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EXPLORING NPR1 GENE IN CROP PLANTS

Opinion Article

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Living organisms always need to confront biotic and abiotic stresses and this remains utmost important and evident in case of plants. Among the biotic stresses, plant diseases have played significant role in crop production. Many approaches have been accomplished for studying the nature of disease thus facilitating resistance development strategies. Large numbers of experiments have been conducted based on Pathogenesis-related (PR) proteins that bring about the plant defence response. Among the many PR genes, NPR1 (Non-Expressor of Pathogenesis) has been one of the important master regulator switch involved in stress responsive PR gene expression.

NPR is a gene family consisting of homologous copies within the genome of plants like Arabidopsis, Rice, Sugar beet, Soybean and Grapes etc. They play significant role in the salicylic acid (SA) induced Systemic acquired resistance (SAR) during pathogen infection in plants. The first NPR1 gene was isolated by mutant screens in Arabidopsis (Cao *et al*., 1994). The NPR1 gene encodes BTB/POZ (broad-complex, tramtrack, and bric-a-brac/poxvirus, Zinc finger domain) and ankyrin repeat domain, both of which are involved in protein-protein interactions. The absence of DNA binding domains in this gene shows that it is a regulatory molecule that interacts with other proteins to bring about PR gene expression.

Having known about this switch nodule that regulates whole set of PR genes expression downstream the signal transduction pathway, here are a few approaches for isolation of NPR1 gene in crop plants.

With NPR1 playing important role in disease resistance, map based molecular markers such as SNPs, SSRs, CAPS could be developed and screened for genic variation in the mapping populations and germplasm. Since the coding sequence of NPR1 is known in many crops, this could be taken as tool to design degenerate primers from the conserved region to identify the NPR1 gene in uncharacterized genome, further, PCR amplification, sequencing of amplified product, sequence analysis, genome walking and 5′ and 3′ RACE could lead to isolation of full length gene of NPR1.Another way is to screen the genomic / cDNA library using the homologous NPR1 gene as a probe. Once the gene is isolated next step should be to identify the copies of NPR1 gene in the genome, as it occurs in family and contain several members. Southern hybridization and Quantitative real time PCR are the commonly used methods to identify the gene copy numbers.

After isolation of gene and knowing its copy number, the next challenge is to know about its structure and function. The structure of gene can be dissected using genomics tools. The complete gene structure could be characterised for introns, exons, splice sites and other signals using GENEID or GENEPARSER, FGENESH softwares (Salamov *et al*., 2000). The promoter region with regulatory sequence motifs are predicted using PLACE and PLANTCARE softwares. The regulatory elements thus identified can be validated using GUS or GFP reporter system via transient or transgenic approach. Further, deletion analysis is required to identify the essential elements for promoter activity. Sequence variations such as functional SNPs within the gene can be mined by multiple sequence alignment using ClustalX/W (Thomson *et al*., 1994), Bioedit or MEGA software (Tamura *et al*., 2011). Mining involves the sequence

comparison of genes with known heterologous genes present in databases like NCBI, TAIR (Arabidopsis),LIS(Legume information system), IRIS(Rice), IPhIS(Beans), ICASS(Cassava), ICHIS (Chickpea), IVIS (Cowpea), IGnIS (Groundnut), IMIS (Maize), ISgIS (Sorghum), IMIS (Wheat). Further phylogenetic relationships can be analyzed using PHYLIP, MEGA , MOLPHY or PAUP softwares.

In Silico functional characterization of the gene can be done using bioinformatics tools like transeq that can translate the gene sequences and identify the functional domains. Further the variations in protein sequence leading to differential protein functions can be analysed using SMART or PROSITE softwares. The results of *in silico* analysis can be validated or confirmed for their function by developing transgenics using suitable expression cassettes.

Functional validation of NPR1 gene

Gene complementation in mutated/ disease susceptible lines can be done to confirm the gene function. The role of NPR1 in stress mediated signal transduction is studied using T-DNA insertion mutants *or* RNAi knockdown line and NPR1 gene overexpression line. The NPR1 genes fused with GFP or GUS constructs are used for protein localisation and expression studies *in vivo*. Protein- protein interactions of NPR1 with other transcription factors can be studied using yeast two hybrid system. The Y2H system helps in identifying the protein partners of *NPR1* that can be targeted for further studies. Further, proteomics study with respect to *NPR1* gene would help to establish its networks and modules in the cellular system and metabolomics and ionomics study would reveals its role in bringing about changes in the cellular conditions.

Some of the NPR genes that have been isolated and studied from different plants are AtNPR1(At1g64280), AtNPR2 (At4g26120), AtNPR3 (AT5G45110), and AtNPR4 (AT4G19660) from *Arabidopsis thaliana*.

The role of NPR1 in stress mediated signal transduction is studied using Arabidopsis mutants called *npr1*. The NPR1 genes fused with GFP or GUS constructs are used for protein localisation and expression studies In Vivo. BGL2-GUS transgenic plants of Arabidopsis were mutagenized with EMS and the M2 plants were screened for SA or INA non-responsive mutants. NPR1 was one of the mutants that exhibited significant reduction in the expression of the GUS, BGL2 and PR-1 genes compared to the wild type (Cao *et al*., 2004). OsNPR1 (AAX18700.1) from rice – five rice NPR1 like genes were retrieved by BLASTP search using the conserved ankyrin repeat domain of the Arabidopsis NPR1 gene (Yuan *et al*., 2007); GmNPR1-1 (ACJ45013.1) and GmNPR1-2 (ACJ45015.1) from soybean – A soybean EST (Gm-c1004-4231) showing high identity to Arabidopsis NPR1 was used to develop primer pair to amplify GmNPR1 from Williams 82 genomic DNA. Further DNA gel blot analysis using GmNPR1 probe revealed that there are two copies of NPR1 like sequences in the soybean genome (Sandhu *et al*., 2009); MpNPR1(ACC77697.1) from apple- Malnoy *et al*., 2007 have cloned the MpNPR1 using AtNPR1 probes for screening an apple cDNA library; MdNPR1 (ACJ04030.1) from banana was isolated using the technique of RACE-PCR from cv. Dongguan Dajiao (Musaspp. ABB) by Zhao *et al*., 2009; RcNPR1 (EEF48081.1) from castor bean, BjNPR1 (DQ359129.3) from *Brassica juncea*, BnNPR1 (EF613226.1) from *Brassica napus* and PtNPR1 (XP_002322351.1) from poplar; PpNPR1 (ABK62792.1) from pear. LeNPR1(AAT57637.1) from tomato; ZmNPR1 (NP_001147587.1) from maize; NtNPR1 (AAM62410.1) from tobacco have also been cloned using gene specific primers and probes.

Thus, we have seen how to work with any candidate gene like that of NPR1 and derive at scientific conclusions regarding the structure and function of such genes. The techniques described above can be implemented in achieving the results with respect to candidate gene analysis.

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