 - 10µg/ml). The total phenolic content was expressed based on gallic acid equivalent (GAE). The results were expressed as µg of gallic acid equivalents (GAE)/g fruit weight.

Table 1

Classification of genotypes of the association panel based on *percent* disease index

% Disease index	Disease reaction category
< 5	Highly resistant (HR)
5 - 20	Resistant (R)
20 - 40	Moderately resistant (MR)
40 - 64	Moderately susceptible (MS)
>64	Highly susceptible (HS)

***ChiLCV* and Anthracnose Screening**

Genotypes were evaluated for responses to *ChiLCV* by following augmented inoculation methodology through starved viruliferous whiteflies maintained separately in the glass house through challenge inoculation in two years namely, 2019 and 2021 summer seasons following the procedure given by Barchenger *et al*. (2019) in the experimental plots of Main Research Station (MRS), Hebbal, Bengaluru, Karnataka, India. Test plants were quantified for their

responses to *ChiLCV* infection after 60 days post inoculation using the standard scale (Table 1). Disease scores were then converted into *Percent* Disease Index (PDI) (Mckinney, 1923).

During 2020 and 2021 rainy seasons, genotypes were evaluated for responses to *Colletotrichum trancatum* infection employing microinjection of detached fruit method (AVRDC, 2003). Ten randomly selected fruits were harvested from each genotype at color break stage from five tagged plants that are retained till maturity. Subsequently the harvested fruits were brought to laboratory in HPI Unit, Department of GPB, GKVK, UAS, Bangalore for artificial inoculation with 1µL of calibrated pathogen spore suspension under sterilized conditions. Without infection, normal shelf life of the detached fruits would be upto 10 days (Edusei *et al.,* 2012). While, upon infection lesions are developed on the detached fruits, whose diameter was recorded as Overall Lesion Size (OLS) and True Lesion Size (TLS) in millimetre after eight days of inoculation.

Statistical Analysis

The genotypes were classified into three classes, namely high, medium and low based on capsaicin and phenol contents. Each of the genotypic classes classified based on capsaicin content ranged from 134.63 - 715.88 µg/ml, 719.94 - 1292.13 µg/ml and 1302.13 - 1879.63 µg/ml for low, medium and high classes, respectively. Similarly low, medium and high classes based on phenols ranged from 2.01 - 2.56 mg GAE/ml, 2.68 - 3.28 mg GAE/ml and 3.31 - 3.93 mg GAE/ml, respectively. Significance / otherwise of differences among the genotypes classified based on capsaicin and phenolic content for PDI, OLS and TLS was examined using F-test (Fisher, 1950). Non-significance and otherwise of F tests indicate lack of influence and significant influence of capsaicin and phenols on responses to *ChiLCV* and anthracnose (Ranjitha *et al.,* 2023). Strength of the association between secondary metabolites (capsaicin and phenols) and responses to *ChiLCV* and anthracnose diseases was then examined through correlation analysis in R software.

Results and Discussion

Based on the content of capsaicin and phenols present in chilli fruit, 144 genotypes were grouped into three different classes *i.e.,* 33, 96 and 15 genotypes with high, medium and low capsaicin content while, 14, 75 and 55 genotypes with high medium and low total phenolic content, respectively.

The genotypes (High, Medium and Low) categorized based on the capsaicin content differed significantly with respect to responses of *ChiLCV* infection, measured as PDI (Table 2). On the other hand, they did not differ significantly for *Colletotrichum capsici* infection response quantified through TLS and OLS (Table 3). These results suggest the influence of capsaicin content on *ChiLCV* disease development in chilli. However, though correlation coefficient between PDI due to infection by *ChiLCV* and capsaicin content was significantly negative (Fig. 2), the strength of the relationship is not strong enough. Thus, it is possible to develop cultivars resistant to *ChiLCV* with high levels of capsaicin content.

Table 2

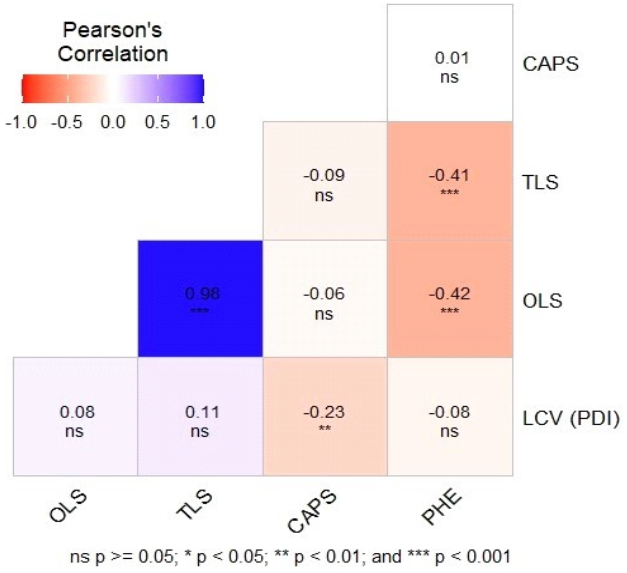
Single factor analysis of variance of Percent Disease Index (PDI) due to infection by *ChiLCV* among chilli genotypes classified based on capsaicin

Capsaicin	Mean *ChiLCV* (PDI)	Source of variation	Degrees of freedom	Mean sum of squares	P value
				ChiLCV (PDI)	
Low	46.91	Between groups	2	4371.254	0.003325
Medium	53.27	-	-	-	-
High	27.63	Within groups	141	735.44	

TABLE 3

Single factor analysis of variance of Overall Lesion Size (OLS) and True Lesion Size (TLS) due to infection by anthracnose among chilli genotypes classified based on capsaicin

Capsaicin	Mean		Source of variation	Degrees of freedom	Mean sum of squares		P value
	OLS	TLS			Overall Lesion Size (OLS)	True Lesion Size (TLS)	
Small	24.06	24.66	Between groups	2	38.81	59.39	OLS0.5269
Medium	22.73	22.91					
Large	21.47	21.58	Within groups	141	60.31	53.81	TLS0.3350



(CAPS : Capsaicin, TLS : True Lesion Size, OLS : Overall Lesion Size, LCV (PDI) : Percent Disease Index due to *ChiLCV* and PHE : Phenols)

Fig. 2 : Heat map of Phenotypic correlation coefficients of PDI (*ChiLCV)* and TLS & OLS (Anthracnose) with secondary metabolites

TABLE 4

Single factor analysis of variance of Overall Lesion Size (OLS) and True Lesion Size (TLS) due to infection by anthracnose among chilli genotypes classified based on phenols

Phenols	Mean		Source of variation	Degrees of freedom	Mean sum of squares		P value
	OLS	TLS			Overall Lesion Size (OLS)	True Lesion Size (TLS)	
Small	28.98	28.99	Between groups	2	424.67	357.29	OLS0.0006
Medium	23.49	23.58					
Large	20.55	21.13	Within groups	141	54.84	49.58	TLS0.0010

TABLE 5

Single factor analysis of variance of Percent Disease Index (PDI) due to infection by ChiLCV among chilli genotypes classified based on phenols

Phenols	Mean *ChiLCV* (PDI)	Source of variation	Degrees of freedom	Mean sum of squares *ChiLCV* (PDI)	P value
Low	50.81	Between groups	2	268.69	0.7132
Medium	52.84	-	-	-	-
High	48.73	Within groups	141	793.07	

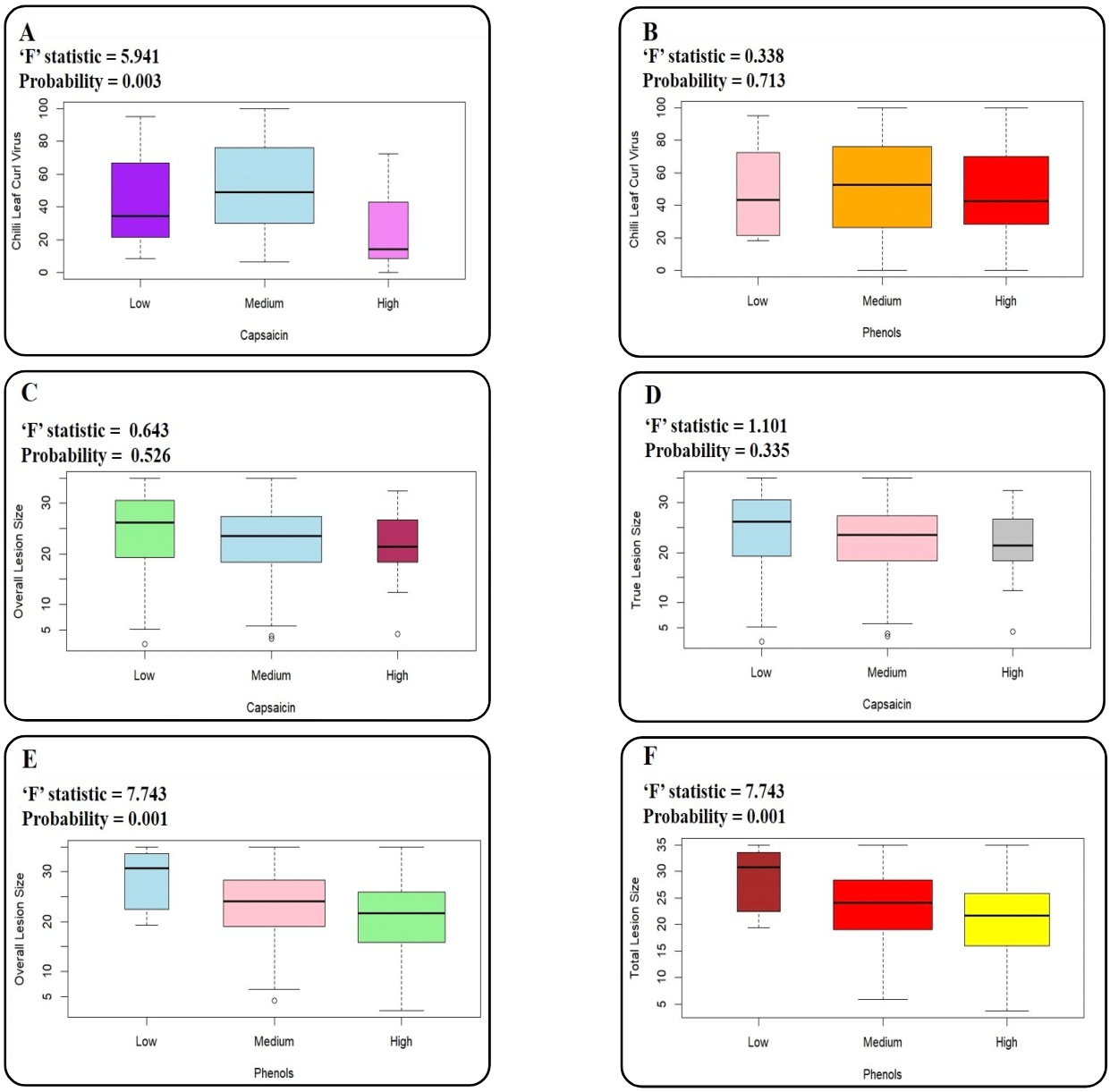


Fig. 3 : Box plots depicting the (A) effect of capsaicin on *ChiLCV* (B) phenols on *ChiLCV,* capsaicin on (C) OLS (D) TLS, phenols on (E) OLS (F) TLS in 144 genotypes of chilli

Based on phenolic content, chilli genotypes were grouped into three distinct classes, differing significantly with respect to *Colletotrichum trancatum* infection (Table 4), suggesting the influence of phenols on responses to anthracnose disease. However, the same was not true with respect to responses to *ChiLCV* disease. Though correlation of response to anthracnose diseases and phenols was significant and negative, the strength was not strong enough. These results are in agreement with those of Prasath and Ponnuswami (2008) and Srideepthi *et al.* (2017). Phenolic attributes such as antimicrobial nature, cell wall strengthening capacity, Reactive Oxygen Species (ROS) Scavenging and phytoalexin production might support in the development of resistance to this fungal disease (Kaur *et al.,* 2022 and Chrpova *et al.,* 2021) in chilli.

Implications in Plant Breeding

In chilli, breeding for resistance to diseases such as anthracnose and *ChiLCV* involves selecting and developing chilli cultivars that are less susceptible to these diseases. Capsaicin and phenols are the group of secondary metabolites found in chilli, possessing several implications in this context of resistance breeding. Our study suggested that capsaicin and phenols influence resistance to *ChiLCV* and anthracnose diseases, respectively.

A breeder can select for those chilli cultivars with high phenols and capsaicin, as this can enhance the plants natural defense mechanism. Selection for high phenols and capsaicin can be done either by phenotypic screening, following spectrophotometric or High Performance Liquid Chromatography (HPLC) based protocols or by genotypic screening, employing genetic markers that are associated with pathways involved in the synthesis of above mentioned secondary metabolites (Nimmakayala *et. al*., 2016, Han *et al.,* 2018 and Rosa-Martínez *et al.,* 2023). Nevertheless, breeders must also consider other important traits such as yield, flavour, pungency level and fruit morphological traits.

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