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Genome Wide Screening, Identification and Characterization of NAC Transcription Factors from Capsicum Species: An *In Silico* Approach

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A B S T R A C T

NAM, ATAF, and CUC (NAC) transcription factors comprise a large plant-specific gene family and a few members of this family have been characterized for their roles in plant growth, development, and stress tolerance. Although recent genome sequencing of Capsicum annum aid in identification of few NAC transcription factors but which is yet to be complete in terms of other capsicum species. So the present work was undertaken to screen and identify NAC transcription factors from two Capsicum genome sequences i.e., *Capsicum ang Zunla and Capsicum annum Chileptein* through comparative genomics approach using known NAC transcription factors from Solanaceae family and various plant species as reference. Further attempt was made elucidate NAC domain architecture, conserved motifs, localization, trans-membrane region elucidation followed by phylogenetic studies and three-dimensional structure prediction. Extensive genome wide screening resulted a total of 186 non-redundant (46 from *chileptein* and 46 from *zunla*) NAC transcription factors from Capsicum species. Domain architecture and phylogenetic analysis showed that NAC gene family are highly diverse in nature even though they possess highly conserved N-Terminal region. Most members contained a complete NAC DNA-binding domain and a variable transcriptional regulation domain. Furthermore, most of the gene found in the nuclear region and dominated by the possession of helix region. Distinct compositions of the putative motifs were revealed on the basis of NAC protein sequences in Capsicum. Sequence analysis, together with the organization of putative motifs, indicated distinct structures and potential diverse functions of NAC family in Capsicum species. Considering the fact that a very limited number of genes of the NAC family have been characterized, our results provide a very useful reference for further functional characterization of this family in Capsicum species in near future.

Introduction

Plants grow in a dynamic and uncontrolled environment which can frequently impose adverse effect on growth and development, resulting in considerable losses to the yields

of highly important crops. Among the adverse abiotic factors commonly encountered by plants are extreme temperature, water deficit, high salinity and submergence that affect plant growth and productivity. To deal with challenging conditions, many communities Breeders take a relatively traditional approach. They grow and cross varieties then evaluate how the progenies vary in their ability to deal with stresses. Scientific communities, meanwhile, have taken advantage of recent advances in functional genomics and biotechnology to genetically engineer crops which can give better yield than the unmodified ones in adverse conditions (Katerji N*et al.,* 2012). The best-adapted plants will then be selected for growing in fields exposed to stresses. In order to cope with stresses, which vary in timing and severity from place to place, season to season, plants activate a number of defense mechanisms that function to increase tolerance to the unfavorable conditions imposed by the stress conditions. The regulatory group includes genes encoding various transcription factors (TFs), which can regulate various stress-inducible genes cooperatively or separately, and may constitute gene networks. The functional group contains genes encoding metabolic components such as sugar, sugar alcohols and amines, which play an important role in stress tolerance. Recent efforts have provided vital information for improving stress tolerance of important crops by increasing root growth and development as well as enhancing various physiological and metabolic responses through genetic engineering of transcriptional networks (Nakashima K *et al.,* 2012).

Transcription Factors

Transcription Factors are molecules involved in regulating gene expression. They are usually proteins, although they can also consist of short, non-coding RNA. Transcription factors are also usually found working in groups and complexes, forming multiple interaction that allow for varying degree of control over rates of transcription. In eukaryotes, genes are usually in a default "off" state, so Transcription factors serve mainly to turn gene expression "on". In bacteria, the reverse is often true, and genes are expressed constitutively until a Transcription factors turns it off. Transcription factors work by recognizing certain nucleotide sequences (motifs) before or after the gene on the chromosome (up- and downstream) (Phillips, T *et al., 2011*). Eukaryotes often have a promoter region upstream from the gene or enhancer regions up or downstream from the gene, with certain specific motifs that are recognized by the various types of Transcription factors. The Transcription factors bind, attract other Transcription factors and create a complex that eventually facilitates binding by RNA polymerase, thus beginning the process of transcription. Transcription factors are only one of the means by which cells express different combinations of genes, allowing for differentiation into the various types of cells, tissues and organs. Transcription factors can also control gene expression by creating a "cascade" effect; where in the presence of small amounts of one protein triggers the production of larger amounts of a second, which triggers production of even larger amounts of a third, and so on (Phillips, T *et al.,* 2011).

Role of Transcription Factors in *Solanancea* Family Plants

The transition from a functional photosynthetic organ to an actively degenerating and nutrient-recycling tissue in a leaf's life history represents the onset of

leaf senescence. This onset is a developmental switch that involves dramatic differential gene expression. Differential gene expression is believed to play an important role in leaf senescence. In a senescing leaf, many genes that are expressed in green leaves, including those genes involved in photosynthesis, are down regulated, while a subset of genes, generally referred to as senescence-associated genes (*SAG*s), are up regulated. Leaf senescence is under direct nuclear control, and *SAG* expression is required for senescence to proceed. Inhibitors of transcription or translation prevent leaves from senescing (Buchanan-Wollaston *et al.*, 2003; Guo and Gan, 2005; Hadfield and Bennett *et al.*, 1997; Lim and Nam, 2005 *et al.*, 1994). For the past decade, much effort has been made to isolate *SAG*s, and hundreds of *SAG*s have been cloned from various plant species including Arabidopsis, barley, *Brassica*, maize, cucumber, rice, tobacco, radish, asparagus and soybean (Buchanan-Wollaston *et al.*, 2003; Gepstein *et al.*, 2003; He and Gan *et al.*, 2003).

Role of NAC Transcription Factors in plants

Several members of the NAC family were initially described more than a decade ago (Olsen AN *et al.*,). Since then a great number of NAC TFs have been identified and functionally characterized in both model and crop plants such as Arabidopsis, rice, soybean (*Glycine max*) and wheat (*T. turgidum* ssp. durum) (Nakashima K *et al.*, 2012). Proper characterization of particular TFs often requires a detailed study in the biological context of a whole TF family since functional redundancy is a common occurrence within TF families (Tran L-SP *et al.*,). Furthermore, since TFs control the expression of the genome, it is impossible to completely understand their function without performing detailed functional studies at a genome-wide level (Reichmann JL *et al.*, 2010). The identification, characterization and classification of TFs at the genome-wide level will provide an important resource for researchers who are studying the regulation of gene expression and the functions of the genes. Complete genomic sequences of several model plants and crops, including Arabidopsis, rice and soybean are now available. Genome-wide analyses of their genomes indicated that there are more than 60 reported families of TFs in these plants (Reichmann JL *et al.*, 2010).

Capsicum annum

Pepper (Capsicum) is an economically important genus of the Solanaceae family, which also includes tomato and potato. The genus includes at least 32 species native to tropical America, of which C. annuum L., C. baccatum L., C. chinense Jacq. C. frutescens L., and C. pubescens (Ruiz & Pavon *et al*, 2010) were domesticated as far back as 6000 B.C. by Native Americans. Peppers have a wide diversity of fruit shape, size, and color. Pungent peppers are used as spices, and sweet peppers are used as vegetables. Pepper global production in 2011 reached 34.6 million tons fresh Fruit and 3.5 million tons dried pods harvested in 3.9 million hectares (Qina C *et a*l., 2012) Despite the growing commercial importance of pepper, the molecular mechanisms that modulate fruit size, shape, and yield are mostly unknown. Since the 1990s, genetic diversity and allelic shifts among cultivars, domesticated landraces, and wild accessions have been partially explored using restricted sets of anonymous or neutral molecular markers and annotated DNA sequences. Hot pepper (*Capsicum annuum*), one of the oldest domesticated crops in the Americas,

is the most widely grown spice crop in the world. Whose genome has been sequenced by Mexican landrace of *Capsicum annuum* cv. CM334 whole-genome sequencing and assembly of the hot pepper at 186.6× coverage.

Rationale of the work

NAM, ATAF, and CUC (NAC) transcription factors comprise a large plant-specific gene family and a few members of this family have been characterized for their roles in plant growth, development, and stress tolerance. Although recent genome sequencing of *Capsicum annum* aid in identification of few NAC transcription factors however, little is known in other Capsicum species. So the present study was undertaken to screen and identify NAC transcription factors from Capsicum species through comparative genomics approach using known NAC transcription factors from plant species as reference. Considering the fact that a very limited number of genes of the NAC family in Capsicum species have been characterized, our results is expected provide a very useful reference for further functional characterization of this family in Capsicum species in near future

Keeping the importance of NAC TFs in Capsicum species, the present research work was designed with the following three objectives:

Screening and identification of NAC transcription factors from Capsicum genome i.e., *Capsicum annum* L. Zunla1 and *Capsicum annum* var. *glabriusculum* Chiltepin.

Elucidation of conserved motifs, localization, trans-membrane region, domain architecture and phylogenetic analysis of the identified NAC TFs.

Physico-chemical characterization, secondary structure analysis followed by 3-Dimensional architecture prediction of NAC domains.

Review of Literature

Drought, cold, and salinity are major forms of stress from abiotic sources that adversely affect plant growth and productivity (Nakashima *et al.,* 2012), of which drought is considered as the most devastating. Water is one of the significant limiting factors and affects maize at all stages of its growth. Maize is especially sensitive to drought at the reproductive stage, particularly between tassel emergence and early grain-filling (Grant *et al.,* 1989). Drought stress during this period reduces kernel size and thus lowers grain yield significantly (Bolanos and Edmeades, *et al.,* 1993). Plants adapt to drought stress at physiological, biochemical, and molecular levels by activating a number of defense mechanisms that increase the plant's tolerance to water deficit. Transcription factors (TFs) are key proteins that regulate gene expression at the transcription level by interacting with promoter elements of stress genes resulting in over-expression of many functional genes.

Biological functions of NAC proteins

Embryonic, floral and vegetative development the striking appearance of mutant phenotypes first indicated the importance of the NAC gene family in plant biology. Most petunia (*Petunia hybrida*) nam (no apical meristem) mutants lack the shoot apical meristem (SAM) and die at the seedling stage. Cotyledon fusions occur in these mutant seedlings, and plants developed from occasional escape shoots display aberrant floral development. NAM was the first NAC gene to be characterized but was

soon followed by the characterization of the Arabidopsis CUC2 (CUP-SHAPED COTYLEDON 2) gene. When combined, mutations in the CUC1 and CUC2 genes cause defects similar to the nam phenotype. The cuc1 cuc2 double mutants have fused cotyledons and the embryonic SAM is absent. When shoots are induced by regeneration from mutant calli, abnormal flowers are formed. The mutant phenotypes and the expression patterns of NAM, CUC1 and CUC2 suggested a function for the gene products in boundary specification and SAM formation. Thorough studies of the Arabidopsis CUC genes have since provided further information about the roles of NAC proteins in development. CUC1 was found to encode a NAC-domain protein with high sequence similarity to CUC2. Functional redundancy was further demonstrated by the recent discovery that a third Arabidopsis NAC gene, CUC3, is involved in the cotyledon boundary and the shoot meristem. The cuc mutant phenotype prompted an investigation of the interaction between the CUC1 and CUC2 genes and the STM (SHOOT MERISTEMLESS) gene. STM is a KNOTTED 1-like homeo box (KNOX) gene involved in SAM formation and maintenance as well as in cotyledon separation. CUC1 and CUC2 were found to be required for STM expression during embryonic SAM formation. Furthermore, overexpression of CUC1 was shown to induce adventitious shoots on cotyledons through STM expression.(Ken-ichiro Hibara *et al* , 2009) examined the genetic interaction between CUC1 and the AS1 (ASYMMETRIC1) and AS2 genes, which are also important in SAM formation. Their results indicated that CUC1 also promotes SAM formation through an STM-independent pathway that is negatively regulated by AS1 and AS2. AS1 encodes a MYB (*Myeloblastosis*) domain transcription factor, and it is thus apparent from this single example of NAC gene function that research into the roles of NAC proteins in plant biology will contribute to an unravelling of transcription factor networks (K. Yamaguchi ,Nakashima *et al* , *2012*). Indeed, a recent study of cup (*cupuliformis*) mutants in Antirrhinum (*snapdragon*) showed that the CUP protein is involved in the establishment of aboveground organ boundaries and that it interacts with a TCP (TB1, CYC, PCF) domain transcription factor. Members of the TCP family of transcription factors are involved in the regulation of plant growth and development. Furthermore, expression of an Arabidopsis NAC gene called NAP (NAC-like, activated by APETALA 3/PISTILLATA) has been shown to be directly activated by a heterodimer of the APETALA 3 and PISTILLATA proteins, both of which are MADS (MCM1, AGAMOUS, DEFICIENS and SRF) box transcription factors essential for the specification of floral organ identities. Other regulators of NAC gene expression have been identified without evidence of immediate regulation. CUC1 and CUC2 spatial expression is affected by mutations in STM and in PIN1 (PIN-FORMED 1), PID (PINOID) and MP (MONOPTEROS), genes involved in auxin signalling. Moreover, NAC1 transcription is activated by NAC1and CUC3 transcription is stimulated by CUC1 and CUC2. Lateral root formation and auxin signalling A role in a different developmental program, the formation of lateral roots, has been demonstrated for NAC1. NAC1 was initially examined because of its predominant expression in the root tip and in lateral root initiation points. NAC1 expression was shown to be induced by the hormone auxin, which is involved in lateral root production. Over- and under expression of NAC1 increased or reduced lateral root formation, respectively. In addition, the auxin responsive genes AIR3 (AUXIN-INDUCED

IN ROOT CULTURES 3) and DBP (DNA-BINDING PROTEIN) were identified in a screen for downstream targets of NAC1 (Figure 1c). DBP encodes a DNA-binding protein and AIR3 encodes a subtilizing-like protease that might weaken cell-to-cell connections to facilitate lateral root emergence. Detailed studies have demonstrated a specific activation of the AIR3 promoter by NAC1. Defence and abiotic stress Several NAC proteins have been identified because they interact with other proteins of biological importance. The wheat (Triticum sp.) gemin virus RepA-binding (GRAB) proteins GRAB1 and GRAB2 were identified because of their ability to interact with the wheat dwarf Gemini virus RepA protein, and Arabidopsis turnip crinkle virus (TCV)-interacting protein (TIP) was identified because of its binding to the TCV capsid protein (CP). TCV induces a hypersensitive response and systemic resistance in TCV-resistant Arabidopsis. The ability of TCV to induce resistance was dependent on interaction between TCV CP and TIP, suggesting that TIP is essential for the TCV resistance response pathway. A function of NAC proteins in biotic stress responses has also been suggested by the induction of the potato (Solanum tuberosum) StNAC gene by Phytophthora infestans infection and induction of several Brassica napus (rape) NAC genes by insect herbivory and fungal infection. The expression of several of these genes was also induced by abiotic stress such as wounding, cold shock and dehydration. Recently, it was reported that transgenic plants overexpressing three different Arabidopsis NAC genes (ANAC019, ANAC055 and ANAC072) showed significantly increased drought tolerance. Furthermore, ANAC072 {referred to as RD26 (Kim *et al., 2012*) was shown to function in a novel abscisic acid (ABA) - dependent stress-signalling pathway. The ANAC genes belong to a subgroup of NAC genes defined by the wound-inducible ATAF1 and ATAF2 genes, and were upregulated by dehydration, high salinity and ABA, and some also by methyl jasmonate. In addition, the expression of ANAC072 was shown to be induced by reactive oxygen species. Overexpression of the ANAC genes in transgenic plants revealed potential, stress related target genes. ANAC072 Trans activated the promoter of one of these genes, encoding a glyoxalase I family protein.

Various Functions of plant-specific NAC transcription factors

NAC proteins are plant-specific TFs which have been shown to function in relation to plant development and also for abiotic and/or biotic stress responses. (Ookaa *et al.,* 2012) The cDNA encoding a NAC protein was first reported as the responsive to dehydration gene in Arabidopsis. The NAC domain was identified based on consensus sequences from Petunia NAM and Arabidopsis ATAF1/2 and CUC2 proteins (the domain was named from their first letters of the genes). Many NAC proteins, including Arabidopsis CUC2, have important functions in plant development. Some NAC genes are up-regulated during wounding and bacterial infection, whereas others mediate viral resistance. NAC proteins were thought to be transcriptional activators as the Arabidopsis ATAF1/2 proteins can activate the CaMV 35S promoter in yeast cells. The Arabidopsis AtNAM (NARS2) protein was confirmed by (Duval *et al.,* 2011) to function as a transcriptional activator in a yeast system swell. In rice, Kikuchi *et al.,* Described the molecular properties of the eight NAC genes in rice (OsNAC1 to OsNAC8) which encode proteins with a single NAC domain. Each OsNAC gene has a unique tissue-specific

expression pattern, suggesting this family regulates the development of rice. (Ooka *et al.*, 2012) performed a comprehensive analysis of NAC family genes in rice and Arabidopsis. As a result of their studies, they identified 75 predicted NAC proteins in full-length cDNA datasets of rice and 105 predicted genes in the Arabidopsis genome. Recently, (Nuruzzaman *et al.*,) conducted a genome-wide analysis of the NAC transcription factor family in rice and Arabidopsis by investigating 151 non-redundant NAC genes in rice and 117 in Arabidopsis. Furthermore, it is suggested that the prototypes of SNAC-A and SNAC-B subgroups may have emerged after the separation of lycophytes and other vascular plants but prior to the separation of monocots from dicots. Transcription factors acting upstream of NAC genes is still limited but recent reports have supplied interesting examples of regulation at the transcriptional level. Studies of the transition from leaf cells to protoplasts have suggested that the acquisition of pluri potentiality involves the activation of several silent NAC genes. A role in dedifferentiation is in accordance with NAC gene function in meristem development. Another example is the maize (Zea mays) endosperm NAC gene nrp1 (NAM-related protein 1), which is regulated by gene specific imprinting. Thus, paternally transmitted alleles are silenced, allowing maternal control of endosperm development .Post transcriptional control miRNAs are small regulatory RNAs that pair with target mRNAs, thereby providing post-transcriptional repression of the targets. Informatics analyses suggested that transcription factors involved in cell-fate determination are the predominant targets of miRNAs in plants. A subset of Arabidopsis NAC mRNAs, including CUC1, CUC2, NAC1, At5g07680 and At5g61430, was initially predicted to be targeted by members of the miR164 gene family, and CUC1 and CUC2 mRNAs were shown to be cleaved within their miR164 complementary site. In addition, expression of miR164-resistant versions of CUC1 and CUC2 mRNAs, and overexpression of miR164 proved that miR164 is necessary for proper regulation of CUC1 and CUC2. MiR164-directed cleavage of NAC1, At5g07680 and At5g61430 was also detected.

Structural feature of NAC proteins

The N-terminus of NAC proteins is a highly homologous region containing the DNA-binding NAC domain. The NAC domain is approximately 150 amino acids in length and contains five conserved regions (A to E). The structure of the DNA-binding NAC domain of Arabidopsis ANAC019 (Arabidopsis thaliana NAC019) has been determined by X-ray crystallography. The NAC domain was reported to lack a classical helix–turn–helix motif; instead it was revealed to possess a new-type of TF fold consisting of a twisted beta-sheet that is surrounded by a few helical elements. The functional dimer formed by the NAC domain was identified in the structural analysis. Recently, the NAC domain structure of a rice stress-responsive NAC protein (SNAC1; STRESS-RESPONSIVE NAC 1) was also determined. The structure of the SNAC1 NAC domain shares a structural similarity with the NAC domain from Arabidopsis ANAC019. The dimerization of the SNAC1 NAC domain has also been demonstrated. Regarding additional features in NAC proteins, the N-terminal NAC domain also contains a nuclear localization signal and the C-terminal region is highly variable and contains a transactivation domain. In some NAC proteins, the C-terminal domains exhibit protein-binding activity and others contain transmembrane (TM) motifs.

NAC proteins related to abiotic stress response in Arabidopsis

Our group isolated abiotic stress-responsive NAC proteins as factors regulating the expression of the EARLY RESPONSIVE TO DEHYDRATION 1 (ERD1) gene in Arabidopsis. Firstly, (Kiyosue *et al*, 2010). Isolated the ERD1 cDNA encoding a Clp protease regulatory subunit (ClpD) from a cDNA library derived from one-hour-dehydrated Arabidopsis plants. Nakashima *et al.*, subsequently isolated the promoter region of the ERD1 gene and demonstrated that ERD1 is not only induced by dehydration but is also up-regulated during senescence and dark induced etiolation. Promoter analysis of ERD1 in transgenic plants showed that two different novel cis-acting elements, a MYC-like sequence (CATGTG) and a rps1 site 1-like sequence (CACTAAATTGTCAC), are involved in induction by dehydration stress. Tran *et al.*, used the yeast one-hybrid screening method and isolated three kinds of homologous SNAC group NAC proteins (ANAC019, ANAC055 and ANAC072 (RD26)) that bind to a region containing the MYC-like sequence of the ERD1 promoter. The expression of these genes is induced by drought, high salinity and by the phytohormones ABA and methyl jasmonic acid (MeJA). A detailed DNA-binding assay of these NAC proteins revealed that "CACG" is a core sequence of the NAC Recognition Sequence (NACRS). Microarray analysis of transgenic plants overexpressing either ANAC019, ANAC055, or ANAC072 revealed that several stress-inducible genes, including the glyoxalase gene, were up-regulated in the transgenic plants. Furthermore, the plants were shown to have significant improvements in their drought tolerance. However, the expression of the ERD1 gene was not up-regulated in the plants overexpressing these NAC genes. CDNAs for the TF that binds to the rps1 site 1-like sequence was isolated by using the one hybrid screening method. These cDNAs encoded zinc-finger homeo domain (ZFHD) proteins and one of these genes (ZFHD1) was shown to function as a transcriptional activator in response to dehydration stress. Overproduction of both the NAC and ZFHD proteins increased the expression of ERD1, indicating that both cis-acting elements are necessary for the expression of ERD1. The NAC proteins are capable of functioning as transcription activators in cooperation with the ZFHD proteins or by functioning alone. Recently, Wu *et al.*, reported that Arabidopsis plants overexpressing the abiotic stress-responsive gene ATAF1, which is homologous to RD26, showed improved drought tolerance. These reports indicate that NAC factors have important roles for the control of abiotic stress tolerance and that their overexpression can serve as a potential biotechnological application for improving the stress tolerance of plants. In addition, they also provided evidence that positive ABA-regulatory functionality is associated with both the ANAC019 TRD and the DBD. Recently, an ABA perception and core signalling module, including the ABA receptor (PYR/PYL/RCAR), group a 2C-type protein phosphatase (PP2C), and class III SnRK2 protein kinases was identified. Our group and another laboratory generated a srk2d srk2e srk2i (srk2dei) triple mutant to elucidate the function of class III SnRK2 protein kinases in Arabidopsis. Microarray experiments revealed that the up that the expression of stress-inducible NAC genes, including RD26, were under the control of the central ABA perception and signalling module containing PYR/PYL/RCAR, PP2C, and SnRK2.On the other hand, some NACs function in relation to abiotic stresses in addition to osmotic stresses including

drought and high salinity. For example, RD26 was reported to be responsive to cold stress. Morishita *et al.,* also reported that ANAC078 in the NAC group TIP (Fig. 1) is responsive to a combination of high-light and heat-stress. They also demonstrated that ANAC078 regulates flavonoid biosynthesis, leading to the accumulation of anthocyanins under high-light conditions. These reports indicate that various NAC factors function in relation to numerous types of abiotic stresses. Transcription factors (TF) are an essential part in the transcription machinery and the analysis of transcription factor families is one of the major areas of research. TFs are involved in the control of transcription, an essential step in gene expression. By and large, transcriptional control leads to phenotypic divergence in species, i.e. it brings about a change in key physiological functions and thereby regulates development. Though there are several TF that are common to plants and animals, NAC, and AP2/ERF TF are plant specific while the WRKY transcription factor family had origin in early eukaryotes and greatly expanded in plants and they have all been implicated in conferring tolerance to different biotic and abiotic stresses . Among the unique transcription factor families, the NAC family is one with probably the largest number of members of plant specific TF. NAC (for NAM, ATAF1, and CUC) was initially identified as a gene essential for pattern formation in embryo and flowers (NAM) in Petunia and organ separation (CUC) in Arabidopsis. In subsequent years it was found that NAC was involved in various developmental process like the formation of adventitious shoots, shoot apical meristem flower development; defence responses like conferring resistance to turnip crinkle virus, response to wounding and fungus infection, abiotic stress responses like induction of dehydration responsive genes, ABA cell signalling pathway and attenuation of auxin signalling (Sathesh *et al.,* 2010).

33.3 million tons of hot pepper planted on 3.8 Mha (United Nations Food and Agriculture Organization (FAO) statistics; see URLs). In the last decade, world production of hot pepper increased by 40%. The pungency of hot pepper is due to the accumulation of capsaicinoids, a group of alkaloids that are unique to the Capsicum genus. The heat sensation created by these capsaicinoids is such a defining aspect of this crop that the genus name Capsicum comes from the Greek kapto, which means ‘to bite’. Capsaicin, dihydrocapsaicin and nordihydrocapsaicin constitute the primary capsaicinoids, which are produced exclusively in glands on the placenta of the fruit. The organoleptic sensation of heat caused when capsaicin binds to the mammalian transient receptor potential vanilloid 1 (TRPV1) receptor in the pain pathway7 can be argued to be a sixth taste along with sweet, sour, bitter, salty and umami (savory). M lation of the pathway is not fully understood. With more than 22 capsaicinoids isolated from hot pepper, this genus provides an excellent example for exploring the evolution of secondary metabolites in plants2. Capsaicinoids have been found in nature to have antifungal and antibacterial properties, to act as a deterrent to animal predation when ingested and to have inherent properties that aid in avian seed dispersal. Capsaicinoids have many health benefits for humans: they are effective at inhibiting the growth of several forms of cancer8–10, are an analgesic for arthritis and other pain11, reduce appetite and promote weight loss12–14. It is surprising that a complete understanding of the capsaicinoid pathway at the molecular level is lacking, considering the economic and cultural importance of capsaicinoids. Here we report a high-quality genome

sequence for hot pepper. C. annuum cv. CM334 (Criollo de Morelos 334), a landrace collected from the Mexican state of Morelos, has consistently exhibited high levels of resistance to diverse pathogens, including Phytophthora capsici, pepper mottle virus and root-knot nematodes. This landrace has been extensively used in hot pepper research and cultivar breeding. We also provide resequencing data for two cultivated peppers and for a wild species, C. chinense. Comparative genomics of members of the Solanaceae family, which includes hot pepper, provides an evolutionary view into the genome expansion, origin of pungency, distinct ripening process and disease resistance of hot pepper. This high-quality reference genome of hot pepper will serve as a platform for improving the horticultural, nutritional and medicinal values of Capsicum species (Kim s *et al.,* 2011). Agricultural productivity and yields are dependent upon the environment a crop encounters during its growth. In order to burgeon, a species must learn adaptive approaches against these recurrent challenges. Grasses belonging to the genus Setaria which have a world-wide existence, provide a fine example of such species. Particularly, Setaria italica, which was domesticated from the problematic weed Setaria viridis. 8700 years ago, is amongst the oldest cultivated crops. This abiotic stress-tolerant grass has presented itself as an ideal model for understanding biological processes in potential biofuel grasses such as switch grass, napier grass and pearl millet as they have closely-related but comparatively complex genome. Considering this importance, its genome has been recently sequenced by two independent groups viz., Joint Genome Institute, Department of Energy, USA and Beijing Genomics Institute, China .Preliminary analysis of the draft genome has revealed that the stress-adaptive characteristics which foxtail millet possesses have yet not been evolved in other plants. Stress adaptation is a complex incident as stress may occur at diverse stages of plant development and often multiple stresses concurrently affect the plant. Comprehensive investigations have revealed the molecular stress adaptation mechanisms which are governed by processes that allow regulated gene expression by an accurate signalling and tight transcriptional control. This entails binding of specific transcription factors (TFs) to cis-regulatory sequences in promoter of a stress-responsive gene. A corollary of this fact is that plants donate a large part of their genome (?7%) to encode TFs belonging several families, such as AP2/ERF, bZIP, NAC, MYB, MYC, Cys2His2, zinc-finger and WRKY, each with a dedicated binding site through which they can activate or repress the expression of their respective target genes (puranik s *et al* 2012). As a crucial form of TFs, the well-known NAC gene family has emerged as a complex plant-specific superfamily. The ellipsis, NAC, derives its name from three earliest characterized proteins from petunia NAM (no apical meristem), Arabidopsis ATAF1/2 and CUC2 (cup-shaped cotyledon). This family has been noted for the presence of numerous members in the model plant Arabidopsis (Arabidopsis thaliana: 117), crops such as rice (Oryza sativa: 151), soybean (Glycine max: 152), tree species like poplar (*Populus trichocarpa*: 163), grape (*Vitis vinifera*: 79) and tobacco (*Nicotiana tabacum*: 152). It is characterized by the presence of a highly conserved NAC domain at N-terminal of the protein, however some exceptions have also been noted. Although this domain confines the ability of DNA binding (DB), it shows great variation in recognizing DB sites in the target genes and atleast 5 different arrays have been identified. This feature allows

them to regulate spatial and temporal expression of a variety of downstream genes towards governing multiple cellular or molecular processes. The highly diverged C-terminal end functions as a transcription regulatory region, by conferring either activation or repression activity. In some NAC proteins, these N- or C-terminal domains may modulate protein-protein interactions. As an additional feature, some NAC proteins comprise an a-helical transmembrane (TM) motif for anchoring to plasma membrane or endoplasmic reticulum. Genes encoding NAC proteins can be regulated (i) transcriptionally by upstream TFs such as ABREs (ABA-responsive elements) and DREs (Dehydration-responsive elements), (ii) post transcriptionally by micro-RNAs or alternative splicing, and (iii) post translationally by ubiquitinization, dimerization, phosphorylation or proteolysis. These regulatory steps assists the functional involvement of NAC proteins in majority of plant processes including orchestration of organ, fiber and secondary wall development, cell cycle control , and senescence . Their multi-functionality has also been implicated in the regulation of molecular pathways that govern abiotic and biotic stress responses through mediation by hormones. Although about one fourth (20–25%) NAC genes functions in at least one or the stress-response, very few candidate genes have been functionally characterized for enhancement of stress tolerance correspondingly, till now foxtail millet invited little research with respect to development of genetic, genomic and functional resources although it is potentially better stress tolerant when compared to other staple cereals. The recent release of its genome sequence facilitates the prediction and systematic analysis of important genes families, including the multi-functional plant specific NAC TFs. In this context, we conducted a genome-wide survey and identified a comprehensive and non-redundant set of 147 NAC genes from foxtail millet (internally annotated as Setaria italica NAC; SiNAC) and classified into eleven classes on basis of the conserved motifs and sequence phylogeny. Sequence comparison of SiNAC genes with themselves and with other monocots like sorghum, maize and rice facilitated the detection of presence and distribution of paralogous and orthologous NAC genes between the grasses. The experimental outcomes have paved a way for further comparative genomic and phylogenetic analyses of NAC TFs among members of grass family. Subsequently, quantitative real time PCR (qRT-PCR)-based gene expression profiling displayed temporal and stress-specific expression pattern of selected candidate SiNAC genes. Three-dimensional structure determination and molecular simulation of a stress-responsive protein SiNAC128 was performed for understanding the basis of its molecular function. This study provides the first information about foxtail millet NAC genes, which would serve as potential candidates for dissecting NAC-mediated regulatory pathways in this significant yet neglected crop species (Puranik s *et al.*, 2012).

Materials and Methods

Materials used

Databases

Plant Transcription Database

The Plant Transcription Factor Database (PlnTFDB; http://plntfdb.bio.uni-potsdam.de/v3.0/) is an integrative database that provides putatively complete sets of transcription factors (TFs) and other transcriptional regulators (TRs) in plant species (*sensu lato*) whose genomes have

been completely sequenced and annotated. The complete sets of 84 families of TFs and TRs from 19 species ranging from unicellular red and green algae to angiosperms are included in PlnTFDB, representing >1.6 billion years of evolution of gene regulatory networks. For each gene family, a basic description is provided that is complemented by literature references, and multiple sequence alignments of protein domains. TF or TR gene entries include information of expressed sequence tags, 3D protein structures of homologous proteins, domain architecture and cross-links to other computational resources online. Moreover, the different species in PlnTFDB are linked to each other by means of orthologous genes facilitating cross-species comparison.

Peeper genome database

Capsicum, commonly referred to as pepper, is an economically important genus of the Solanaceae family which includes tomato and potato. The genus includes at least 32 species native to tropical America, of which *C. annuum L.*, *C. baccatum L.*, *C. chinense Jacq.*, *C. frutescens L.*, and *C. pubescens* (Ruiz & Pavon) were domesticated as far back as 6000 BC by Native Americans. Peppers have a wide diversity of fruit shape, size and color. Pungent peppers are used as spices and sweet peppers as vegetables. Following the return of Columbus from America in 1492 and subsequent voyages of exploration, peppers spread around the world because of their adaptation to different agroclimatic regions and food, medical and ornamental uses. Global production of pepper in 2010 reached 29.4 million tons of fresh fruit and 3.1 million tons of dried pods produced on 3.9 million hectares (http://www.fao.org). In spite of the growing commercial importance of pepper, the molecular mechanisms that modulate fruit size, shape and yield are mostly unknown. We sequenced the complete genomes of wild Mexican pepper accession "Chiltepin" and a Chinese inbred derivative "Zunla-1" using the Illumina platform. As one of the most important vegetable crops, pepper genome will provide an invaluable new resource for biological research and breeding of Capsicum. To better manage the pepper genome data and facilitate public academic users to access the genome data and related information, we developed the Pepper Genome Database.

Software tools

Web Servers

Reduce redundancy tool

This program allows you to reduce the redundancy in a set of aligned or unaligned sequences. The algorithm used by this program was developed by Cédric Notredame and is unpublished. The trim algorithm works as follow:

Computes all the pairwise alignments (PAM250, gop=-10, gep=-1) or use a multiple alignment.

Measure the %id (number id/number matches) of each pair

If a minimum identity *min%* is set: all the sequences with less than *min%* identity with ANY sequence in the set will be removed so that in the remaining set ALL the pairs of sequences have more than *min%* identity. The removal will stop uncompleted if the set becomes smaller than *n*.

Remove one of the two closest sequences until either *n* is reached or until all the sequences have less than *max%* identity.

Return the new set.

ProtoParam tool

ProtParam (References / Documentation) is a tool which allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered protein sequence. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY)

Pfam

Pfam version 28.0 was produced at the European Bioinformatics Institute using a sequence database called Pfamseq, which is based on UniProt release 2014_07. Pfam is freely available under the Creative Commons Zero ("CC0") licence. Pfam is powered by the HMMER3 package written by Sean Eddy and his group at the Howard Hughes Janelia Research Campus, and built by the Pfam consortium.

Smart

SMART (a Simple Modular Architecture Research Tool) allows the identification and annotation of genetically mobile domains and the analysis of domain architectures. More than 500 domain families found in signalling, extracellular and chromatin-associated proteins are detectable. These domains are extensively annotated with respect to phyletic distributions, functional class, tertiary structures and functionally important residues. Each domain found in a non-redundant protein database as well as search parameters and taxonomic information are stored in a relational database system. User interfaces to this database allow searches for proteins containing specific combinations of domains in defined taxa. For all the details, please refer to the publications on SMART.

Interproscan

The European Bioinformatics Institute is part of EMBL, Europe's flagship laboratory for the life sciences. EMBL-EBI provides freely available data from life science experiments covering the full spectrum of molecular biology. While we are best known for our provision of bioinformatics services, about 20% of our institute is devoted to basic research. Our extensive training programme helps researchers in academia and industry to make the most of the incredible amount of data being produced every day in life science experiments. We are a non-profit, intergovernmental organisation funded by EMBL member states. Our 500 staff represent 43 nationalities, and we welcome a regular stream of visiting scientists throughout the year. We are located on the Wellcome Genome Campus in Hinxton, Cambridge in the United Kingdom.

Psipred

The PSIPRED protein structure prediction server allows users to submit a protein sequence, perform a prediction of their choice and receive the results of the prediction both textually via e-mail and graphically via the web. The user may select one of three prediction methods to apply to their sequence: PSIPRED, a highly accurate secondary structure prediction method; MEMSAT 2, a new version of a widely used transmembrane topology prediction method; or GenTHREADER, a sequence profile based fold recognition method. Psipred is available athttp://globin.bio.warwick.ac.uk/psipred/.

Concord

Most of the protein structure prediction methods use a multi-step process, which often includes secondary structure prediction, contact prediction, fragment

generation, clustering, etc. For many years, secondary structure prediction has been the workhorse for numerous methods aimed at predicting protein structure and function. This paper presents a new mixed integer linear optimization (MILP)-based consensus method: a Consensus scheme based on a mixed integer linear optimization method for secondary structure prediction (CONCORD). Based on seven secondary structure prediction methods, sspro, DSC, PROF, PROFphd, PSIPRED, Predator and GorIV, the MILP-based consensus method combines the strengths of different methods, maximizes the number of correctly predicted amino acids and achieves a better prediction accuracy. The method is shown to perform well compared with the seven individual methods when tested on the PDBselect25 training protein set using six fold cross validation. It also performs well compared with another set of 10 online secondary structure prediction servers (including several recent ones) when tested on the CASP9 targets (http://prediction center.org/casp9/). The average Q3 prediction accuracy is 83.04 per cent for the six fold cross validation of the PDBselect25 set and 82.3 per cent for the CASP9 targets. We have developed a MILP-based consensus method for protein secondary structure prediction. A web server, CONCORD, is available to the scientific community at http://helios.princeton.edu/CONCORD .

Pred

JPred is a Protein Secondary Structure Prediction server and has been in operation since approximately 1998. JPred incorporates the Jnet algorithm in order to make more accurate predictions. In addition to protein secondary structure JPred also makes predictions on Solvent Accessibility and Coiled-coil regions (Lupas method).

Standalone tools

BLAST

BLAST for Basic Local Alignment Search Tool is an algorithm for comparing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences. A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold.

MEGA

MEGA is an integrated tool for conducting automatic and manual sequence alignment, inferring phylogenetic trees, mining web-based databases, estimating rates of molecular evolution, inferring ancestral sequences, and testing evolutionary hypotheses.

i) MODELLER 9.15

MODELLER is used for homology modelling of protein three dimensional structures .The user provides an alignment of a sequence to be modelled with known related structures and MODELLER automatically calculates a model containing all non-hydrogen atoms. MODELLER implements comparative protein structure modelling by satisfaction of spatial restraints and can perform many additional tasks; MODELLER is available for download for most Unix/Linux systems, Windows, and Mac and is available at salilab.org/modeller.

PyMOL

PyMOL is a molecular modelling program that is particularly effective for the construction and 3D visualization of macromolecules, including proteins and

protein –ligand complexes. PyMOL can be used to visualize .pdb files, which contain a refinement of a crystal structure It is available at http://www.pymol.org/ under "download."

Discovery Studio

Discovery Studio is a suite of software for simulating small molecule and macromolecule systems. It is developed and distributed by Accelrys. The product suite has a strong academic collaboration programme, supporting scientific research and makes use of a number of software algorithms developed originally in the scientific community, including CHARMM, MODELLER, DELPHI, ZDOCK, DMol3 and more.

Methods

Sequence retrieval from pepper genome database

To identify members of the Capsicum NAC gene family, Protein coding sequences from two capsicum genomes i.e., *Capsicum annum* L. Zunla1 and *Capsicum annum* var. *glabriusculum* Chileptin were downloaded from Pepper genome database.

Reference transcription factors sequences

Initially, 746 amino acid sequences encoding NAC transcription factors from Solanaceae were retrieved from plant transcription factor database 3.0 (plntfdb.bio.uni-potsdam.de/). These sequences were used as reference to identify putative NAC transcription factors in Capsicum protein coding gene sequences. Moreover, the HMM profiles of the NAM and NAC domains in the Pfam database (http://pfam.sanger.ac.uk/) were searched against the Capsicum protein coding sequences.

Screening and identification of NAC transcription factors

BLAST was locally configured and BLAST search (E-value cut-off 1.0) was performed using NAC transcription factors as query against protein sequences of Capsicum genome to screen putative NAC TFs.Further, attempt was made to analyze the domains of all of the Capsicum proteins using a Hidden Markov Model (HMM) profile of the NAM domain retrieved from Pfam 26.0 (http://Pfam.sanger.ac.uk/) with an expected value (e-value) cut-off of 1.0. All identified protein models were subjected to Pfam analysis to confirm the presence of the NAM domain with an e-value cut-off of 1e-3.Combining the results from BLAST search and PFAM, putative NAC TFs (from both the genomes) were screened and subjected to redundancy removal using Reduce redundancy tool of EBI.

Primary characterization of NAC TFs

Each non-redundant sequence was checked for the presence of the conserved NAC domain by SMART (http://smart.emblheidelberg.de/) and Pfam (http://pfam.sanger.ac.uk/) searches.Physico-chemical parameters including molecular weight, isoelectric point, instability index, alihphatic index and GARVY were elucidated using Protparam tool of ExPASy (http://web.expasy.org/protparam/).
Transmembrane motifs in the sequences were identified with TMHMM Server v.2.0 (http://www.cbs.dtu.dk/services/TMHMM/) using default parameters. The conserved motifs in full-length NAC proteins were identified using Multiple Expectation Maximization for Motif Elicitation (MEME) v. 4.9.0 (http://meme.nbcr.net/meme3/meme.html). Analysis was performed

with the following parameters: number of repetitions, any; maximum number of motifs, 20; and optimum width of the motif, ≥50.Discovered MEME motifs (≤1E-30) were searched in the InterPro database with InterProScan (http://www.ebi.ac.uk/Tools/pfa/iprscan5/).

Phylogenetic study of NAC domains

Phylogenetic analysis

To clarify the phylogenetic relationships among the Capsicum NAC proteins, phylogenetic trees were constructed using NAC domain and an un-rooted tree was generated by constructing a multiple sequence alignment with a gap open and gap extension penalties of 10 and 0.1, respectively using ClustalW Neighbor-joining method was used for unrooted tree construction with bootstrap analysis (1,000 replicates) in MEGAv6.0.

Homology Modelling of NAC Domains

To elucidate the three-dimensional architecture of NAC domains, comparative modelling approach was employed where suitable templates were identified using BLASTP, PSI-BLAST and DELTA-BLAST search against PDB database. From each genome only representative 5 NAC domains were selected for homology modelling. Identified templates were aligned with respective target NAC domains. Based on the target-template alignment, Modeller facilitated in the development 50 rough models of NAC domains in Capsicum species. These theoretical structural models of Cry1Id were ranked based on their normalized discrete optimized protein energy (DOPE) scores. The model with the lowest value of the normalized DOPE score is considered as the best model for energy minimization in Discovery Studio3.5 (Accelrys, Inc. San Diego, USA).

Energy minimization

The best model (in each case) with lowest DOPE score was subjected to energy minimization by DS3.5 with the minimization protocol. The minimization protocol employs the steepest descent and conjugate gradient methods of minimization algorithms with a generalized born implicit solvent model. In the present study the following parameters are considered for the structural minimization: distance-dependent dielectric constant=1, non-bonded radius of 14 Å with CHARMM force field, spherical electrostatic cut-off, and the steepest descent algorithm to remove close van der Waals contacts for a maximum steps of 5000 with 0.1 minimizing RMS gradient. Finally the potential energy, van der Waals energy and electrostatic energy for the minimized models was determined using the calculate energy protocol in DS3.5.

Model quality assessment

The refined models were evaluated by a number of tools to test the internal consistency and reliability of the model. PROCHECK analysis which quantifies the amino acid residues in the available zones of Ramachandran plot, was used to assess the stereo chemical quality of the models. ERRAT tool, which finds the overall quality factor of the protein, was used to check the statistics of non-bonded interactions between different atom types. The VERIFY-3D program was used to determine the compatibility of the atomic models (3D) with its own amino acid sequence (1D). The average magnitude of the volume irregularities in the model was calculated using PROVE program. PROVE program uses an algorithm which treats the atoms

like hard spheres and calculates a statistical Z-score (i.e., deviation) for the model from highly resolved (2.0 Å or better) and refined (R-factor of 0.2 or better). Standard bond lengths and bond angles of the model were determined using WHAT IF web server. The estimated energy of the models was calculated by the ANOLEA server. Furthermore, the stereo-chemical calculations were also performed using the MetaMQAP, ProQ, and Mod FOLD version 4.0 servers. Also MolProbity web server (http://molprobity.biochem.duke.edu/) was used in the model validation process which provides a detailed atomic contact analysis of any steric problems within the molecules as well as the dihedral-angle diagnostics. Subsequently the Protein structure analysis (ProSA-web) (https://prosa.services.came.sbg.ac.at/prosa.php) tool was employed in the refinement and validation process to check the native protein folding energy of the model by comparing the energy of the models with the potential mean force derived from a large set of known protein structures. Structural superimposition of proposed 3-D model with its closest homologue was performed in iPBA (http://www.dsimb.inserm.fr/dsimb_tools/ipba/) web server. The iPBA web server presented the root mean square deviation (RMSD) between the Cα-atoms and all atoms of the homology model and template. To have a knowledge on the conservedness in the secondary structure of the refined model and the template, the pair-wise 3-D structural alignment was performed in the pair-wise 3-D alignment tool MATRAS (Markovian transition of Structure evolution) (http://strcomp.protein.osaka-u.ac.jp/matras/). So as to ensure the accuracy in the assignment of secondary structure elements in the proposed models, the results of secondary structure elements assigned by STRIDE (http://webclu.bio.wzw.tum.de/stride/) and DSSP (http://swift.cmbi.ru.nl/gv/dssp/) was compared with the results of CONCORD web server.

Ramachandran Plot

A Ramachandran plot (also known as a Ramachandran diagram or a (φ,ψ) plot), originally developed in 1963 by G. N. Ramachandran, C. Ramakrishna , and V. Sasisekharan, is a way to visualize backbone dihedral angles ψ against φ of amino acid residues in protein structure. The figure at left illustrates the definition of the φ and ψ backbone dihedral angles (called φ and φ' by Ramachandran). The ω angle at the peptide bond is normally 180°, since the partial-double-bond character keeps the peptide planar. The figure at top right shows the allowed φ,ψ backbone conformational regions from the Ramachandran *et al.*, 1963 and 1968 hard-sphere calculations: full radius in solid outline, reduced radius in dashed, and relaxed tau (N-Calpha-C) angle in dotted lines. Because dihedral angle values are circular and 0° is the same as 360°, the edges of the Ramachandran plot "wrap" right-to-left and bottom-to-top. For instance, the small strip of allowed values along the lower-left edge of the plot are a continuation of the large, extended-chain region at upper left.

Structure Validation and Functional Region Identification

PROSA

ProSA is a tool widely used to check 3D models of protein structures for potential errors. Its range of application includes error recognition in experimentally determined structures theoretical models and protein engineering. ProSA-web, that encompasses

the basic functionality of stand-alone ProSA and extends it with new features that facilitate interpretation of the results obtained. The overall quality score calculated by ProSA for a specific input structure is displayed in a plot that shows the scores of all experimentally determined protein chains currently available in the Protein Data Bank (PDB). This feature relates the score of a specific model to the scores computed from all experimental structures deposited in PDB. Problematic parts of a model are identified by a plot of local quality scores and the same scores are mapped on a display of the 3D structure using color codes. It is available at https://prosa.services.came.sbg.ac.at/prosa.php.

Results and discussion

Methods

Sequence Retrieval and Identification of NAC Domain Proteins from Capsicum annum

Three different approaches were applied to identify putative NAC domain containing proteins from Capsicum annum. Initially, 898 amino acid sequences encoding NAC transcription factors from four plants (Arabidopsis thaliana, Oryza sativa, Zea mays, solanum tubersum, solanum lypersicum and Sorghum bicolor) were retrieved from plant transcription factor database 3.0 (plntfdb.bio.uni-potsdam.de/). These sequences were used to identify homologous peptides from sollanaci by performing a BLASTP search at PHYTOZOME v8.0 database (www. phytozome.net/) using default parameters. In addition, the database was searched using the keywords 'NAC', 'no apical meristem' or 'NAM''. Moreover, the HMM profiles of the NAM and NAC domains in the Pfam database (http://pfam.sanger.ac.uk/) were searched against the PHYTOZOME database of Capsicum annum. Similarity searches were also performed through TBLASTN at NCBI database against the EST sequences of Capsicum annum genome to eliminate possible exclusions of any additional NAC member. All hits with expected values less than 1.0 were retrieved and redundant sequences were removed using the decrease redundancy (web.expasy.org/decrease_redundancy).
Each non-redundant sequence was checked for the presence of the conserved NAC domain by SMART (http://smart.emblheidelberg. de/) and Pfam (http://pfam.sanger.ac.uk/) searches. Transmembrane motifs in the sequences were identified with TMHMM Server v.2.0 (http://www.cbs.dtu.dk/services/ TMHMM/) using default parameters.

Chromosomal Location, Gene Structure and Estimation of Genomic Distribution

Specific chromosomal position of the genes encoding these SiNAC proteins were determined by BLASTP search of the Capsicum annum sequences against the PHYTOZOME database using default settings. The genes were plotted separately onto the nine sollanaci chromosomes according to their ascending order of physical position (bp), from the short arm telomere to the long arm telomere and finally displayed using Map Chart. As a gene family may be expanded through tandem and segmental duplication events, we intended to identify the mechanisms involved for expansion of NAC members in sollanaci. Segmental duplications were identified based on the method of Plant Genome Duplication Database. Briefly, BLASTP search was executed against all predicted peptide sequences of Capsicum annum and top 5 matches with E-value, 1e-

05 were identified as potential anchors. Collinear blocks were evaluated by MCScan and alignments with E-value, 1e-10 were considered as significant matches. Tandem duplications were characterized as adjacent genes of same sub-family located within10 predicted genes apart or within 30 kb of each other. The exon-intron organizations of the genes were determined using Gene structure display server (gsds.cbi.pku.edu.cn/) through comparison of their full-length cDNA or predicted coding sequence (CDS) with their corresponding genomic sequence.

Step 2: Secondary structure prediction and multiple alignments

To discover the conserved regions of 96 *NAC* genes, we performed Sequence alignment by using PRALINE, default parameter were set to BLOSUM62 weight matrix , associated gap penalty 12 open and 1 extension , and secondary structure was predicted by applying psipred server with default parameters setting NR PSI-BLAST and DSSP TRAINED neural nets, we also executed motif search by applying meme suite. After getting the results of multiple sequence alignment, secondary structure prediction and motif search, we have come to conclude that all 96 *NAC* genes have highly conserved domains and most of the sequences are dominated by helix possession.

STEP 3 Domain search of NAC protein

After the final 92 sequences were retrieved Arbitrary chosen NAC domain useing SMART, INTERPROSCAN and Pfam, Was used for the domain search.

Primary Characterisation of NAC TFs

Physico-chemical parameters including molecular weight, isoelectric point, instability index, alihphatic index and GARVY were elucidated using Protparam tool of ExPASy (http://web.expasy.org/protparam/).

Phylogenetic Analysis

The amino acid sequences were imported into MEGA 6.0 and multiple sequence alignments were performed using ClustalW with a gap open and gap extension penalties of 10 and 0.1, respectively. The alignment file was then used to construct an uprooted phylogenetic tree based on the neighbour - joining method and after bootstrap analysis for 1000 replicates, the tree was displayed using Interactive tree of life.

STEP 4: Secondary structure prediction

SOPMA

Steps for SOPMA

The secondary structure prediction was done by SOPMA

By seeing the alpha value, beta value and coil turn sheet secondary structure prediction has been done.

The table 4.5 and 4.6 shows the SOPMA results.

STEP-5: Templates identified using BLASTP, PSI-BLAST and DELTA_BLAST search against PDB to model NAC domains identified from NAC TFs of Capsicum genome

After secondary structure prediction the value of α, β and turn coil have been predicted by SOPMA.
Then the best template search has been done by protein BLAST against PDB id.

Then the BLAST sequence has been modelled

The result of BLAST has been shown in table 4.7 and 4.8.

The best template found are been highlighted.

Tertiary Structure Identification

After finding the best five template from Zunla 1 and chileptein are being modelled using MODELLER 9.5 the model have been generated are shown below.

Extensive genome wide screening resulted a total of 186 non-redundant (37 from *Capsicum annum L.* Zunla_*1* and 42 from *Capsicum annum* var. *glabriusculum* Chiltepin) NAC transcription factors from Capsicum species. Domain architecture and phylogenetic analysis showed that NAC gene family are highly diverse in nature even though they possess highly conserved N-Terminal region. Most members contained a complete NAC DNA-binding domain and a variable transcriptional regulation domain. Furthermore, most of the gene found in the nuclear region and dominated by the possession of helix region. Distinct compositions of the putative motifs were revealed on the basis of NAC protein sequences in Capsicum. Sequence analysis, together with the organization of putative motifs, indicated distinct structures and potential diverse functions of NAC family in Capsicum species.

Table.1 Statistics of hot pepper genome and gene annotation

Number of scaffolds	37,989
Total length of scaffolds	3.06 Gb
Anchored scaffolds	2.63 Gb (86.0%)
N50 of scaffolds	2.47 Mb
Longest (shortest) scaffolds	18.6 Mb (264 bp)
Number of contigs	337,328
Total length of contigs	2.96 Gb
N50 of contigs	30.0 kb (24,618th)
Longest (shortest) contigs	442.1 kb (71 bp)
GC content	35.03%
Number of genes	34,903
Average/total coding sequence length	1,009.9/35.2 Mb
Average exon/intron length	286.5 bp/541.6 bp

Table.2 Species collected

Capsicum annum L.	***Glabriusculum Chiltepin***
3536	4597
12.1 MB	14.94 MB

Table.3 Reference Genome

Plant Name	No. of transcription factor
Solanum Tubersum	131
Solanum Lypopersicum	101
Oryza sativa subsp. Japonica	170
Oryza barthii	140
Oryza brachyantha	118
Oryza glaberrima	117
Oryza punctate	142
Nicotina tabacum	42
TOTAL	**1004/8133**

Table.4 NAC domain position with NAC TFs identified from Zunla_1 (C. annuum L.) and its wild progenitor Chiltepin (C. annuum var. glabriusculum)

Zunla-1		Chiltepin	
Sequence ID	**Domain position**	**Sequence ID**	**Domain position**
>Capang06g001635	9-132	>Capana03g002678	59-198
>Capang00g001342	33-161	>Capana12g002456	2-81
>Capang02g000055	6-134	>Capana01g004489	12-139
>Capang06g002229	7-115	>Capana06g001739	9-132
>Capang05g002025	10-138	>Capana00g001918	34-160
>Capang07g002464	31-160	>Capana02g000057	7-133
>Capang02g002410	5-139	>Capana05g002476	11-137
>Capang06g001474	6-134	>Capana07g002471	32-159
>Capang11g001762	9-135	>Capana06g001560	7-133
>Capang03g003256	10-138	>Capana11g001813	10-134
>Capang06g000574	29-156	>Capana03g003315	11-137
>Capang04g001450	8-133	>Capana06g000625	30-155
>Capang05g000480	13-138	>Capana05g001292	1-80
>Capang03g000984	3-129	>Capana05g001365	49-191
>Capang01g000591	19-145	>Capana04g001537	9-132
>Capang06g000704	18-147	>Capana05g000569	14-137
>Capang04g000043	22-150	>Capana03g001027	4-128
>Capang06g002270	6-134	>Capana06g000752	19-146
>Capang03g000737	19-145	>Capana04g000414	10-118
>Capang02g003111	14-142	>Capana04g000051	23-149
>Capang07g001770	82-223	>Capana06g002441	7-133
>Capang12g001767	4-132	>Capana03g000802	20-144
>Capang06g000116	10-140	>Capana02g003374	15-141
>Capang07g002241	16-144	>Capana07g001769	83-222
>Capang06g001635	9-132	>Capana12g002058	5-131
>Capang01g001704	14-141	>Capana04g000417	10-118
>Capang03g001717	27-156	>Capana06g000136	11-139
>Capang00g000431	14-140	>Capana07g002220	17-143
>Capang01g004302	32-159	>Capana01g002000	15-140
>Capang02g000055	6-134	>Capana03g001780	28-155
>Capang03g002576	5-134	>Capana09g000936	15-139
>Capang02g002041	6-134	>Capana08g001727	32-157
>Capang02g003111	14-142	>Capana02g002277	7-133
>Capang02g003090	16-142	>Capana02g003352	15-141
>Capang02g003279	53-196	>Capana02g003557	56-197
>Capang05g001291	7-134	>Capana05g001593	17-144
>Capang11g002132	102-240	>Capana11g002231	102-240
>Capang05g001119	48-192	>Capana05g002477	19-145
>Capang05g002025	10-138	>Capana06g003012	11-137

>Capang05g002026	44-170	>Capana01g002406	10-137
>Capang06g002772	11-137	>Capana06g001387	9-136
>Capang06g002229	7-115	>Capana07g002159	11-137
>Capang06g001635	9-132	>Capana01g001228	12-137
>Capang01g004302	32-159	>Capana12g002457	49-175
>Capang07g002241	16-144	>Capana11g000346	47-172
>Capang03g000737	19-145	>Capang03g000737	19-145
>Capang11g000319	47-152	Capang11g000319	47-152
>Capang02g000055	6-134	>Capang02g000055	6-134
>Capang05g002026	44-170	>Capang05g002026	44-170
>Capang05g002025	10-138	>Capang05g002025	10-138
>Capang06g002772	11-137	>Capang06g002772	11-137
>Capang06g000704	18-147	>Capang06g000704	18-147
>Capang12g001767	4-132	>Capang12g001767	4-132
>Capang01g001065	12-137	>Capang01g001065	12-137
>Capang12g002104	11-140	>Capang12g002104	11-140
>Capang12g002106	48-174	>Capang12g002106	48-174
>Capang05g001291	7-134	>Capang05g001291	7-134
>Capang03g002576	5-134	>Capang03g002576	5-134
>Capang01g000591	19-145	>Capang01g000591	19-145

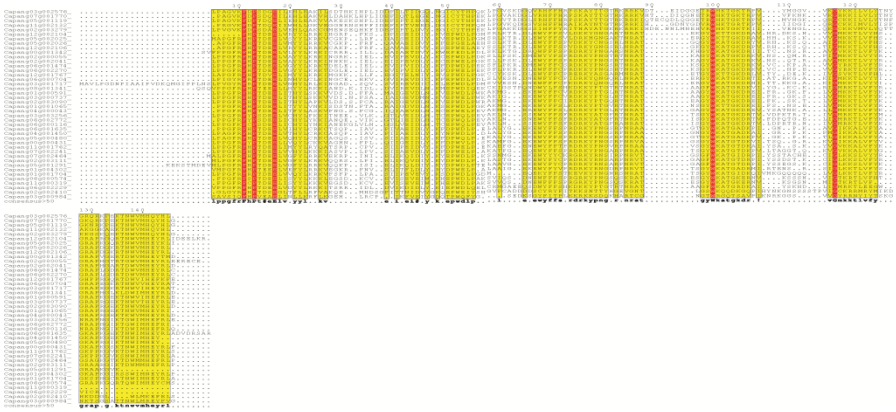

Fig. 4.1: NAC Domain alignment of *Chileptein*

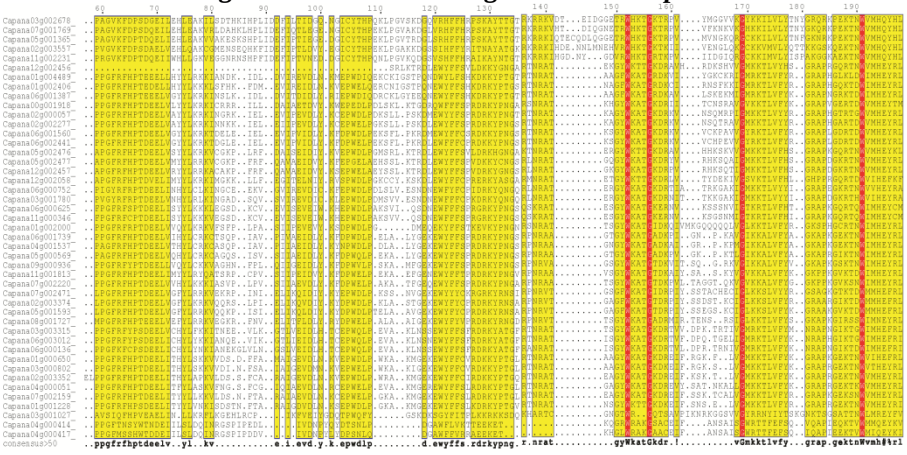

Fig. 4.2: NAC Domain alignment of *Zunla_1 Chileptein*

Table.5 Physico-chemical characterisation of NAC transcription factors identified from two genomes of Capsicum species using ProtParam tool of ExPaSy Zunla_1 (C. annuum L.)

Sequence ID	No. of amino acid	Molecular weight (kDa)	PI	Instability index	Aliphatic index	GRAVY
Capang03g002576	290	33.0666	8.46	48.61	76.86	-0.657
Capang12g002104	528	58.9613	4.78	33.91	70.42	-0.577
Capang08g001341	313	35.9111	6.11	46.00	64.76	-0.785
Capang06g001635	294	33.6662	6.26	54.98	67.69	-0.660
Capang00g00132	598	65.8700	4.84	45.81	66.06	-0.610
Capang02g000055	577	65.9552	5.09	46.61	63.55	-0.705
Capang06g002229	316	36.6281	6.11	42.20	66.36	-0.806
Capang05g002025	543	60.6187	4.68	38.22	70.42	-0.468
Capang07g002464	323	37.2216	7.13	50.81	60.99	-0.796
Capang02g002410	296	33.9693	6.60	57.08	68.82	-0.706
Capang06g001474	342	38.4157	5.63	48.75	55.56	-0.618
Capang001762	294	33.9183	6.66	30.45	67.59	-0.652
Capang003256	290	33.3445	5.86	47.06	65.17	-0.650
Capang00054	408	45.9157	9.10	47.15	70.44	-0.552
Capang004003	459	51.2797	5.98	49.69	52.46	-0.936
Capang05g1119	486	53.9603	6.45	48.75	65.62	-0.739
Capang001450	290	33.3169	8.10	49.60	66.62	-0.610
Capang09g001941	132	15.2614	9.37	33.60	64.24	-0.645
Capang05g000480	306	34.9414	6.96	37.23	62.84	-0.694
Capang03g000984	284	32.0148	4.71	29.48	78.20	-0.553
Capang01g000591	356	39.6510	7.62	33.23	68.38	-0.372
Capang06g000704	637	70.1060	4.77	37.43	76.50	-0.505
Capang04g000383	373	41.5550	5.05	46.94	59.95	-0.643
Capang04g000043	402	45.3429	6.11	28.81	65.30	-0.618
Capang06g002270	334	37.8961	5.38	43.01	51.95	-0.700
Capang03g000737	332	38.1330	7.08	30.81	65.15	-0.669
Capang02g003111	397	44.8412	6.14	43.73	65.31	-0.570
Capang07g001770	325	36.5264	7.70	34.03	75.51	-0.550
Capang12g001767	719	79.5033	4.70	38.67	77.16	-0.288
Capang04g000385	371	40.9581	4.67	56.14	63.69	-0.662
Capang06g000116	287	33.3714	5.67	40.00	71.32	-0.671
Capang04g002241	339	37.9185	8.56	42.37	65.60	-0.727
Capang06g001635	294	33.6662	6.26	54.98	67.69	-0.660
Capang01g001704	247	28.1949	9.53	40.67	59.96	-0.730
Capang03g001717	632	69.8746	4.83	39.15	69.72	-0.588
Capang12g002106	425	47.5245	5.35	53.43	72.73	-0.626
Capang00g000431	350	39.6168	9.11	32.70	72.14	-0.644
Capang02g000055	577	65.9552	5.09	46.61	63.55	-0.705
Capang05g001292	411	46.2679	6.64	51.28	67.64	-0.561
Capang02g002041	632	72.5194	5.83	43.54	70.02	-0.619
Capang02g003090	326	37.2779	7.58	44.92	57.70	-0.786
Capang02g003279	385	42.9157	5.03	45.05	64.83	-0.752
Capang00g001342	586	65.8700	4.84	45.81	66.06	-0.610
Capang05g001291	411	46.2679	6.64	51.28	67.64	-0.561
Capang05g002026	420	47.4850	5.70	50.32	74.48	-0.473
Capang06g002772	290	33.3967	5.88	46.35	69.62	-0.575
Capang06g002229	316	36.6281	6.11	42.20	66.36	-0.806
Capang06g001635	294	33.6662	6.26	54.98	67.69	-0.660
Capang01g004302	409	46.4232	6.63	55.66	68.70	-0.787
Capang07g002241	339	37.9185	8.56	42.37	65.60	-0.727
Capang11g000319	414	46.5895	8.86	49.47	70.14	-0.731
Capang02g0055	577	65.9552	5.09	46.61	63.55	-0.705
Capang01g001065	348	38.9664	7.20	42.51	49.89	-0.770

(b) *Chiltepin* (*C. annuum* var. *glabriusculum*)

Sequence ID	No. of amino acid	Molecular Weight (kDa)	PI	Instability index	Aliphatic index	GRAVY
Capana03g002	290	33.0666	8.46	48.61	76.86	-0.657
Capana12g0022456	476	52.8764	4.60	32.05	69.92	-0.556
Capana01g004489	313	35.9411	6.11	46.28	64.44	-0.793
Capana06g001739	213	24.5653	9.10	47.18	70.56	-0.697
Capana00g001918	586	65.8360	4.80	44.93	66.21	-0.603
Capana02g000057	577	66.0173	5.09	47.38	62.88	-0.708
Capana05g002476	551	61.6118	4.69	40.20	69.93	-0.479
Capana07g002471	323	37.1936	7.15	50.78	61.58	-0.802
Capana11g001813	294	33.8462	7.05	31.38	67.59	-0.642
Capana03g003315	290	33.3445	5.86	47.06	65.15	-0.650
Capana05g001292	459	51.2655	5.81	49.97	52.46	-0.934
Capana04g001537	290	33.3169	8.10	49.60	66.62	-0.610
Capana03g001027	284	32.0409	4.90	28.97	78.20	-0.558
Capana04g00414	356	39.5315	4.91	46.51	59.24	-0.769
Capana04g000051	390	44.1115	6.28	29.54	66.31	-0.618
Capana07g001769	325	36.5724	7.22	34.36	74.31	-0.582
Capana04g000417	371	40.9581	4.67	56.14	63.69	-0.662
Capana06g000136	287	33.3714	5.67	38.86	71.32	-0.669
Capana01g002000	248	28.3391	9.59	41.55	58.15	-0.766
Capana02g002277	632	72.5675	5.89	43.65	70.02	-0.615
Capana02g003557	387	43.1040	5.03	44.87	64.50	-0.744
Capana01g002406	303	34.9318	7.79	36.78	60.20	-0.865
Capana06g000625	408	45.9046	9.00	48.64	69.49	-0.565
Capana07g002159	323	36.2277	8.80	43.87	60.71	-0.531
Capana03g001780	632	69859.6	4.88	38.45	69.72	-0.589
Capana11g002231	506	57.5254	5.51	48.24	72.79	-0.752
Capana02g003374	397	44.8412	6.14	43.73	65.31	-0.570
Capana02g003352	326	37.3040	7.58	44.92	58.90	-0.771
Capana09g000936	350	39.6168	9.11	32.70	72.14	-0.644
Capana05g1365	486	53.9603	6.45	48.75	65.62	-0.739
Capana06g002441	334	37.9091	5.38	42.92	51.95	-0.708
Capana08g001727	408	46.3112	6.64	55.77	68.87	-0.787
Capana07g002220	340	37.9915	8.56	42.84	65.12	-0.734
Capana03g000802	332	38.0949	7.08	30.27	64.58	-0.674
Capana11g000346	414	46.5895	8.86	49.47	70.14	-0.731
Capana05g002477	396	44.6363	5.41	52.75	68.66	-0.629
Capana06g003012	290	33.3826	5.88	46.61	69.62	-0.575
Capana06g001387	322	37.5936	5.85	46.51	52.70	-0.937
Capana06g000752	637	70.1060	4.77	37.43	76.50	-0.505
Capana12g002058	719	79.4972	4.68	40.07	77.02	-0.289
Capana01g001228	352	39.1947	7.20	43.54	49.32	-0.765
Capana12g002457	424	47.6276	5.35	53.60	72.32	-0.619
Capana05g001593	410	46.2109	6.64	51.08	67.80	-0.561
Capana05g000569	306	34.9419	7.08	37.23	62.84	-0.694
Capana01g000650	356	39.6530	7.08	33.60	68.40	-0.376

Table.6 Secondary structure prediction
(a) Chileptein

Sequence id	SOPMA		
	Heli (%)	Beta sheet %	Turn coils %
Capang03g002576	10	11.72	44.14
Capang12g002104	28.60	6.63	45.64
Capang08g001341	20.45	9.90	47.60
Capang06g001635	20.41	6.12	55.10
Capang00g001342	26.45	7.85	45.05
Capang02g000055	29.98	8.15	45.41
Capang06g002229	32.59	8.23	45.57
Capang05g002025	26.52	6.63	47.33
Capang07g002464	29.55	6.50	51.08
Capang02g002410	28.38	10.81	39.53
Capang06g001474	13.16	7.89	56.73
Capang11g001762	21.09	7.14	49.32
Capang03g003256	16.00	9.66	47.59
Capang06g000574	25.98	7.60	45.59
Capang00g004003	15.69	7.84	61.0
Capang05g001119	16.87	7.61	56.17
Capang04g001450	20.34	7.59	52.07
Capang09g001941	13.64	15.91	35.61
Capang05g000480	24.84	8.50	48.37
Capang03g000984	29.23	8.45	36.97
Capang01g000591	21.91	9.83	44.66
Capang06g000704	23.23	8.16	49.14
Capang04g000313	35.92	4.29	47.18
Capang04g000043	25.02	11.19	40.80
Capang06g002270	7.78	8.88	61.08
Capang03g000737	28.61	6.33	44.28
Capang02g003111	22.92	9.07	50.38
Capang07g001770	20.62	10.15	46.15
Capang12g001767	21.28	7.79	47.29
Capang04g000385	37.20	4.58	44.74
Capang06g000116	21.60	10.80	40.42
Capang07g002241	15.34	10.03	55.16
Capang06g001635	20.41	6.12	55.10
Capang01g001704	22.67	11.34	38.87
Capang03g001717	25.32	7.12	47.94
Capang12g002104	28.60	6.63	45.64
Capang00g00431	28.86	8.57	42.29
Capang01g004302	25.43	5.62	50.12
Capang02g000055	29.98	8.15	45.41
Capang03g002576	10.00	11.72	44.14
Capang02g002041	25.47	8.39	47.63
Capang02g003090	20.25	8.90	48.47
Capang02g003279	24.16	7.27	47.27
Capang00g001342	26.45	7.85	45.05
Capang05g001291	33.58	7.79	44.28
Capang11g002132	31.60	8.73	37.84
Capang05g002025	26.52	6.63	47.33
Capang05g002026	31.19	8.83	42.62
Capang06g002772	22.41	13.10	34.66
Capang06g002229	32.59	8.23	45.57
Capang06g001635	20.41	6.12	55.10
Capang01g004302	25.43	5.62	50.12
Capang11g000319	28.02	9.66	46.86
Capang07g002241	15.34	10.03	55.16
Capang12g001767	21.28	7.79	47.29
Capang01g001065	25.86	6.90	46.55
Capang12g002106	35.53	7.53	43.06
Capang05g001291	33.58	7.79	44.28
Capang00g001342	26.45	7.85	45.05
Capang01g001591	21.91	9.83	44.60

(b) *Zunla_1*

Sequence id	SOPMA		
	Helix (%)	Beta sheet %	Turn coils %
Capana03g002678	10.00	11.72	44.14
Capana12g002456	28.78	6.93	43.49
Capana01g004489	20.45	9.90	47.60
Capana06g001739	23.00	7.04	51.64
Capana00g001918	26.28	7.51	46.25
Capana02g000057	29.64	8.67	45.06
Capana05g002476	27.04	6.53	47.19
Capana07g002471	26.93	6.50	51.70
Capana11g001813	20.41	7.48	50.0
Capana03g003315	16.90	9.66	47.59
Capana05g001292	15.69	7.84	61.00
Capana04g001537	20.34	7.59	52.07
Capana03g001027	29.23	8.45	37.32
Capana04g000414	33.71	4.78	50.84
Capana04g000051	26.15	11.03	41.03
Capana07g001769	18.77	10.77	47.08
Capana04g000417	37.20	4.58	44.74
Capana06g000136	21.60	10.80	40.42
Capana01g002000	22.18	10.48	39.92
Capana02g002277	25.32	7.91	48.42
Capana02g003557	23.77	7.24	47.80
Capana01g002406	23.43	11.22	46.86
Capana06g000625	25.98	8.82	47.61
Capana03g001780	25.16	7.28	47.78
Capana07g002159	23.53	6.50	49.54
Capana11g002231	33.20	8.30	36.96
Capana02g003374	22.92	9.07	50.38
Capana02g003352	21.78	9.51	46.93
Capana09g000936	28.86	8.57	42.29
Capana05g001365	16.87	7.61	56.17
Capana06g002441	7.78	8.68	60.78
Capana08g001727	25.49	5.64	50.25
Capana03g000802	28.01	5.72	44.88
Capana07g002220	15.29	9.12	56.18
Capana11g000346	28.02	9.66	46.86
Capana05g002477	28.28	9.09	45.20
Capana06g003012	23.10	13.79	37.93
Capana06g001387	18.94	6.83	51.86
Capana06g000752	23.23	8.16	49.14
Capana12g002058	21.56	7.65	46.45
Capana01g001228	25.57	7.10	47.16
Capana12g002457	35.12	7.98	42.96
Capana05g001593	33.66	8.05	43.90
Capana05g000569	24.84	8.50	48.37
Capana01g000650	21.07	9.83	45.22
Capana03g002678	10.00	11.72	44.14
Capana12g002456	28.78	6.93	43.49
Capana01g004489	20.45	9.90	47.60
Capana06g001739	23.00	7.04	51.64
Capana00g001918	26.28	7.51	46.25
Capana02g000057	29.64	8.67	45.06
Capana05g002476	27.04	6.53	47.19
Capana07g002471	26.93	6.50	51.70
Capana11g001813	20.41	7.48	50.0
Capana03g003315	16.90	9.66	47.59
Capana05g001292	15.69	7.84	61.00
Capana04g001537	20.34	7.59	52.07
Capana03g001027	29.23	8.45	37.32
Capana04g000414	33.71	4.78	50.84
Capana04g000051	26.15	11.03	41.03

Table.7 Templates identified using BLASTP, PSI-BLAST and DELTA_BLAST search against PDB to model NAC domains identified from NAC TFs of Capsicum genome(The highlighted with yellow band are been modelled according to their best e-value)

(a) Zunla_1

Sequence id	PDB ID	BLAST P			PSI BLAST			DELTA BLAST		
		Score	% of identity	e-value	Score	% of identity	e-value	Score	% of identity	e-value
Capang03g002576	3ULX A	61.2	29	4e-11	61.2	29	4e-11	32.0	19	0.67
Capang08g001341	3ULX A	160	42	2e-47	160	42	2e-47	244	45	4e-88
Capang05g002025	3SWMA	160	47	2e-45	160	28	2e-45	246	27	1e-77
Capang07g002464	1UT4 A	164	49	1e-48	164	49	1e-48	244	49	1e-79
Capang02g002410	3ULX A	34.3	52	0.075	34.3	52	0.075	165	25	2e-49
Capang06g001474	1UT4 A	171	47	2e-51	171	47	2e-51	244		1e-79
Capang11g001762	3ULX A	222	53	1e-71	222	53	1e-71	272	53	6e-91
Capang03g003256	1UT4 A	159	51	47	159	51	3e-47	242	52	2e-79
Capang06g000574	1TU4 A	155	37	1e-44	155	37	1e-44	239	35	7e-77
Capang00g004003	3SWMA	77.0	21	6e-16	77.0	21	6e-16	164	21	2e-47
Capang05g001119	3ULX A	68.6	30	5e-13	68.6	30	5e-13	206	30	4e-63
Capang04g001450	3ULX A	245	55	1e-80	245	55	1e-80	264	55	5e-88
Capang09g601941	3SWM A	68.6	44	9e-15	68.6	44	9e-15	140	58	4e-42
Capang03g000984	3ULX A	49.3	42	4e-07	49.3	42	4e-07	185	56	2e-57
Capang03g000383	1UT4 A	46.2	38	7e-06	46.2	38	7e-06	177	38	2e-53
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(b) *Chileptin*

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Capana00g001918	3ULXA	167	21	9e-48	167	21	9e-48	243	26	3e-76
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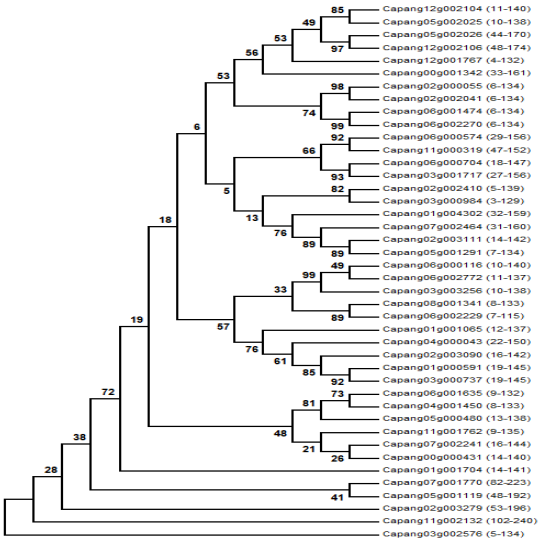


Fig. 4.3: Phylogenetic tree of *Chileptein Zunla_1*

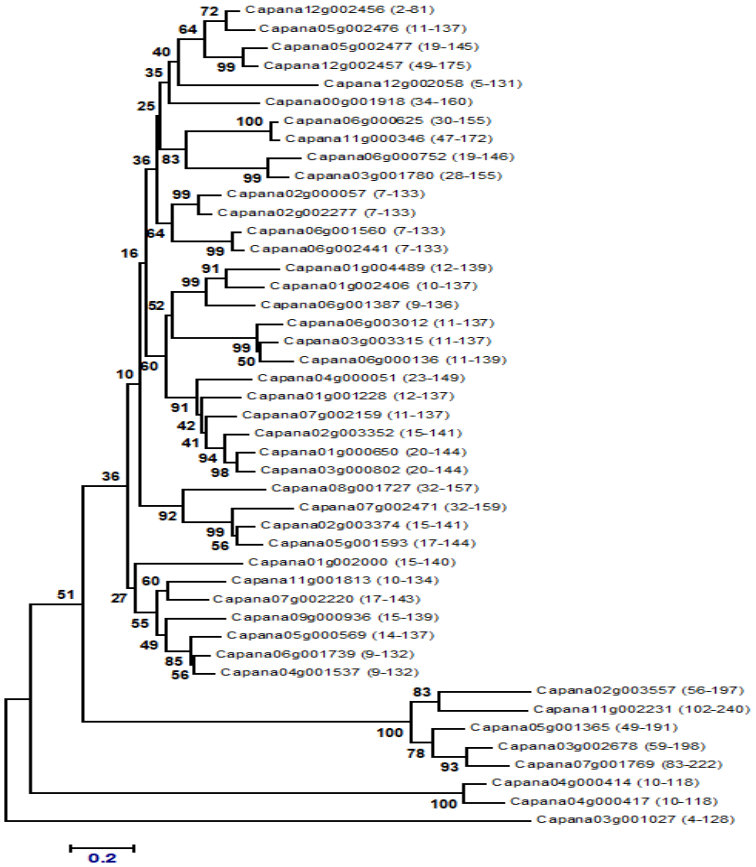
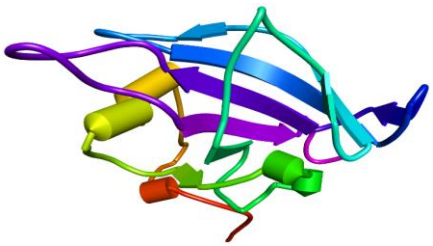
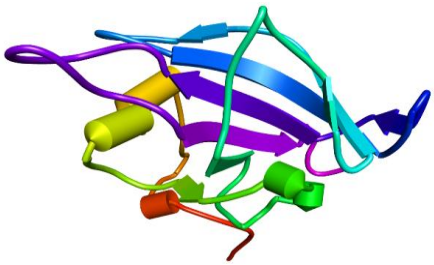
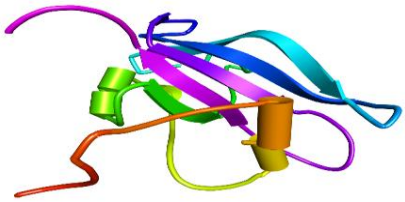


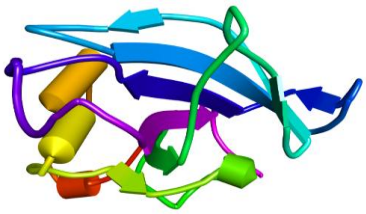
Fig. 4.4: Phylogenetic tree for *Zunla_1 Chileptein*

Fig. 4.5: Capang00g000431Fig. 4.6: Capang05g000480









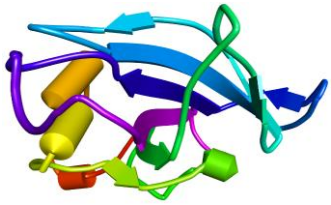
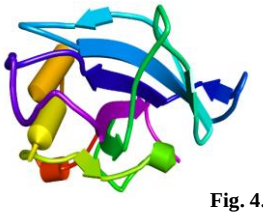


Fig. 4.9: Capang11g001762

Fig. 4.7: Capang06g001474Fig. 4.8: Capang00g00163

Zunla_1



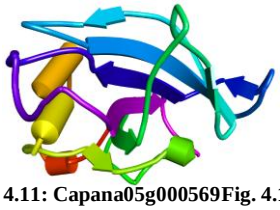
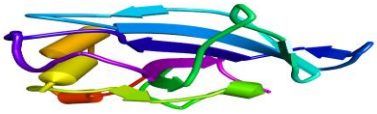


Fig. 4.10: Capana04g001537Fig. 4.11: Capana05g000569Fig. 4.12: Capana01g001739



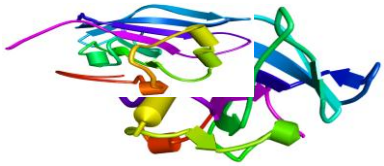
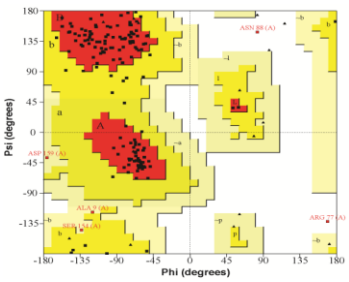


Fig. 4.13: Capana11g001813Fig. 4.14: Capana09g000936

STEP-6

Ramachandran Plot (*chileptein*)



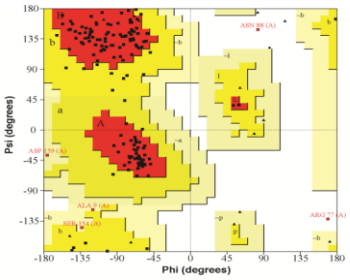
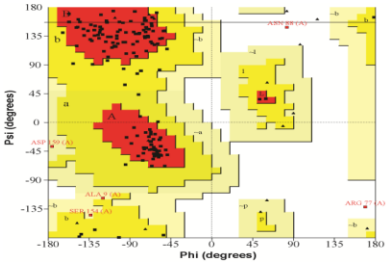


Fig. 4.15: Capang00g000431 **Fig. 4.16: Capang05g000480**



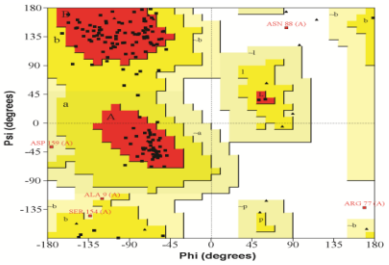


Fig. 4.17: Capang06g001474 **Fig. 4.18: Capang00g001635**

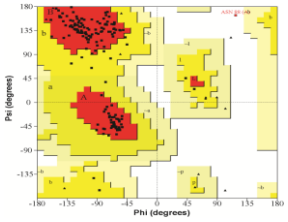
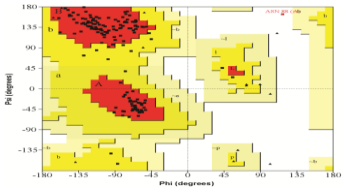


Fig. 4.19: Capang11g001762

Zunla_1



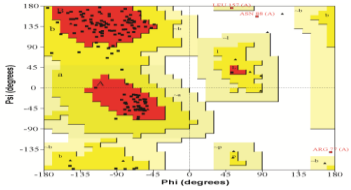
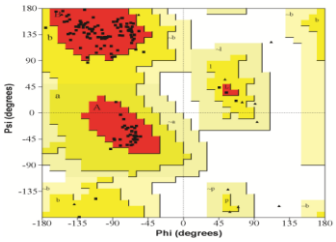


Fig. 4.20: Capana04g001537 **Fig. 4.21: Capana05g000569**



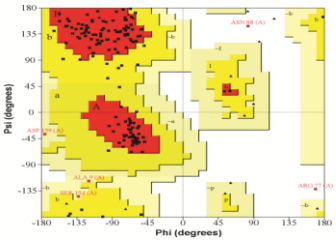


Fig. 4.22: Capana01g001739 **Fig. 4.23: Capana11g001813**

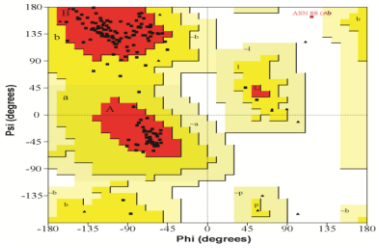
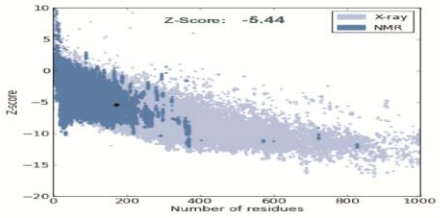


Fig. 4.24: Capana09g000936

Chileptein



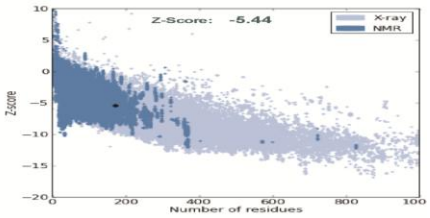
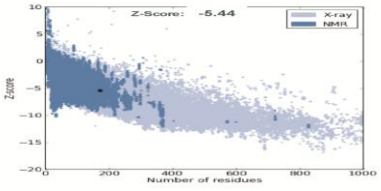


Fig.4.25: Capang00g000431 **Fig.4.26: Capang05g000480**



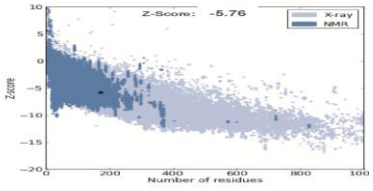


Fig.4.27: Capang06g001474 **Fig. 4.28: Capang00g001635**

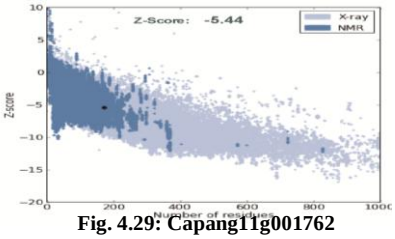
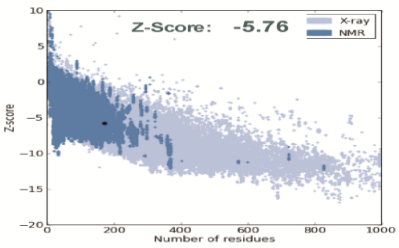


Fig. 4.29: Capang11g001762

ZUNLA_1



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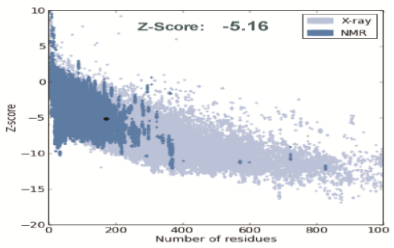

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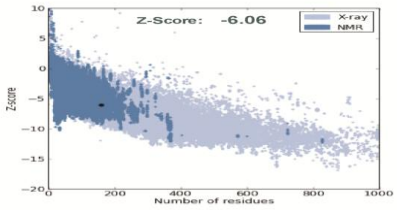

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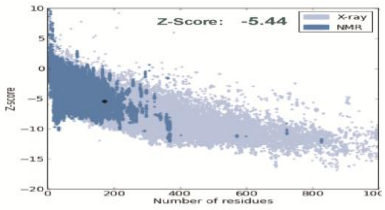

Fig.4.33: Capana11g001813

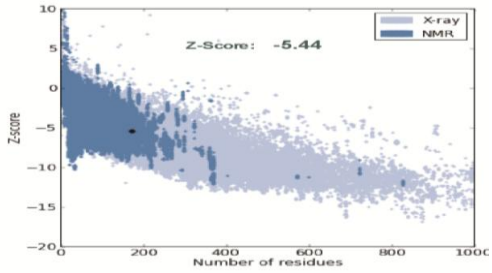

Fig.4.34: Capana09g000936

Taken together, NAC family was comprehensive characterized from Capsicum species may serve as a future genetic resource to elucidate candidate stress-responsive NAC TFs. In the future, a combination of reverse genetics, genomics, and proteomic approaches in various developmental stages and stress conditions will provide us with critical information to elucidate the functional differences of the stress-responsive NAC factors and their relationship in transcriptional control. Since numerous NAC factors have various functions in plant growth and development, including senescence as well as stress responses, the functions of NAC transcription factors and NAC network will also be analysed in relation to the crosstalk between stress responses and plant growth. Overall, our work has laid a solid foundation for further characterization of this important NAC gene family in *Capsicum* species.

Acknowledgement

First of all we want to give our extreme thanks to Dr.Budheswar Dehury for his support and guidance for which the entire work may not be completed. Thenwe would acknowledge to the Department of Biotechnology, Govt. of India, for providing the BIF Centre facility to carry out the

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