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Impact of Salt on Capsaicin Synthesis in Three Capsicum Cultivars.

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ABSTRACT

The pungency, lineament of chilli, is conferred by a set of compounds known as capsaicinoids which are synthesized only in fruits of capsicum genus. Accumulation of capsaicinoids in the fruits may be influenced by various environmental factors beyond their optimal range for the plant. A few studies have investigated the impact of abiotic stress on capsaicinoids production. In present study, the impact of salt stress on capsaicin accumulation was evaluated in three capsicum cultivars. To accomplish this objective three capsicum cultivars (CO1, K2 and G4) having different sensitivity level against salt were subjected to various salt concentrations (25mM, 50mM, 100mM, 150mM and 200mM). However, capsaicin accumulation was depended on stress severity, fruit age as well as the genotypes. Capsaicin accumulation was increased in fruits plants treated with low salt concentration as compared to control plants while it was found to be decreasing in fruits of plant exposed to high salt concentration among all selected cultivars. In addition to this effect, capsaicin accumulation was also exhibited correlating with fruit developmental stages.
Keywords: Capsaicin – capsaicinoids - abiotic stress – chilli - salt stress

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INTRODUCTION

The red chilli belongs to genus Capsicum of tropical plant family Solanaceae. Although there are 25 species under this genus capsicum, hot chilli the most cultivated capsicum crop in its natural habitat tropical and temperate areas and belongs to the species *Capsicum annuum*. The chilli is one of the most widely consumed vegetable in fresh fruit as well as processed form. Currently India is the largest producer of chilli with around one million tons per year. Capsicum is known for its unique pungency and color. Chilli has attained its huge demand in food industry due to its colour, flavour and pungency. The pungency, the common feature of chilli, is due to unique group of alkaloids called as capsaicinoids [1]. Capsaicinoids are derived from phenylpropanoid compound [2] and consists of 12 or more alkaloids with structure of vinyllyl amide of branch fatty acid with 9-11 carbons [3]. Capsaicin and dihydrocapsaicin are responsible for 90% of punginess of spices [4].

Many reports have focused on the accumulation of capsaicinoids in Capsicum fruits in relation to fruit age, size, and stage of development [5, 6]. These findings show that capsaicinoids start to accumulate during the early stages of fruit development with maximal accumulation when the fruit approaches the end of its growth phase. Accumulation continues to increase after the fruit attain its maximum length [7]. Enhancement of phytopharmaceutics biosynthesis by *in vitro* cultured plant cells as well as plant can be accomplished by exposing plant cells or plant to stress condition. Exposure to environmental factors such as osmotic stress, water stress or salt stress induces diverse reactions in plants. Reactive oxygen species are also generated in plant under these stress condition [8, 9]. To assess the impact of these stresses on capsaicin biosynthesis, most of these studies have focused on *C. annuum* spices*.* Application of exogenous salicylic acid and methyl josmonate has inducted synthesis as well as accumulation of capsaicinoids in *C. chinense* [10]. The complication of the plant response to salt stress may result in ion toxicity apart from osmotic effects [11]. Plant cells, in response to osmotic stress, tend to accumulate sugars and compatible solutes in the cytoplasm [12] and other osmotically active low molecular weight compounds [13].

Auspiciously, these various compounds induced under stress condition act as precursor as well as enhancer for secondary metabolites[14,15]. For example, *in vitro* cultured plant cells were examined to synthesize extra amounts of soluble phenolics, anthraquinone [16], Flavonoids, indole alkaloids [17] anthocyanin [18] under various abiotic stress.

These results reflect the fact that pungency levels in peppers are determined by two factors: genetics and plant–environment interactions [19]. The main objective of the present study evaluates to impact of salt treatment on capsaicin biosynthesis in three capsicum cultivars which varied in their tolerance level against salt.

MATERIAL AND METHODS

Salt treatment

Seeds of three capsicum cv. susceptible cultivar (CO1), tolerant (G4) and moderately sensitive (K2) [20] were collected from Coimbatore Agriculture University and allowed to grow in the greenhouse at VIT University, Vellore under controlled conditions. 10 plants were placed in six sets of plants (three replicates) for each cultivar and allowed to grow for 60 days. Then the plants were treated with a 500ml solution of six different concentrations of NaCl such as 25mM, 50mM, 100mM, 150mM and 200mM respectively for 30 days. Control plants were treated with distilled water. Mature green, turning red and fully ripened red chilli fruits were collected and stored at -80°C till further use.

Reagents

Capsaicin standard was purchased from Sigma –Aldrich (India), HPLC grade acetonitrile and methanol were purchased from nice chemicals (India). Analytical grade ethanol was obtained from Changshu Yangyuan Chemicals (China).

Extraction of capsaicin from capsicum fruit

The capsaicinoids were extracted from 5g of samples in 20 ml ethanol by being heated at 80 °C for 4 h. Suspensions were periodically shaken every 30 min throughout the extraction process. The suspended material was allowed to cool and settle. The supernatant was filtered into a 2 ml glass vial by using a whatman filter paper 1 and then supernatant was used for capsaicin estimation.

HPLC analysis

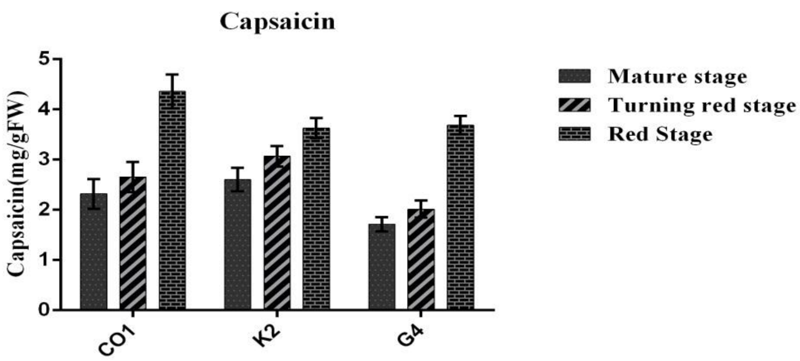
Capsaicin was estimated using [21] with slight modification. The capsaicin content of the samples was quantified using HPLC using a Phenomenex C18 Column on a SHIMADZU HPLC equipped with a manual sampler, a gradient pump, and a UV/vis detector. Capsaicin standards were obtained from Sigma Chemical Co (India) and capsaicin standard was dissolved in HPLC grade methanol. Samples were eluted at a flow rate of 1.5 ml/min with a mobile phase of 40% acetonitrile and 60% deionized water at 30 °C A UV/vis detector at a wavelength of 220 nm and 230nm were used to quantify samples at retention times of 3.37 min for capsaicin when the injection volume of the samples was 20µl.

RESULTS AND DISCUSSION

Pungency of chili fruit is a characteristic feature which results from the interaction between the phenotype and the environmental factors [22, 23]. Salt stress profoundly influenced the accumulation of capsaicin depending upon the genotype and severity of stress. Another important factor that influences capsaicin content is fruit developmental age, because mainly capsaicinoids are accumulated in different ways throughout the plant's development [7, 24].

These evaluations were performed in mature green, turning red and red fruit developmental stages. It is important to note that capsaicin content varied among cultivars [25, 26]. The highest capsaicin content was observed in fully red fruit followed by turning red and mature green fruit among selected cultivars [27] (Figure 1). We also noticed the extent of increase capsaicin in red fruit stage for G4 cultivar was higher than K2. Our result coincides with previous studies emphasizing the changing pattern of capsaicin under different developmental stages of fruit [5, 19, 24].

Figure 1: Capsaicin content in fruit of three developmental stages of three capsicum cultivars (n=3±sd)



Accumulation of capsaicin was found to depending on the severity of salt stress. At low salt concentration capsaicin content was higher than untreated control plant. In case of CO1 and K2 cultivar 50mM salt treatment was observed to be optimal for capsaicin accumulation exhibiting maximum capsaicin concentration, but G4 cultivar expressed the highest capsaicin content in 100mM salt treated plant while high salt concentrations (150mM and 200mM) were found to detrimental for capsaicin synthesis among selected cultivars.

When we investigated the impact of salt on capsaicin content in mature green fruit highest level of capsaicin was observed in 100mM treated G4 cultivar followed by 50mM salt treated K2 and CO1 cultivars. Salt stress severity drastically affected the capsaicin content which as 1.30±0.35 (CO1), 1.83±0.19 (K2) and

2.23±0.30 mg/gFW (G4) (Figure 2). In case of turning red fruit stage the maximum extent of capsaicin is was observed in K2 cultivar treated with 50mM salt (3.92±0.18 mg/gFW) followed by G4 cultivar exposed to 100mM (3.85±0.20 mg/gFW) and CO1 cultivar subjected to 50mM (2.96±0.12 mg/gFW) (Figure 3). We did observed that salt exposure have also affected the capsaicin in fully red fruit. The highest level of capsaicin was detected in K2 cultivar (5.82±0.13 mg/gFW), G4 (5.21±0.20 mg/gFW) and CO1 (4.40±0.20 mg/gFW) in fully ripened fruit (Figure 4). Some of previous studies also conclude about the increase of the capsaicin content under stress condition [28-31].

Figure 2: Capsaicin content in mature green fruit of three capsicum cultivars treated with various salt concentrations (n=3±sd)

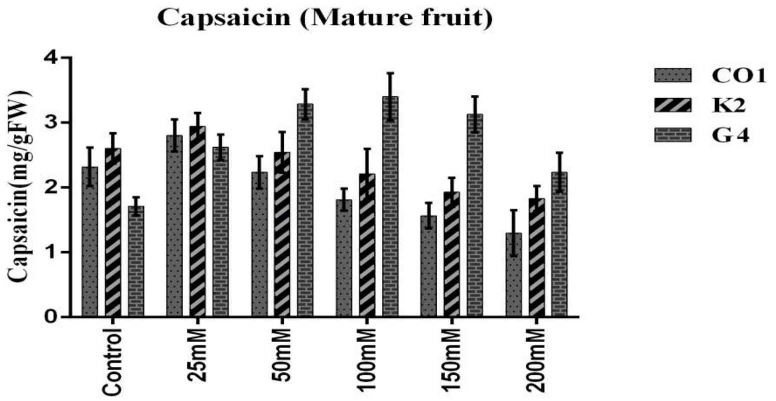


Figure 3: Capsaicin content in turning red fruit of three capsicum cultivars treated with various salt concentrations (n=3±sd)

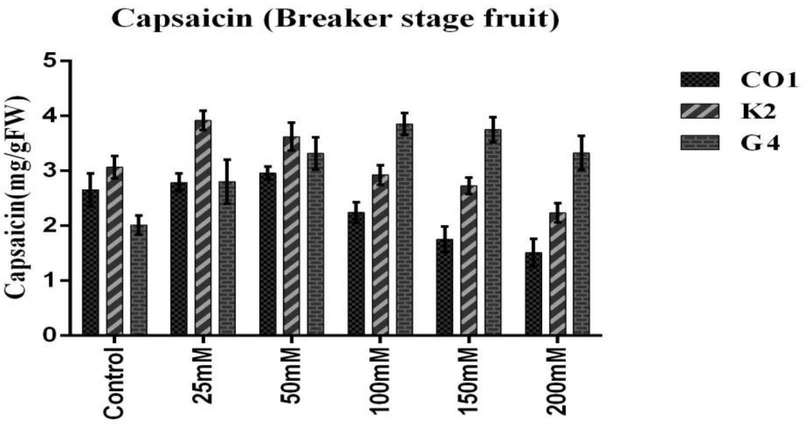
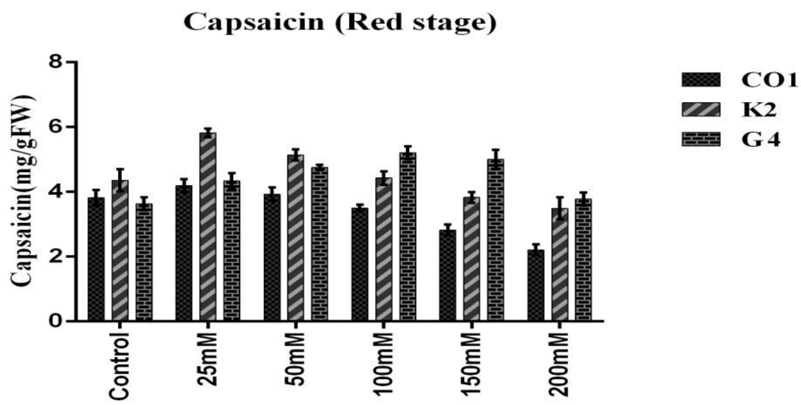


Figure 4: Capsaicin content in fully ripened red fruit of three capsicum cultivars treated with various salt concentrations (n=3±sd)



Among all selected cultivars G4 capsicum cultivar was observed least affected by salt. This discrimination may be because of difference in tolerance level among the cultivars. Degradation of capsaicin in fruit of plants treated with high salt concentration may be due to peroxidases enzymes. Studies in capsicum give the clue for involvement of peroxidases in degradation for capsaicinoids in capsicum cultivar [3, 19]. In addition to that Contreras-Padilla & Yahia, 1998 also revealed that peroxidases are most likely candidates for capsaicinoid degradation [24]. Based on survey on the impact of water stress on accumulation by Ruiz-Lau et al., 2011, degradation were found to be correlated to decreased enzyme activity [29]. This may also be the possible reason for degradation of capsaicin in the high salt exposed plant. These results suggest that the degradation of capsaicin in these fruits could be a response to salt stress condition conditions and needs further investigation to get a proper understanding about this process.

Generally, environmental factors are indispensable part of plant life for its proper growth and development, but beyond its optimal range of plant, these environmental factors influence the plant life. However, salinity, one of the most critical biotic factors, was exhibited positive as well as negative impact on capsaicin accumulation. Low level of saline concentration enhanced the capsaicin accumulation while high salinity causes degradation of capsaicin content among selected cultivar. The highest capsaicin contents were detected in fully ripened fruit among all cultivars. Capsaicin level was observed higher in salt treated plant of tolerant cultivars (G4 and K2) than salt treated plants of the susceptible cultivar (CO1). The results presented in this article are the first report evaluating the impact of salt on capsaicin synthesis and it also provides new insight for the regulation of metabolism of capsaicinoids in response to salt stress in the fruit of capsicum.

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