

Variability in Capsaicinoids content and phylogenetic analysis of *AT3*, and *Acyltransferase* gene in Chilli (*Capsicum annuum* L.)

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Abstract : Capsaicinoids are the alkaloids responsible for pungency in chilli fruits. Estimates of capsaicinoids obtained by colorimetric method and HPLC were highly correlated ($R^2 = 0.996$). Data suggest that colorimetric method an effective means for estimating total capsaicinoids in extracts of fresh chilli fruits. Thirty accessions were screened using colorimetric method and considerable variability was observed for total capsaicinoids (0-1.65 %). In order to see sequence polymorphism associated with pungency level, we cloned and sequenced part of *AT3* gene, an acyltransferase belonging to the BAHD family of acyltransferases. Results revealed that abundant indel (insertion and deletion events) and SNP (single nucleotide polymorphisms) in the cloned *AT3* partial sequence. Both *AT3-1* and *AT3-2* are present in many cultivars that suggest paralogous gene lineages.

Introduction

Pungency (heat) is an important quality attribute of hot pepper besides colour (carotenoids). The nature of pungency has been established as a mixture of seven or more homologous braded-chain alkyl vanillylamides, named capsaicinoids (Torabi, 1997). Capsaicinoids are unique to the genus *Capsicum* and is produced in glands on the placenta of the fruit, they are odourless, colourless, flavourless, non-nutrient compounds. Capsaicin, a major alkaloid among capsaicinoids has wide applications in the food, medicine and pharmaceutical industries. As a medicine, capsaicin is known to kill some types of cancer cells (Min *et al.*, 2004) and provide relief in arthritis and respiratory ailments (Mazzone and Geraghty, 1999). Chillies have been included in ayurvedic medicines and used as tonic toward off many diseases. The

pharmaceutical application of capsaicinoids is attributed to its antioxidant, anticancer, antiarthritic and analgesic properties (Prasad *et al.*, 2006). Pungency in chilli is measured by Scoville scale which was named after Wilbur Scoville. One of the hottest chillies “Naga or Bhut Jolokia” (8, 55, 000 SHU) in the world is found in Tezpur India.

A single locus *C* has been reported to be essential for the control of production of capsacinoids, the pungent allele is dominant over the non-pungent allele (Ben chaim *et al.*, 2001; Blum *et al.*, 2002). Lang *et al.* (2006) isolated two genes characterized as putative *Capsicum* acyltransferase (*Catf-1* and *Catf-2*) from placenta of pungent pepper, expression of *catf-1* coincided with accumulation of capsaicinods suggesting that *catf-1* is a candidate gene differentiating pungent and non-pungent peppers. Through candidate gene analysis, Stellari *et al.* (2009) identified that the mutation results in the loss of pungency is a deletion in the gene *AT-3* (encodes an acyltransferase protein) which belongs to BAHD family. Fruit quality breeding is a major challenge to hot/ bell pepper breeders and to speed up the breeding process, molecular markers associated with quality traits in chilli could be effectively used to rapidly screen a large number of individual plants. With this objective, nucleotide variation at *AT3* (a candidate gene underlying the *Pun1* locus) was studied to identify the possible sequence polymorphisms that could be associated with pungency level that can be used for development of molecular marker for pungency.

Materials and methods

Thirty pepper accessions with varied pungency level were selected from germplasm accessions for analysis of total capsaicinoids (Table 1). The plants were grown in field during 2009 *kharif* season (eastern dry zone of Karnataka state, at 12° 58' north latitude, 77° 45' east longitude and at an altitude of 930 meters above the mean sea level and average rainfall of this area is about 800mm)

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