



Microsatellite identification in Solanaceae crops associated with Nucleoside Diphosphate Kinase (NDK) specific to abiotic stress tolerance through *in silico* analysis

Reena Rosy Thomas, M.K. Chandra Prakash, M. Krishna Reddy¹,
Sukhada Mohandas² and Riaz Mahmood³

Section of Economics & Statistics
Indian Institute of Horticultural Research, Hesaraghatta
Bangalore -560089, India
Email : reenart@hotmail.com

ABSTRACT

Abiotic stress often causes a series of morphological, physiological, biochemical and molecular changes that affect plant growth, development and productivity. To cope with abiotic stresses, it is necessary to understand plant responses to stresses that disturb homeostatic equilibrium at the cellular and molecular level. Genomic information on *Capsicum annuum* has been explored to identify microsatellite markers associated with abiotic stress tolerance and assign them to cognate functional groups related to specific stress responses. Several *in silico* methods have been used to identify simple sequence repeats associated with stress responsive gene candidates in *Capsicum annuum*. In this study, a microsatellite marker has been identified in *Capsicum annuum* associated with Nucleoside Diphosphate Kinase (NDK) having multiple environmental stress tolerance (oxidative, high temperature and salt stress) and which is also highly conserved in crops of Solanaceae. These are house-keeping enzymes that maintain intracellular levels of all nucleoside triphosphates (NTP) with the exception of adenosine triphosphate (ATP). These are also involved in phytochrome A response, UV-B signaling, auxin responses and oxidative stress signaling.

Key words: Nucleoside Diphosphate Kinase (NDK), microsatellite, abiotic stress, Solanaceae

INTRODUCTION

Stress conditions such as drought, high salinity and extreme temperatures, are major factors affecting plant growth and crop productivity (Boyer, 1982) and are often inter-related. Exposure to adverse abiotic environmental conditions causes oxidative stress in plants by rapid and excessive accumulation of reactive oxygen species (ROS) in their cells (Foyer *et al*, 1994). Reactive oxygen species inactivate enzymes and damage important cellular components. ROS are responsible for protein, lipid and nucleic acid modification. As several stress responses are mediated through ROS, plants make use of common pathways that allow them to acclimatize to a range of different stresses and some changes in ROS metabolism cause enhanced tolerance and sensitivity. Therefore, to provide adequate protection against a hazardous environment, a common signaling system has evolved in plants and is known as cross-tolerance (Bowler and Fluhr, 2000).

Plants respond to environmental stresses by activating

related genes, to increase their tolerance to the latter. However, engineering of an individual stress-response gene has not been effective because many kinds of stress responses are necessary for plants to survive under various adverse conditions. An understanding of plant responses to abiotic stresses at the genomic level provides a foundation for identifying genes, microsatellite markers and associated elements. It has been reported in plants that nucleoside diphosphate kinases (NDKs) play a key role in signaling both stress and light. However, little is known about structural elements involved in their function.

NDKs are ubiquitous enzymes that transfer phosphate groups from triphosphate nucleosides to nucleoside diphosphates (NDPs) (Parks and Agarwal, 1973). These enzymes play an important role in phytochrome A response, UV-B signaling, heat stress, and growth (Yano *et al*, 1995). Sequence of the NDK has been highly conserved throughout evolution. A single histidine residue is conserved in all known NDK isozymes and is involved in the catalytic mechanism. NDK2 plays a

regulatory role in H₂O₂-mediated mitogen-activated protein kinase (MAPK) signaling in plants, indicating that plant NDKs also have a diverse array of biological functions (Moon *et al*, 2003). Among various NDKs expressed in *Arabidopsis thaliana*, NDK2 is known to be involved in phytochrome-mediated signal transduction (Yang *et al*, 2003; Im *et al*, 2004).

METERIAL AND METHODS

With whole-genome sequencing initiatives, large amounts of genomic sequence data are available in the public domain that serve as an attractive source of *in silico* mining of microsatellite sequences. However, finding potentially useful microsatellites that occupy specific genomic regions in plants, still remains a challenge. Availability of this information can facilitate discovery of microsatellites associated with abiotic stress tolerance, using *in silico* methods.

Capsicum annuum EST database, consisting approximately 23000 sequences, has been explored for microsatellites having low-complexity repeats, for identification of markers associated with stress tolerance. Of the 23000 EST sequences, 2507 non-redundant EST sequences having repeats comprising of di, tri, tetra and penta SSRs were located using Repeat Finder programs. These 2507 EST sequences were converted to Textual Data in FASTA format. A computer program developed in Microsoft’s Visual studio 2010 in Windows platform has been coded specifically to read large-size of text files. Potential microsatellite markers comprising single, di and tri-nucleotide repeats were located in the text file, based on input data. These sequences were further subjected to *in silico* analysis for classifying the markers and to assign them to cognate functional groups related to specific stress responses.

RESULTS AND DISCUSSION

One of the 2507 non-redundant EST sequences with a length of 755bp from *C. annuum*, found to have a marker associated with Nucleoside Diphosphate Kinase (NDK), was subjected to BLAST analysis. The 755bp sequence of *Capsicum annuum* nucleoside diphosphate kinase mRNA sequence with a single nucleotide repeat sequence of 22bp of A repeats is given below:

GGCACGAGATTTGCTAACTCATTTCAGTAACATCAA
 AGAAGCAAGAAATGGAGCAAACCTTCATCATGATT
 AAGCCTGATGGTGTCCAACGTGGCCTCGTTGGTGA
 GATTATCGGCAGATTTGAAAAGAAAGGTTTCTCTT
 TGAAAGGTTTGAAGCTCATCACTGTGGATCGCGCT

TTTGCTGAGAAGCATTATGCAGATTTGCTGCTAAG
 CCTTTCTTTAATGGGCTTGTGAGTACATTGTTTCT
 GGACCCGTTGTCTCTATGGTCTGGGAAGGTAAGGG
 TGACTTACCACTGGCAGGAAGATCATTGGAGCAA
 CCAACCCCTTGAATCTGCTCCTGGTACCATCCGTG
 GTGATTATGCTATTGACATTGGCAGGAATGTCATTC
 ATGGAAGTGATGCTGTTGAGAGTGCAAGGAAGGAGA
 TTGCTCTTTGGTTCCCGAAGGAGTTGCAGAGTGG
 CAGAGCAGCCTTCACTGTTGGATCTACGAGTAGAAAA
 GTTCTATGAAAGATTCATGGCCAGCCTCTTTGGTTG
 TAACTTATGAGTTTTGTTTGTTCATTTAAGTCCAGAA
 GTA ACTTAAGAGTTTTGTTTCGTCATTTAAGTCCAG
 AAGTTAGATGTTTTTAAGATCTACTAGCGGTTCCCT
 ATTTGAAGAATATTTAAGTTGTGGTGTTTTATCTGTTG
 TGTTCCATGTGTTGCAATTTCTAGTAATTGAGCTTCCA
 CAATTTTTTAGCCGTCAAAAAAAAAAAAAAAAAAAAA

BLAST analysis shown in Fig. 1 revealed that the Nucleoside Diphosphate Kinase (NDK) sequence is evolutionarily conserved, conferring multiple environmental stress tolerance (oxidative, high temperature and salt stress). Further, the associated 22 base pair of single nucleotide ‘A’ repeat is also evolutionarily conserved in most of the Solanaceae crops (shown in green colour). This could be a potential microsatellite marker associated with NDK sequence.

Data in Table 1 indicate that *C. annuum* sequence with a maximum identity of >90% and E value of 0.0

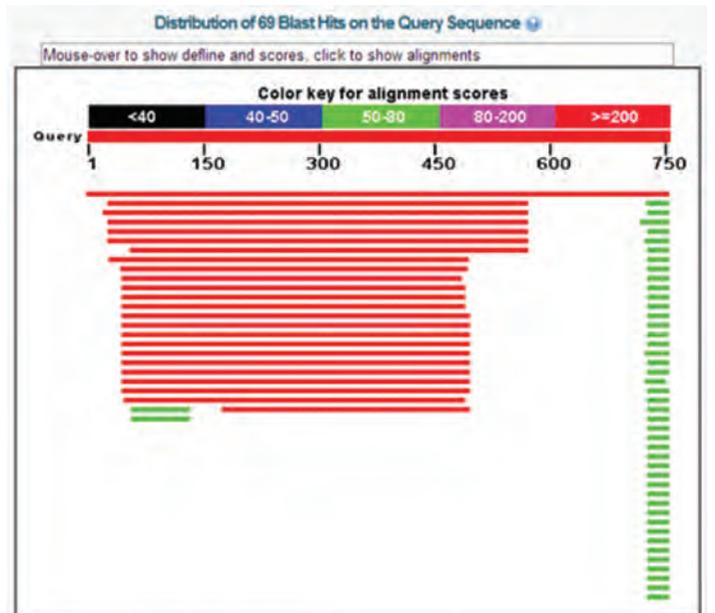


Fig 1. BLAST analysis result shows that NDK sequence is highly conserved in crops of Solanaceae; Red color shows high sequence similarity and green colour shows associated marker of single nucleotide A repeats of 22 base pair length

belongs to Solanaceae crops. It is also highly conserved in *Solanum chacoense*, *Nicotiana tabacum*, *Solanum lycopersicum* and *Lycopersicon esculentum*.

Protein sequence similarity

Protein sequence of NDK also blasted against protein database from NCBI, showed highest similarity with *Arabidopsis thaliana* (Fig. 2). Putative conserved domains are shown as small, red triangles against *C. annuum* sequences. It is seen that the sequence belongs to NDPk superfamily which has been highly conserved through evolution. Molecular graphic structure of NDK in

Arabidopsis thaliana is shown in Fig. 3.

NDK protein sequence of *C. annuum*, given below, has 100% similarity to *Arabidopsis thaliana*.

>gi|7643788|gb|AAF65509.1| nucleoside diphosphate kinase [*Capsicum annuum*]

MEQTFIMIKPDGVQRGLVGEIIGRFEKKGFSLKGLKL
ITVDRAFAEKHYADLSAKPFFNGLVEYIVSGPVVS
MVWEGKGVLTTRRKIIIGATNPLESAPGTIRGDYAI
D IGRNVIHGSDAVESARKEIALWFPEGVAEWQSSLHC
WIYE

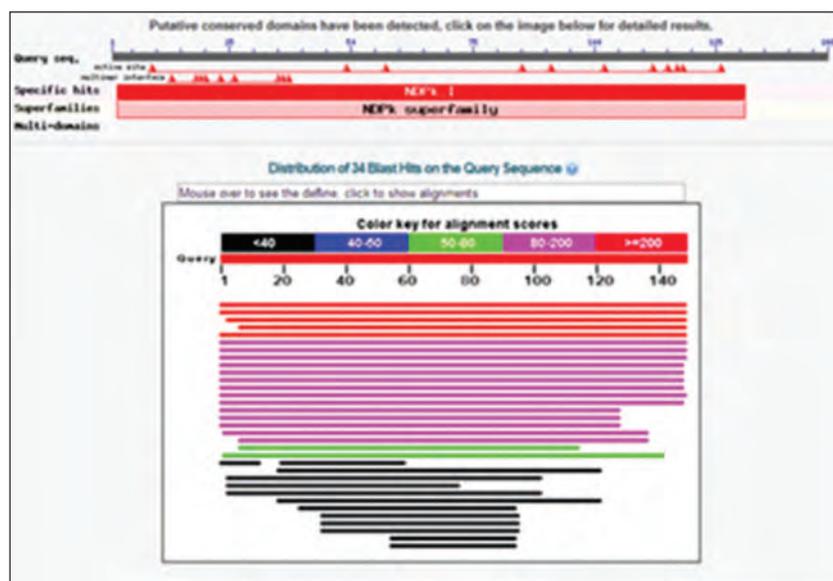


Fig 2. Putative conserved domain and BLAST hits on protein sequence of *C. annuum* that shows high similarity with *Arabidopsis thaliana*



Fig 3. molecular graphic structure of NDK in *Arabidopsis thaliana*

Table 1. Significant hit of nucleotide BLAST results against *C. annuum* 755bp NDK sequence

Description	Max score	Total score	Query coverage	E value	Max identity	Accession number
<i>Capsicum annuum</i> nucleoside diphosphate kinase mRNA, complete cds	1395	1395	100%	0.0	100%	AF108881.1
<i>Solanum chacoense</i> cytosolic nucleoside diphosphate kinase mRNA, complete cds	743	743	72%	0.0	91%	DQ157699.1
<i>Nicotiana tabacum</i> nucleoside diphosphate kinase mRNA, complete cds	719	719	72%	0.0	90%	AY962601.1
<i>Solanum lycopersicum</i> cDNA, clone: LEFL1007CH03, HTC in leaf	676	676	72%	0.0	89%	AK320311.1
<i>Solanum lycopersicum</i> cDNA, clone: FC06DD10, HTC in fruit	676	676	72%	0.0	89%	AK246327.1
<i>Lycopersicon esculentum</i> clone 114282R, mRNA sequence	676	676	72%	0.0	89%	BT013034.1
<i>Solanum lycopersicum</i> nucleoside diphosphate kinase (LOC544095), mRNA >emb X75324.1 <i>L. esculentum</i> (Ailsa Craig) mRNA for nucleoside diphosphate kinase	636	636	68%	9e-179	89%	NM_001247245.

Table 2. Protein BLAST result of *C. annuum* NDK protein sequence against *Arabidopsis thaliana* protein sequences

Description	Max score	Total score	Query cover	E value	Max indent	Accession
Nucleoside diphosphate kinase 1 [<i>Arabidopsis thaliana</i>] >gb AEE82742.1 nucleoside diphosphate kinase 1 [<i>Arabidopsis thaliana</i>]	255	255	100%	7e-87	80%	NP_567346.1
RecName: Full=Nucleoside diphosphate kinase 1; AltName: Full=Nucleoside diphosphate kinase I; Short=NDK I;	254	254	100%	9e-87	80%	P39207.1
Nucleoside diphosphate kinase [<i>Arabidopsis thaliana</i>]	250	250	98%	3e-85	80%	CAA49170.1
Unknown protein [<i>Arabidopsis thaliana</i>] >gb AAL66933.1 unknown protein [<i>Arabidopsis thaliana</i>]	242	242	95%	7e-82	80%	AAK48956.1
Nucleoside diphosphate kinase [<i>Arabidopsis thaliana</i>]	223	223	100%	3e-74	74%	CAA49173.1
Chain A, Crystal structure of Nucleoside Diphosphate Kinase 2 from <i>Arabidopsis</i> > pdb 1S57 B Chain B, Crystal structure of Nucleoside Diphosphate Kinase 2 from <i>Arabidopsis</i>	196	196	100%	1e-63	60%	1S57_A
Nucleoside diphosphate kinase Ia [<i>Arabidopsis thaliana</i>]	196	196	100%	1e-63	60%	AAC14280.1

From Table 2, it is evident that *C. annuum* NDK protein sequence has >98% query coverage and is 80% identical to *Arabidopsis thaliana* sequences. Plant NDK plays a prominent role in plant defense mechanisms and involvement of NDK is associated with various stress mechanisms. It was proved that over expression of NDK resulted in tolerance against several environmental stresses such oxidative stress, high temperature and salt stress. *C. annuum* 755bp sequence having the maximum identity with nucleotide and protein sequences in Solanaceae crops and *Arabidopsis* confirms similar structural and molecular functions. As the sequence of microsatellite marker is evolutionarily conserved across Solanaceae crops, this could be useful in selecting parental lines and developing abiotic-stress tolerant crops.

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