Western European Journal of Medicine and Medical Science
Volume 2, Issue 3, March, 2024
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

OBTAINING A RECOMBINANT FORM OF PLANT DEFENSIN FROM NIGELLA SATIVA

Tsoy V.E., Dalimova D.A., Ibragimova Sh.N., Muminov M.I., Yakubov M.D., Turdikulova Sh.U..
Center for Advanced Technologies, Tashkent, Uzbekistan
e-mail: vladimirs9003@gmail.com

Abstract: Today, one of the challenges facing modern agriculture is crop loss due to various biotic and abiotic factors. We investigated defensins, which are an important component of the plant's defense system and form protective antimicrobial barriers between different types of tissues and organs of plants, as well as around seeds. The aim of our research was to obtain a recombinant form of plant defensin with antimicrobial activity. A recombinant DNA vector construct pRSET_A was obtained with the cloned Ns-D2 defensin gene under a regulatable promoter, a histidine tag, and an enterokinase site. The resulting construct can be used for defensin synthesis, which can be employed to combat pests of agricultural crops.
Keywords: Defensins, Peptides, Fungicidal And Antimicrobial Activity.

One of the current problems of modern agriculture remains crop loss due to various biotic and abiotic factors. Plants have a multi-component defense system, including the formation of protective barriers, activation of hypersensitivity reactions, and synthesis of antimicrobial peptides, which are low-molecular-weight compounds exhibiting a broad spectrum of activity against fungi, bacteria, and viruses. In addition to fungicidal activity, some plant defensins possess antibacterial properties. The main function of defensins is to inhibit fungal infection [6, 8, 9], but antibacterial activity has been described for some peptides [7, 21]. Various damages caused by insects, fungi, viruses, or physical factors result in yield losses. To counteract the negative impact of the environment, plants employ a multi-component system, including hypersensitivity reactions, reinforcement of protective barriers using components of the cell wall, activation of protective protein production, and antimicrobial peptides [5].

Thus, plant defensins (a group of peptides exhibiting antifungal and bactericidal activity and participating in various responses to abiotic stresses) are extremely important in addressing the problem of obtaining resistant varieties of agricultural crops. Representatives of this peptide group are characterized by extremely low similarity in primary structure (except for conservatively located cysteine residues), yet modern computational biology methods combined with sequencing technologies expedite the identification and analysis of defensin-encoding sequences, including those in non-model plant biology objects [23].

For a small number of defensins, involvement in plant adaptation processes to abiotic stresses has been described. For example, elevated concentrations of NaCl influenced changes in the expression of defensin-encoding genes in Arachis hypogaea [14, 17] and Arabidopsis thaliana [14]. The impact of water deficit on changes in the expression of defensin-encoding genes has been demonstrated in Glycine max [16, 18]. Cold also affects the expression of defensin genes [4, 10]. In winter wheat, the Tad1 gene has been identified, the expression of which is specifically induced during acclimatization to cold stress [10]. In the zinc and

Western European Journal of Medicine and Medical Science
Volume 2, Issue 3, March, 2024
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

cadmium hyperaccumulator Arabidopsis halleri, the gene encoding the defensin AhPDF1.1 has been identified and extensively studied [11, 12, 13, 15, 19]. Expression of this gene in A. thaliana and S. cerevisiae enhances zinc resistance but not cadmium, cobalt, iron, or sodium. Zinc-dependent regulation of the defensin pool is observed in A. halleri both at the transcript and peptide levels. Compared to A. thaliana, A. halleri shows an increase in the quantity of defensins in shoots [11].

For some defensins, participation in response to multiple stressors has been demonstrated. In Brassica rapa, the expression of genes encoding the defensins BrDLFP and BrBetvAFP significantly changed under cold stress, drought, and salinity [1]. A study on the influence of pre-treatment of Arabidopsis plants with non-toxic concentrations of silicon and cadmium on Botrytis cinerea infection showed enhanced expression of the gene encoding defensin PDF1.2 [3]. The expression of the defensin gene NbDef2.2 in N. benthamiana increases not only after infection with Pseudomonas syringae pv. tabaci but also after mechanical injury and ethylene treatment [2]. Thus, plant defensins may be components of overlapping systems responding to both abiotic and biotic stresses.

Plant defensins are important components of the plant's defense system, where they form protective antimicrobial barriers between different types of plant tissues and organs, as well as around seeds [20]. These peptides also exhibit other types of activities that are important for application in agriculture as well as in the medical field. It is known that plant defensins possess antifungal activity, antibacterial activity, protease inhibitor activity, and insect amylase inhibitor activity. Among the numerous peptides isolated from plants of various species, a significant number of promising defensins have been identified from the seeds of Nigella sativa L. (Ranunculaceae), an endemic plant of Uzbekistan and China.

Therefore, the aim of our research was to obtain a recombinant form of plant defensin with antimicrobial activity. To obtain defensins, we used the seeds of the plant Nigella sativa L. (Ranunculaceae). Two new defensins named Ns-D1 and D2-Ns were isolated from Nigella sativa seeds by us [22], and their primary sequences were determined. For the synthesis of synthetic defensin, cloning was carried out in the plasmid vector pMK-T. Then, to obtain the producer strain of the Defensin peptide, the vector construct was transformed into E. coli cells for cloning. Optimization of conditions for the expression of recombinant defensin was carried out. Subsequently, recombinant defensin was purified by metal-affinity chromatography, and a method for assessing the antimicrobial and antifungal activity of plant peptides was studied.

As a result of our studies, we found that the peptides obtained by us differ from each other by one amino acid residue and showed high similarity to the sequence of Raphanus Sativus L. defensins RS-AFP1 and RS-AFP2. NS-D1 and D2-Ns defensins exhibited strong and diverse antifungal activity against a range of phytopathogenic fungi. A recombinant DNA vector construct pRSET_A was obtained with the cloned Ns-D2 defensin gene under a regulatable promoter, a histidine tag, and an enterokinase site. The high fungicidal activity of defensins from Nigella sativa makes them promising candidates for the production of genetically engineered plants resistant to pathogens. A recombinant peptide preparation was obtained, which can serve as an alternative to traditional antibiotics.

Due to the increasing microbial resistance to traditional antibiotics, antimicrobial peptide-based drugs are currently in demand in the global pharmaceutical market. Based on the obtained recombinant defensin peptide, medicinal products with antimicrobial and fungicidal action can be developed.

Western European Journal of Medicine and Medical Science
Volume 2, Issue 3, March, 2024
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

References

1. Ahmed N.U., Park J.-I., Jung H.-J., Seo M.-S., Kumar T.S., Lee I.-H., Nou I.-S. Identification and characterization of stress resistance related genes of *Brassica rapa*. *Biotechnol. Lett*., 2012, 34(5): 979-987 (doi: 10.1007/s10529-012-0860-4).
2. Bahramnejad B., Erickson L.R., Chuthamat A., Goodwin P.H. Differential expression of eight defensin genes of *N. benthamiana* following biotic stress, wounding, ethylene, and benzothiadiazole treatments. *Plant Cell Rep*., 2009, 28(4): 703-717 (doi: 10.1007/s00299-009-0672-8).
3. Cabot C., Gallego B., Martos S., Barcely J., Poschenrieder C. Signal cross talk in *Arabidopsis* exposed to cadmium, silicon, and *Botrytis cinerea*. *Planta*, 2013, 237(1): 337-349 (doi: 10.1007/s00425-012-1779-7).
4. Gaudet D.A., Laroche A., Frick M., Huel R., Puchalski B. Cold induced expression of plant defensin and lipid transfer protein transcripts in winter wheat. *Physiologia Plantarum*, 2003, 117(2): 195-205 (doi: 10.1034/j.1399-3054.2003.00041.x).
5. Goyal R.K., Mattoo A.K. Multitasking antimicrobial peptides in plant development and host defense against biotic/abiotic stress. Plant Sci., 2014, 228: 135-149 (doi: 10.1016/j.plantsci.2014.05.012).
6. Fant F., Vranken W., Broekaert W., Borremans F. Determination of the threedimensional solution structure of Raphanus sativus antifungal protein 1 by 1 H NMR. J. Mol. Biol., 1998, 279(1): 257-270 (doi: 10.2210/pdb1ayj/pdb).
7. Franco O.L., Murad A.M., Leite J.R., Mendes P.A., Prates M.V., Bloch C. Identification of a cowpea γ-thionin with bactericidal activity. FEBS J., 2006, 273(15): 3489- 3497 (doi: 10.1111/j.1742-4658.2006.05349.x).
8. Lacerda A., Vasconcelos A.R., Pelegrini P.B., Grossi - de - Sa M.F. Antifungal defensins and their role in plant defense. Front. Microbiol., 2014, 5: 116 (doi: 10.3389/fmicb.2014.00116).
9. Lay F.T., Brugliera F., Anderson M.A. Isolation and properties of floral defensins from ornamental tobacco and petunia. Plant Physiol., 2003, 131(3): 1283-1293 (doi: 10.1104/pp.102.016626).
10. Koike M., Okamoto T., Tsuda S., Imai R. A novel plant defensin-like gene of winter wheat is specifically induced during cold acclimation. *BBRC*, 2002, 298(1): 46-53 (doi: 10.1016/S0006-291X (02)02391-4).
11. 11 Mirouze M., Sels J., Richard O., Czernic P., Loubet S., Jacquier A., François I.E.J.A., Cammue B.P.A., Lebrun M., Berthomieu P., Marquиs L. A putative novel role for plant defensins: a defensin from the zinc hyper-accumulating plant, *Arabidopsis halleri*, confers zinc tolerance. *Plant J*., 2006, 47(3): 329-342 (doi: 10.1111/j.1365 - 313X.2006.02788.x).
12. Mith O., Benhamdi A., Castillo T., Berge M., Mac Diarmid C.W., Steffen J., E ide D.J., Perrier V., Subileau M., Gosti F., Berthomieu P., Marques L. The antifungal plant defensin AhPDF1.1b is a beneficial factor involved in adaptive response to zinc overload when it is expressed in yeast cells. *MicrobiologyOpen*, 2015, 4(3): 409- 422 (doi: 10.1002/mbo3.248).
13. Nguyen N.N., Ranwez V., Vile D., Soulié M.-C., Dellagi A., Expert D., Gosti F. Evolutionary tinkering of the expression of PDF1s suggests their joint effect on zinc tolerance and the response to pathogen attack. *Front. Plant Sci*., 2014, 5: 70 (doi: 10.3389/fpls.2014.00070).

Western European Journal of Medicine and Medical Science
Volume 2, Issue 3, March, 2024
https://westerneuropeanstudies.com/index.php/3
ISSN (E): 2942-1918 Open Access| Peer Reviewed

14. Nishiyama R., Le D.T., Watanabe Y., Matsui A., Tanaka M., Seki M., Yamaguchi - Shinozaki K., Shinozaki K., Tran L.S.P. Transcriptome analyses of a salt-tolerant cytokinin-deficient mutant reveal differential regulation of salt stress response by cytokinin deficiency. *PLoS ONE*, 2012, 7(2): e32124 (doi: 10.1371/journal.pone.0032124).
15. Oomen R.J., Séveno -Carpentie r E., Ricodeau N., Bournaud C., Conéjéro G., P aris N., Berthomieu P., Marquès L. Plant defensin AhPDF1.1 is not secreted in leaves but it accumulates in intracellular compartments. *New Phytol*., 2011, 192(1): 140-150 (doi: 10.1111/j.1469-8137.2011.03792.x).
16. Stolf - Moreira R., Medri M.E., Neumaier N., Lemos N.G., Brogin R.L., Marcelin o F.C., De Oliveira M.C.N., Farias J.R.B., Abdelnoor R.V., Nepomucen o A.L. Cloning and quantitative expression analysis of drought-induced genes in soybean. *Genet. Mol. Res*., 2010, 9(2): 858-867 (doi: 10.4238/vol9-2gmr701).
17. Sui J., Jiang D., Zhang D., Song X., Wang J., Zhao M., Qiao L. The salinity responsive mechanism of a hydroxyproline-tolerant mutant of peanut based on digital gene expression profiling analysis. *PloS ONE*, 2016, 11(9): e0162556 (doi: 10.1371/journal.pone.0162556).
18. Stolf - Moreira R., Medri M.E., Neumaier N., Lemos N.G., Pimenta J.A., Tobita S., Brogin R.L., Marcelino - Guimarres F.C., Oliveira M.C.N., Farias J.R.B., Abdelnoor R.V., Nepomuceno A.L. Soybean physiology and gene expression during drought. *Genet. Mol. Res*., 2010, 9(4): 1946-1956 (doi: 10.4238/vol9-4gmr851).
19. Shahzad Z., Ranwez V., Fizames C., Marquès L., Martret B., Alassimone J., G odé C., Lacombe E., Castillo T., Saumitou - Laprade P., Berthomieu P., Gosti F. Plant Defensin type 1 (PDF1): protein promiscuity and expression variation within the *Arabidopsis* genus shed light on zinc tolerance acquisition in *Arabidopsis halleri*. *New Phytol*., 2013, 200(3): 820-833 (doi: 10.1111/nph.12396).
20. Thomma B.P., Cammue B.P., Thevissen K. Plant defensins. Planta, 2002, 216(2): 193-202 (doi: 10.1007/s00425-002-0902-6).
21. Zhang Y., Lewis K. Fabatins: new antimicrobial plant peptides. FEMS Microbiol. Lett., 1997, 149(1): 59-64 (doi: 10.1111/j.1574-6968.1997.tb10308.x).
22. Yu.I. Oshepkova, O.N. Veshkurova, Sh.I. Salikhov. Antimicrobial activity of cationic and cyclic peptides from Nigella Sativa seeds // DAN RUz, 2015, №1, P.64-67
23. Zhou P., Silverstein K.A., Gao L., Walton J.D., Nallu S., Guhlin J., Young N.D. Detecting small plant peptides using SPADA (small peptide alignment discovery application). BMC Bioinformatics, 2013, 14: 335 (doi: 10.1186/1471-2105-14-335).