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Identification of differentially expressed genes by cDNA-AFLP technique during heat stress in cowpea nodules

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Abstract Legume nodules formed by diazotrophic microorganisms are active sites for biological nitrogen fixation (BNF). In tropical regions, a significant part of N supply for soybean, peanut and bean crops is derived from BNF, which is nevertheless often limited by high temperature stress. In contrast, cowpea nodules are very resistant to high temperatures. To understand the molecular bases of thermotolerance during BNF under heat stress, we have used cDNA-amplified fragment length polymorphism experiments to identify differentially expressed transcripts from cowpea nodules subjected to heat shock treatment. The expression profiles obtained showed approximately 600 bands, 55 up-regulated and nine corresponding to genes repressed by heat stress. Twenty transcript-derived fragments were isolated, cloned and sequenced. The *Vigna unguiculata* nodule and stress response transcripts present similarities to those that encode low molecular weight heat shock proteins, wound-induced proteins, disease resistance protein, and xylan endohydrolase isoenzyme, as well as different housekeeping genes. The differential expression of 15 genes was confirmed by using Northern blot or reverse Northern hybridization experiments.

Key words: Heat stress; Small heat shock protein; Stress-induced protein; Biological nitrogen fixation; cDNA-amplified fragment length polymorphism; *Vigna unguiculata*

1. Introduction

Plants are frequently subjected to environmental stress, which generally impairs plant growth and agricultural productivity [1]. Furthermore, under natural conditions, the interaction of different stress factors tends to maximize the deleterious effect observed for each individual stress condition. In semi-arid regions, the combination of high temperature and nutrient deficiency, such as nitrogen, has a tremendous impact on plant growth and crop yields [2,3].

In legume plants, nitrogen requirements can be supplied by N_2 fixed in root nodules formed during symbiotic association with rhizobia. Within these structures, bacteria reduce N_2 to ammonia, which is then used by the plant [4–6]. Biological nitrogen fixation (BNF) is a complex process requiring several steps and extensive molecular signal exchanges between plant and bacteria, which demands specific gene expression from both partners [4,7]. BNF in several legumes, such as soybean, peanut and bean, is also strongly limited by high temperatures [2,8–10] which affect several steps of the process: rhizobial survival, infection interaction, nodule development [10] and nitrogen fixation. In tropical areas, where soil temperatures often exceed 40°C [11–14], this problem is very relevant since reduction of N_2 fixation has already been observed at temperatures just above 28°C [9,15–17].

Concerning heat shock stress, most research efforts have focused the microsymbiont partner. These studies resulted in the isolation and characterization of efficient and competitive bacterial strains, which are tolerant to several environmental stresses including high temperature [11,18–20]. Although strain selection can increase nodule thermotolerance, the effect seems to be insufficient to produce a high level of BNF under heat stress observed in field conditions. It is probable that limitations caused by high temperature must also be related to plant factors, but little is known about the mechanism involved in the plant symbiosis thermotolerance. Since the BNF process is dependent on the interaction of both partners, plant and bacterium, with environmental factors, the understanding of plant gene expression regulation could help to manage the heat tolerance process in the symbiosis and contribute to reduce the limitations imposed by high temperature stress.

The association between cowpea (*Vigna unguiculata*) and *Bradyrhizobium* sp., well adapted to the Brazilian semi-arid region, was used here as a model to better understand the molecular bases of the response to high temperature in legumes cultivated under symbiosis. Experiments of cDNA-amplified fragment length polymorphism (cDNA-AFLP) [21,22] were used to isolate differentially expressed genes from cowpea nodules subjected to heat shock treatment. The use of cDNA-AFLP has often been described as an extremely efficient method for isolation of differentially expressed genes [22–29]. In this work, several heat shock-induced cDNA fragments were isolated from cowpea nodules, which showed sequence similarity to stress-related genes of different plant species.

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2. Materials and methods

2.1. Plant material, growth conditions and bacterial inoculation procedure

Cowpea seeds (*V. unguiculata*, cv. IPA 206) were surface sterilized and sown in vermiculite/sand mixture (2:1). Four days after emergence (DAE), each seedling was transferred to a plastic recipient (500 ml) containing a 2:1 vermiculite/sand mixture and inoculated with YM liquid broth (1 ml) where *Bradyrhizobium japonicum* strain IPA 201 was allowed to grow for 5 days. The plants were kept in a greenhouse at 28°C with N-free sterile Norris nutrient solution [30] during the entire growth period.

Heat shock treatment was applied to plants at 14 DAE, when they were subjected to 0.5, 1 or 2 h at 40°C in a growth chamber. Plants were harvested, nodules and leaves were collected, immediately frozen in liquid nitrogen and then transferred to −70°C until total RNA extraction.

2.2. cDNA-AFLP experiments

2.2.1. Preparation of poly(A)$^+$ RNA and cDNA synthesis. Total RNA was isolated from each sample of frozen tissue (0.5 g) according to a method adapted from Ragueh et al. [31]. The poly(A)$^+$ RNA was isolated from total RNA (20 μg) using oligo(dT) coupled to paramagnetic beads (Dynal A.S., Oslo, Norway). Double-stranded cDNA was synthesized from poly(A)$^+$ RNA according to cDNA Synthesis Module RPN1256 (Amersham), extracted with phenol/chloroform/isoamyl alcohol, precipitated with ethanol and resuspended in water (20 μl).

2.2.2. Template preparation, AFLP reaction and PAGE analysis of products. The template was prepared as described by Bachem [21,22]. The cDNA was first digested with *Eco*RI and *Mse*I for 2 h at 37°C and then ligated to *Eco*RI and *Mse*I double strand adapter, according to the procedure described in the Core AFLP kit (Gibco-BRL). The preamplification reaction was carried out using 28 cycles (94°C, 30 s; 60°C, 1 min; 72°C, 1 min) using primers corresponding to *Eco*RI and *Mse*I adapters without extension and 1/10 of template volume. Following the preamplification step, the product was diluted (10×) with TE buffer and 5 μl were used for selective amplification, using 42 cycles including 14 touchdown cycles comprising a reduction of the annealing temperature from 65°C to 56°C, in 0.7°C steps, which was then maintained for 28 cycles. Six primer combinations were used for selective amplification: A: *Eco*RI-AAG and *Mse*I-0; B: *Eco*RI-AAG and *Mse*I-A; C: *Eco*RI-AAG and *Mse*I-T; D: *Eco*RI-AGC and *Mse*I-0; E: *Eco*RI-AGC and *Mse*I-A; F: *Eco*RI-AGC and *Mse*I-T. Selective amplification products were denatured in formamide (50%) at 95°C, and separated by electrophoresis in polyacrylamide gel (5%) containing urea and TBE according to Sambrook et al. [32]. Gels were dried onto 3MM Whatman paper (Whatman, Maidstone, UK) and then exposed to Kodak Biomax film (Sigma) for at least 24 h at −70°C.

2.2.3. Transcript-derived fragment (TDF) isolation. The bands of interest were selected, removed from the gel and soaked in water (10 μl). DNA was purified by precipitation as described by Reuber and Ausubel [33] and then reamplified using the same primers as described for the selective amplification totaling 36 cycles, but touchdown conditions were carried out as follows: reduction of the annealing temperature from 65°C to 59°C, in 1.0°C steps, which was then maintained for 30 cycles.

2.3. Sequence analysis

The reamplified TDFs were cloned into plasmid pCRII® (Invitrogen) using the TA Cloning kit (Invitrogen) or plasmid pGEM-T Easy® Vector System I (Promega). Sequencing of the cloned TDFs was carried out on Perkin Elmer Applied Biosystems apparatus (ABI Prism 370, 373 and 377). Database searches were performed using the BLAST Network Service (NCBI, National Center for Biotechnology Service). Each TDF sequence was compared against all sequences in the non-redundant databases using the BLASTX program [34], which compares translated nucleotide sequences with protein sequences. Sequences that returned with no significant homology were compared again against the EST databases using the BLASTN program. To analyze the putative protein domain encoded by TDF, Pfam programs were used [35]. ClustalW (1.7) from the BCM Search Launcher and GeneDoc [36] were programs used for the alignment analysis and edition. Program default parameters were used for all the analyses.

The sequences of TDFs appear in the GenBank data bank under accession numbers: BM279558, BM279559, BM279560, BM279561, BM279562, BM279563, BM279564, BM279565, BM279566, BM279567, BM279568, BM279569, BM279570, BM279571, BM279572, BM279573, BM279574, BM279575 corresponding to *Vu*NSR1 and *Vu*NSR4–20, respectively.

2.4. Northern blot and reverse Northern

For Northern analysis, 20 μg of total RNA from each sample were subjected to electrophoresis in formaldehyde-containing agarose gels (1.5%) as described by Sambrook et al. [32]. After electrophoresis, RNA was blotted onto nylon membranes and hybridized with TDF cloned fragments labeled with ^{32}P as a probe. About 1 μg of the TDFs was used for the reverse Northern analyses. Plasmid DNA from each cloned TDF was digested with *Eco*RI restriction enzyme, the total volume of each digestion was then separated in 1% TAE agarose gel. Gels were denatured, neutralized, blotted into a Hybond-N$^+$ (Amersham) nylon membrane and hybridized using a cDNA complex probe derived from heat-shocked and control cowpea nodules. The cDNA probes were generated with Superscript reverse transcriptase (Life Technologies) with 25 μg total RNA as described by Charon et al. [37]. The membranes were hybridized overnight, one set for each probe, in 0.5 M Na-phosphate pH 7.2, 7% sodium dodecyl sulfate (SDS) buffer at 65°C [38]. After hybridization, the membranes were washed twice for 30 min each with 2×SSC/0.1% and 1×SSC/0.1% SDS and exposed overnight on Kodak films.

3. Results and discussion

3.1. Isolation of differentially expressed genes during heat shock

To analyze genes involved in the thermotolerance of the legume and rhizobium symbiosis, cDNA-AFLP was carried out on nodules of cowpea plants subjected to heat stress. cDNA-AFLP templates were prepared from nodules of plants maintained at 40°C for up to 2 h, as well as control plants. Fig. 1 shows an example of the expression pattern obtained. Number and length, varying from 100 to 500 bp, of the observed TDFs were dependent on primer combination. Many TDFs displayed an altered expression pattern in response to heat stress and were selected for further analysis. A total of 600 bands were generated, 55 of them were up-regulated and nine down-regulated. Arrows in Fig. 1 show examples of each class of TDFs obtained. Twenty of these TDFs were cloned, sequenced and the hypothetical proteins deduced from six frame-translated fragments were analyzed by BLAST. The sequence similarities found are shown in Table 1. The majority of the TDFs characterized showed significant homology with known proteins, such as *Phaseolus vulgaris* low molecular weight heat shock protein, *Medicago sativa* putative wound-induced protein, disease resistance protein, xylan endohydrolase isoenzyme, and pherophorin protein from *Arabidopsis thaliana*. Some of these genes are known to be related to response to biotic and abiotic stress, while others seem to be associated with early events regarding the oxidative stress response, which most likely has regulatory and signal transduction functions. Three TDFs did not show significant matches to any known gene or EST sequence from databases. They may represent yet uncharacterized genes, but this result has to be considered carefully since the DNA fragment lengths are too short which may limit the homology search analysis. Similar results were found by Durrant et al. [25] using cDNA-AFLP to study the interaction between fungal pathogen and tobacco cell. The clones corresponding to different TDFs were renamed *Vu*NSR for *V. unguiculata* nodule and stress response (Table 1).

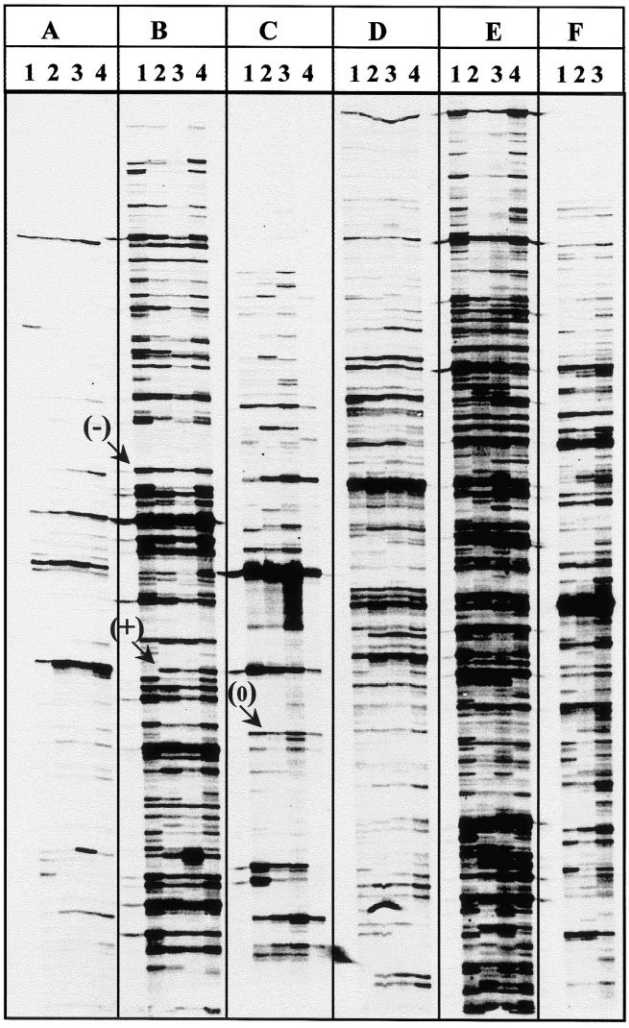

Fig. 1. cDNA-AFLP autoradiography showing the TDF pattern from cowpea nodules subjected to high temperature stress. A–F are the primer combinations used (see Section 2). Each lane corresponds to amplified template from unstressed plants (lane 1) or stressed plants (30, 60 and 120 min after the onset of the heat treatment, lanes 2, 3, and 4, respectively). Arrow-labeled bands correspond to *Vu*NSR2 (induced gene '+'), *Vu*NSR12 (unaltered gene '0') and *Vu*NSR6 (down-regulated gene '−').

3.2. Expression analysis

The relative number of transcripts hybridizing with a specific TDF was analyzed by reverse Northern blot as a way to confirm if expression levels of TDFs were different in treated nodules when compared to control nodules. These experiments, repeated at least twice for each TDF, proved that 13 isolated genes are differentially expressed under the conditions tested. Reverse Northern blots have shown that genes corresponding to TDFs A8.2 (*Vu*NSR1), B5.4 (*Vu*NSR2), C3.4 (*Vu*NSR3), A9.4 (*Vu*NSR8), F7.1 (*Vu*NSR13), F8.1 (*Vu*NSR14), D3.1 (*Vu*NSR15), D9.1 (*Vu*NSR16), C3.5 (*Vu*NSR19) are up-regulated in cowpea nodules subjected to heat stress, while in contrast TDFs B2.2 (*Vu*NSR6), B3.3 (*Vu*NSR9), B1.7 (*Vu*NSR10) and C2.8 (*Vu*NSR20) are down-regulated. In these analyses, TDFs F4.15 (*Vu*NSR7) and C3.3 (*Vu*NSR12) presented comparable expression levels in both treated and control nodules (Fig. 2), in spite of their up-regulated pattern obtained in cDNA-AFLP experiments.

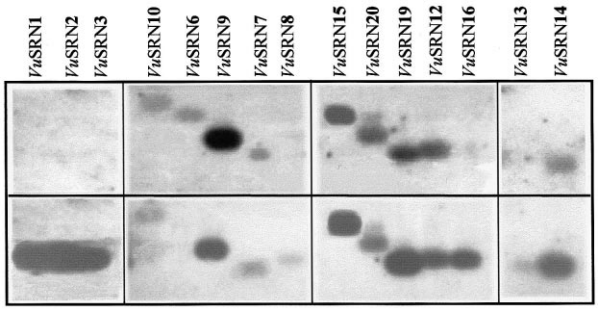

Fig. 2. Expression pattern of TDFs in cowpea nodules during heat stress analyzed by reverse Northern TDF hybridization profiles in cowpea nodules under heat shock (40°C up to 2 h) and control (28°C). Filter containing arrays of TDFs clones isolated by cDNA-AFLP were hybridized with cDNA probes obtained from control (top) or heat-shocked (bottom) cowpea nodules harvested 20 DAE.

Three TDFs encoding different classes of proteins were analyzed by traditional Northern blot hybridization and their kinetics of transcript accumulation in response to heat stress are shown in Fig. 3. *Vu*NSR2 (sHSP), *Vu*NSR4 (wound-induced protein), and *Vu*NSR11 (xylanase) present a coordinated induction of mRNA accumulation in response to heat stress. As a positive control to the heat stress treatment mem-

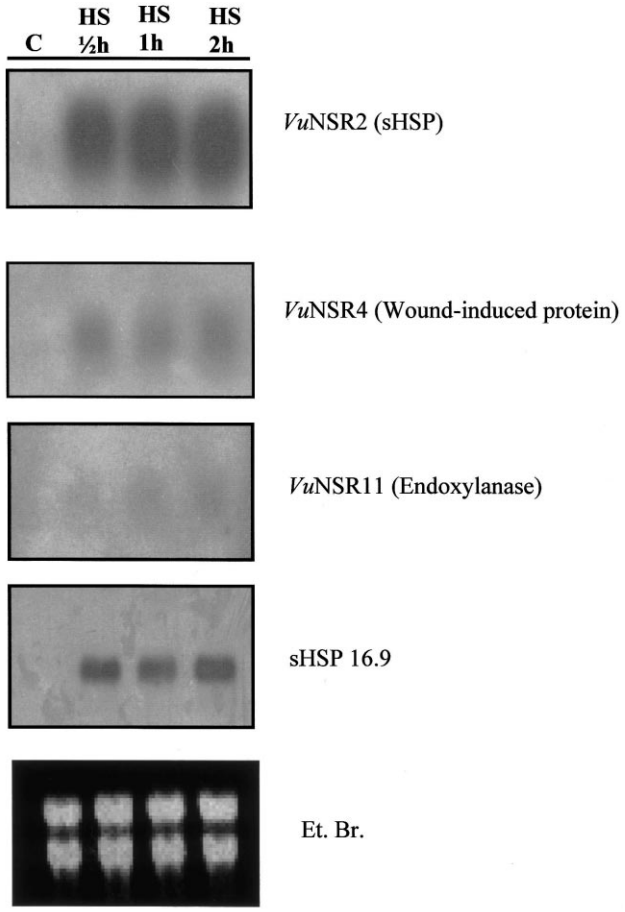

Fig. 3. Induction of transcript accumulation after heat shock treatment analyzed by Northern blot. Control: 28°C; heat shock treatment (HS) at 40°C. Clones used as probes were *Vu*NSR2, *Vu*NSR4, *Vu*NSR11 and wheat sHSP 16.9. Ribosomal RNAs were stained with ethidium bromide (Et. Br.).

Table 1
Analyses of TDF sequences similarity using BLASTX and BLASTN and their expression pattern in cowpea nodules subjected to heat stress

Clone name	TDF	Length (bp)	Homology	Expression profile[c]	E value
Stress-related					
*Vu*NSR1	A8.2	217	Low molecular weight HSP (*Phaseolus vulgaris*)[a]	+	$2e^{-26}$
*Vu*NSR2	B5.4	215	Low molecular weight HSP (*P. vulgaris*)[a]	+	$2e^{-23}$
*Vu*NSR3	C3.4	217	Low molecular weight HSP (*P. vulgaris*)[a]	+	$2e^{-27}$
*Vu*NSR4	C2.1	297	Putative wound-induced protein (*Medicago sativa*)[b]	+	$2e^{-8}$
Signal transduction					
*Vu*NSR5	B7.4	166	Disease resistance protein (*Arabidopsis thaliana*)[b]	nd	$9e^{-25}$
Housekeeping					
*Vu*NSR6	B2.2	476	Glutaminyl-tRNA synthetase (*Lupinus luteus*) [b]	–	$4e^{-66}$
*Vu*NSR7	F4.15	181	Cysteine synthase (*Citrullus vulgaris*)[b]	0	$5e^{-15}$
*Vu*NSR8	A9.4	207	L1 ribosomal protein (*A. thaliana*)[b]	+	$1e^{-29}$
*Vu*NSR9	B3.3	292	40S ribosomal protein S15a (*A. thaliana*)[b]	–	$7e^{-12}$
Cell wall structure or metabolism					
*Vu*NSR10	B1.7	401	Pherophorin-like protein (*A. thaliana*)[b]	–	$3e^{-17}$
*Vu*NSR11	F9.14	150	Xylan endohydrolase (*A. thaliana*)[b]	+	$4e^{-07}$
EST homology					
*Vu*NSR12	C3.3	214	*Glycine max* EST[a]	0	$1e^{-49}$
*Vu*NSR13	F7.1	166	7–10 dpa fiber library cDNA (*Gossypium arboreum*)[a]	+	$2e^{-04}$
*Vu*NSR14	F8.1	153	*G. max* EST similar to B2 protein (*Daucus carota*)[a]	+	$1e^{-11}$
*Vu*NSR15	D3.1	334	cDNA clone young plants (*Lotus japonicus*)[a]	+	$1e^{-131}$
Unknown protein homology					
*Vu*NSR16	D9.1	224	Unknown protein (*A. thaliana*)[b]	+	$1e^{-09}$
*Vu*NSR17	F5.2	214	Unknown protein (*A. thaliana*)[b]	nd	$2e^{-06}$
No significantly homology					
*Vu*NSR18	F6.4	218	No match [a,b]	nd	–
*Vu*NSR19	C3.5	194	No match [a,b]	+	–
*Vu*NSR20	C2.8	300	No match [a,b]	–	–

[a]Similarity analyses of TDF sequences using BLASTX.
[b]Similarity analyses of TDF sequences using BLASTN.
[c]Expression profiles obtained by reverse Northern or Northern blot analyses.

branes were also hybridized with small heat shock protein (sHSP) 16.9 from wheat as a probe [39].

It remains to be confirmed the induction of *Vu*NSR5, *Vu*NSR17 and *Vu*NSR18. In spite of the numerous essays to characterize the expression pattern of these clones by reverse Northern or traditional Northern blot, we were not able to demonstrate the accumulation of transcripts corresponding to these genes. We speculated that their expression level is lower than the limit of detection of these techniques.

3.3. Gene sequence analyses

Although only six primer combinations have been used, the cDNA-AFLP technique allowed the identification of several genes potentially involved in the heat stress response in cowpea/rhizobium symbiosis. Eleven *Vu*NSR clones encode proteins that present similarity to previously described sequences in databases (Table 1). *Vu*NSR1, *Vu*NSR2 and *Vu*NSR3 are up-regulated by heat stress and possess homology to α-crystallin domain characteristic of the sHSP family. *Vu*NSR1 and *Vu*NSR2 differ from *Vu*NSR3 in only one nucleotide residue suggesting that these three TDFs may correspond to the same gene. Presumptively, this protein family acts as chaperones, protecting other proteins against heat-induced denaturation and aggregation [40,41]. The sHSPs can form large multimeric structures and are responsible for a wide range of cellular functions; amongst these, they are able to increase thermotolerance in vivo [41]. The expression of plant class I sHSP in *Escherichia coli* has been shown to be associated with the enhancement of resistance to high temperature stress [42]. Furthermore, modification of sHSP 17.7 expression in carrot resulted in an altered thermotolerance response [43]. However, the thermotolerance mechanism elicited by this protein family has not yet been completely elucidated. Fig. 4 shows the sequence alignment of the *Vu*NSR1 with other plant sHSPs. The sHSP protein family is structurally characterized by the presence of a conserved C-terminal domain of about 100 residues. The *Vu*NSR1 encodes 29 amino acid residues, which are well conserved among all plant sHSPs analyzed (Fig. 4a). This substantial homology of the C-terminus suggests that *Vu*NSR1 encodes a sHSP and represents the first low molecular weight HSPs isolated from cowpea plants. The isolation of its full-length cDNAs will permit the characterization of its complete primary structure and the understanding of how this protein is related to other low molecular weight HSPs.

*Vu*NSR4, which was also induced in response to heat stress, is homologous to the nodule-specific wound-induced gene (clone *Ms*Nod660) isolated from young root nodules of *M. sativa* subsp. x varia [44] (Table 1 and Fig. 4B). This protein group was first characterized as being related to mechanically injured plants but other stresses in plants are also related to it. Wounding, like other abiotic stresses, produces signals that propagate from injured into adjacent non-injured tissues, inducing de novo synthesis of specific wound-induced proteins. Wide ranges of functional classes of proteins are related to wound induction, such as: enzymes of phenolic metabolism [45], proteinase inhibitors [46] and wound-induced protein kinase [47]. Sequence analysis showed that *Vu*NSR4 is also homologous to wound-regulated genes isolated from tomato fruits. The regulation of transcript accumulation in tomato plants depends on the interactions among different factors such as development, ethylene and light. It suggests the combination between developmental and environmental factors affecting gene expression [48]. It is possible that the *Vu*NSR4,

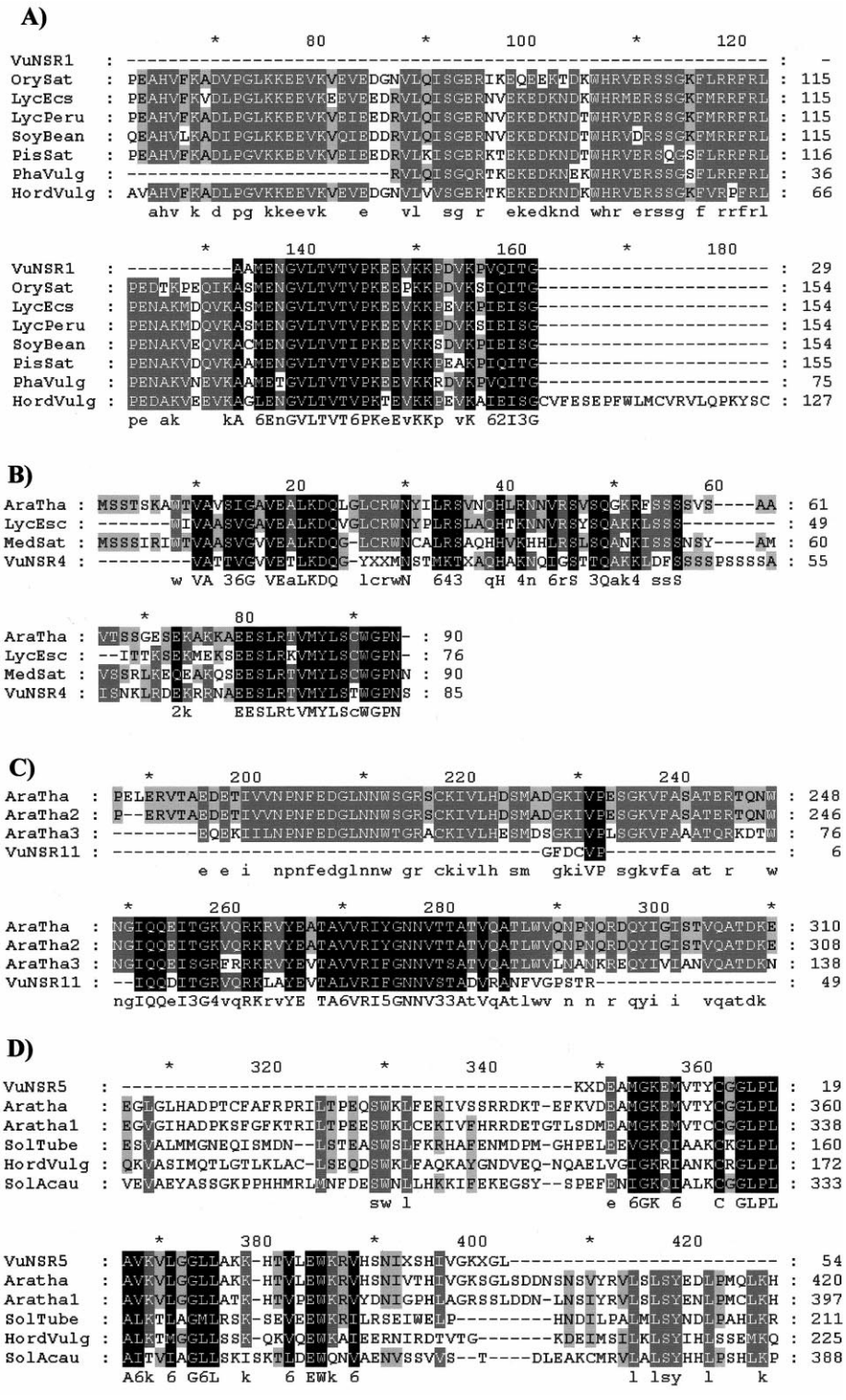

Fig. 4. Alignment of deduced amino acid of each *Vu*NSR clone sequence. After BLASTX analysis, the protein sequences were aligned using ClustalW 1.8 and edited with GeneDoc programs. A: Comparison of *Vu*NSR1 with sHSPs from plants: **PisSat**, probable sHSP from *Pisum sativum* (T06449); **PhaVulg**, class I PvHSP17–19 from *Phaseolus vulgaris* (T12080); **LycEsc**, class I sHSP 17.8 from *Lycopersicon esculentum* (AAD30453); **LycPeru**, sHSP 20.1 from *Lycopersicon peruvianum* (CAA12387); **SoyBean**, class I sHSP 17.6-L from *Glycine max* (P04795); **OrySat**, four class I sHSP 17 from *Oryza sativa* (P31673); **HordVulg**, sHSP18 from *Hordeum vulgare* (T05740). B: Comparison of *Vu*NSR4 with wound-induced proteins (WIP): **AraTha**, WIP from *A. thaliana* (NP_192765); **LycEsc**, WIP from *L. peruvianum* (S19773); **MedSat**, putative WIP from *M. sativa* subsp. x varia (CAB65284). C: Comparison of *Vu*NSR11 with xylan endohydrolase: **AraTha**, putative xylan endohydrolase from *A. thaliana* (NP_176133); **AraTha1**, xylan endohydrolase isoenzyme from *A. thaliana* (AAG50641); **AraTha2**, putative xylanase containing two cellulose binding domains from *A. thaliana* (BAB39757). D: Comparison of *Vu*NSR5 with gene containing NB-ARC domain: **AraTha**, putative viral resistance protein from *A. thaliana* (NP_175742); **AraTha1**, disease resistance protein RPM1 from *A. thaliana* (NP_172561); **SolTube**, disease resistance protein homolog 11 (fragment) from *Solanum tuberosum* (T07755); **HordVulg**, NBS-LRR type resistance protein from *H. vulgare* (T04394); **SolAcau**, NBS-LRR protein from *Solanum acaule* (CAB56299).

encoding a wound-induced protein, could be involved in heat stress adaptation allowing nodule maintenance.

*Vu*NSR10 and *Vu*NSR11 are homologous to proteins related to cell wall metabolism (Table 1, Fig. 4C). *Vu*NSR11 presents homology to xylan endohydrolase (EC 3.2.1.4), which is expressed in barley (*Hordeum vulgare*) during seed germination confined largely to the aleurone layer, but the transcripts were also detected in young vegetative tissue. Its expression is being associated with degradation of cell walls in the starchy endosperm of germinated seeds [49]. Besides that, proteins from the xylanase group have also been reported to be cellulolytic enzymes capable of degrading microbial cellulose-producing fragments that can act as elicitors [50,51]. Lotan [52] showed that endoxylanase induced PR proteins when applied directly to tobacco leaves. Therefore, the induction of a xylan endohydrolase in cowpea nodules in response to heat stress could be considered part of the signal perception of the stress leading to the plant's thermotolerance.

Analysis of the *Vu*NSR5 sequence revealed the presence of a NB-ARC domain, which is found in numerous ATP- and GTP-binding proteins [53]. Plant resistance gene products (R genes) and regulators associated with programmed cell death in animals also share this domain [54]. The R genes are classified regarding the presence of different putative domains. The largest class reported contains a nucleotide-binding site (NBS) and a carboxy-terminal leucine-rich repeat (LRR). A number of genes conferring resistance to different pathogens have been cloned [55] suggesting that this structure is involved in the recognition of a wide range of signals. The typical response mediated by these genes is a rapid oxidative burst. Heat stress as well as plant–pathogen interaction can increase the activated oxygen species (AOS) [56]. Furthermore, legume root nodules are strongly damaged by AOS [57]. Therefore *Vu*NSR5 might be involved in the signaling cascades as a consequence of the AOS generated by high temperature stress.

In this work, cDNA-AFLP allowed the identification of several TDFs from cowpea nodule subjected to high temperature stress. The TDFs characterized so far all have homology to genes related to stress defense, suggesting that they might play a part in the thermotolerance mechanism. The data obtained here will provide the first clues for guiding further functional studies of biological nitrogen fixation. The interaction between *Rhizobium* and the common bean (*P. vulgaris*) is of special interest in tropical countries. A great goal is to understand why this association is so sensitive to high temperatures. It would be essential for the understanding of the molecular basis of thermotolerance during BNF, to compare the expression pattern of several *Vu*NRS in different genotypes with different levels of tolerance to high temperature stress. Thus, the genes here characterized will be used as probes to assess the expression profile following heat shock stress of their homologous genes in distinct bean genotypes that present differences regarding thermotolerance levels. Furthermore, the characterization of new genes involved in this process will permit us to transfer them to bean plants to improve this crop toward resistance to abiotic stress when growing under BNF. Alternatively, this work can also contribute with new markers for the assisted selection of bean varieties adapted to tropical conditions.

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