

PAPER • OPEN ACCESS

## Structure Analysis of a Pathogenesis-Related 10 Protein from *Gardenia jasminoides*

To cite this article: Lan Gao 2019 *IOP Conf. Ser.: Earth Environ. Sci.* **242** 042005

View the [article online](#) for updates and enhancements.



**IOP | ebooks™**

Bringing you innovative digital publishing with leading voices to create your essential collection of books in STEM research.

Start exploring the collection - download the first chapter of every title for free.

# Structure Analysis of a Pathogenesis-Related 10 Protein from *Gardenia jasminoides*

**Lan Gao**

School of Life Sciences and Biopharmaceutics, Guangdong Pharmaceutical University, Guangzhou, Guangdong, 510006, China

glgdp@aliyun.com

**Abstract.** The PR-10 proteins belongs to the huge family of pathogenesis related proteins ubiquitous in the plant kingdom, and it includes major food and tree pollen allergens, major latex proteins (MLPs) and cytokinin-specific binding proteins(CSBPs). In this work, a fruit cDNA library of *Gardenia jasminoides* was constructed, and the GjPR10 cDNA was isolated from the library by sequencing method. The GjPR10 cDNA contains a predicted 486 bp open reading frame, the putative protein contains 161 amino acids, and the protein has a calculated molecular mass 17.7kDa and a theoretical pI 6.25, these characters consistent with usual plant PR10 protein features. A bioinformatics analysis was conducted with previously characterized PR-10 proteins from other plant species. A three-dimensional model of GjPR10 was built. The bioinformatics analysis suggests that the GjPR10 present the conserved P-loop motif. The three-dimensional structure of GjPR10 has the significant common feature of PR10 proteins, it is composed of three alpha-helices and a seven-stranded antiparallel beta-sheet arranged to form a large internal hydrophobic cavity. The results suggest that GjPR10 is a typical PR10 protein, GjPR10 might be involved in plant defence response, developmental processes and in secondary metabolism.

## 1. Introduction

PR (pathogenesis-related) proteins were first identified as defence molecules produced in response to pathogen attack. Many studies have reported their induction under a great variety of abiotic stress conditions as well as possible constitutive or developmentally regulated expression [1, 2]. PR proteins are classified into 17 different families based on sequence homology, and biological activities [3]. The PR-10 subfamily is the largest family with members reported in numerous plant species and it includes major food and tree pollen allergens [4].

PR-10 proteins are share common features such as low-molecular weight (15-20kDa, 154-163 amino acids) with slightly acidic pI, resistant to proteases, similar three-dimensional structure as well as conserved P-loop region, and usually cytosolic location [5]. PR10 have conserved sequence features: three amino acids E97,E149 and Y151 (as positioned in Bet v 1a) possibly involved in ribonuclease activity [6]; E46 is important for IgE binding to the birch pollen [7, 8]; and two other remarkable domains comprising the motif G(D/N)GG(V/P)G(T/S) (aa 47-53 in Bet v 1) that forms a Gly-rich loop (or P-loop) supposed to have NTPase activity, it's the most conserved motif of PR-10 proteins; and the Bet v 1 motif characteristic of proteins from the Bet v 1 superfamily [9]. A significant common feature of PR10 proteins is a large Y-shaped hydrophobic cavity, and PR10 proteins show more divergent amino acids sequences in the cavity region [10]. The hollow cavity in the molecular core, surrounded by a seven-stranded antiparallel  $\beta$ -sheet crossed by a long C-terminal  $\alpha$ -helix (H<sub>3</sub>), resting on a V-



shaped support formed by two additional helices (H<sub>1</sub>, H<sub>2</sub>) [11]. It's strongly suggestive of small-molecule binding in the cavity; the ligands should be rather hydrophobic because the cavity interior has a mostly hydrophobic character. This internal cavity could be responsible for the intracellular transport of apolar ligands, diverse as fatty acids, flavonoids, cytokinins or brassinosteroids [12]. The phytohormones belong to several divergent groups but usually have a hydrophobic part (such as cytokinins), they are signalling molecules in plant development and response to stress [4, 13].

The first PR-10 protein was established from parsley [13]. PR-10 proteins are coded by multigene families, includes the Classic PR-10 proteins yellow lupine; common allergens found birch pollen [14] and some fruits (with ~50% sequence identity); major latex proteins (MLPs, with ~25% sequence identity) were found in *Arabidopsis thaliana*, ginseng and some fruits (bell pepper, melon, soybean, strawberry, peach) [15], MLPs show less conservation in the glycine-rich loop; and Cytokinin-specific binding proteins (CSBPs, with ~25% sequence identity) were structurally confirmed as a PR-10 subclass. To date, over 100 PR-10 members have been identified in flowering plants of more than 70 species [4]. There are eighteen Mal d 1 genes in apple [16], ten Bet v 1 genes in birch [17], fourteen VvPR10 gene in grape [18], eight Fra a 1 genes in strawberry [19], eight in yellow lupine [20], five in rice [21], four HpPR10 in *Hypericum perforatum* [22], and eight Pru p 1 and Pru d 1 genes in peach and almond, respectively [23]. Some PR-10 genes were shown to form physical clusters.

PR-10 genes are differentially regulated in flowering plants in response to pathogen infection, cold, drought, and salinity. Such as pathogen infection by viruses [24], bacteria [25] and fungi [26]. They are induced by abiotic factors such as cold, salinity, drought, oxidative stress [2, 27]. During Xcb infection, the pepper (*Capsicum annuum*) PR10 along with the positive regulator leucine-rich repeat protein1 (LRR1), induces early defence responses, including callose accumulation, SA and ROS burst, and defence-related gene expression [28]. Phytohormones such as cytokinins, ABA, jasmonic acid (JA), salicylic acid (SA), auxin and ethylene induce PR-10 genes in flowering plants [2, 28]. The transcript expression of JcPR-10a (*Jatropha curcas*) was upregulated in response to NaCl, SA, methyl jasmonate, JcPR-10a might be working in coordination with cytokinin signaling in mitigating the stress induced damage by regulating different stress signaling pathways, leading to enhanced stress tolerance. Physical wounding also promotes the expression of PR-10 proteins.

PR-10 proteins may play an important role in plant growth and development as they are constitutively expressed in many organs, which is indicative of their more general biological role in plant development. PR-10 is essential for storage and transport of small molecules [29]. The PR-10 homolog obtain from the Andean crop oca is expressed specifically in the tubers where it accounts for 40-60% of total soluble proteins. The protein level gradually increases with the development of the tubers from 20 to 100 days, and decreases at later stages, under storage and upon sprouting.

Members of the strawberry PR-10 protein family play an important role in the control of phenylpropanoids and flavonoids biosynthesis. Which may related to the cell wall composition referring plant resistance against pathogens [30]. The protein Hyp-1 from *Hypericum perforatum*, it has structure analogous to typical PR10 protein, was crystallized in complex with melatonin [31]. The L1PR-10.2B protein from *Lupinus luteus* was also reported to bind cytokinins [32]. Cytokinin molecules also interact with birch PR-10c and peach Pru p 1.01.

*Gardenia jasminoides* originates in Asia and has been in cultivation for at least a thousand years. The fruit of *G. jasminoides* is used in Asian countries as a natural colorant, and as a traditional herbal medicine. Crocin, crocetin and geniposide are the main secondary metabolites in the fruit, and they all exhibit a wide range of pharmacological activities [33]. In this paper, we identified and analyzed a Pathogenesis related-10 proteins (GjPR10) in *G. jasminoides*.

## 2. Materials and Methods

### 2.1. Plant and Growth Conditions

Gardenia.jasminoides plants cultivated at Guangdong Pharmaceutical University were used as materials. Fruits were collected at development stageII, closed with yellowish green exocarp and orange mesocarp. The samples were stored at  $-80^{\circ}\text{C}$  until required.

## 2.2. CDNA Library Construction, ESTs Sequencing and Cloning of GjPR10

Total RNA was extracted from Gardenia fruit (stageII), using a modified CTAB (hexadecyl trimethyl ammonium bromide) based extraction protocol [34]. From total RNA, the cDNA library construction and amplification were performed following the manual of the Creator<sup>TM</sup> SMART<sup>TM</sup> cDNA Library construction Kit (Clontech, USA). The SMART cDNAs were ligated into SfiI-digested pDNR-LIB vector and transformed into Escherichia coli strain DH5 $\alpha$ . Colonies were randomly picked, inoculate each colony to a separated PCR reaction solutions. The colony was lysised by heating the mixed solutions at  $95^{\circ}\text{C}$  in a PTC-200 Thermocycler (MJ Research, USA) for 5 min. After then, went to PCR amplification procedure with M13 primers provided by the Creator<sup>TM</sup> SMART<sup>TM</sup> cDNA Library construction Kit. The amplified PCR products (ESTs, expressed sequence tags) were analyzed by 1.2% agarose gel electrophoresis. When the amplified PCR products were longer than 1000 bp, incubated the isolated colonies and sequenced the ESTs. There are 40 ESTs were sequenced. After sequencing and analysis, the colony containing the predicted pDNR-LIB-GjPR10 was isolated.

## 3. Results and discussion

We identified novel PR10 homologues in Gardenia jasminoides and (named GjPR10) by exploiting the fruit cDNA library of G. jasminoides. The full-length GjPR10 cDNA (Genbank accession No. KM371231) was obtained. The cDNA contains a predicted 486 bp ORF that encodes 161 amino acids. Multiple sequence alignment was performed using the program Clustal Omega with six PR10 from other plants (Fig 1), the best homology was found to PR10 related protein SPE-16 of *Pachyrhizus erosus*. The two proteins share 60.5% identical amino acids. And the homology to PR10 proteins from other species are VvPR10.3 from *Vitis vinifera* 59%, Pru p 1.01 from *Prunus dulcis*  $\times$  *Prunus persica* 57.4%, Fra a 3 from strawberry 54.6%, Bet v 1a from *Betula pendula* (49.3%), L1PR 10.1A from *Lupinus luteus* (39.1%). GjPR10 have a common Gly-rich (P-loop) domain between amino acid residues 46 and 53. The three conserved residues Glu96, Glu148 and Tyr150 (in Bet v 1) important for the RNase function have counterpart in GjPR10 as Glu99, Glu151, and the His153 instead of Tyr151. The E46 is important for IgE binding to the birch pollen [8], the counterpart in GjPR10 is Asp46. The GjPR10 secondary structure was predicted by Swiss-model software (Figure 1).



Figure 1. Sequence alignment of GjPR10 with Fd amino acid sequences from other organism. Amino acids are numbered at the right of the sequence. The abbreviation and Genbank accession number (or PDB code) are: GjPR10 (*Gardenia jasminoides*, AIX10939), Pac SPE (*Pachyrhizus erosus* 1TW0), VvPR10.3 (*Vitis vinifera*, ACA58119), Pru P 1 (*Theobroma cacao*, EOY06624), Fra a 3 (*Fragaria*  $\times$  *ananassa*, 4C94), Bet v 1a (*Betula pendula*, 1bv1), L1PR10.1A (*Lupinus luteus* p, Licx). Stars indicate residues implied in possible RNase activity. # indicate residue important for IgE binding. Predicted alpha helices and beta sheets by Swiss-model of GjPR10 are marked with H and S, respectively.

GjPR10 is composed of seven  $\beta$ -strands and three  $\alpha$ -helices. The main differences are at the C-terminal. Phylogenetic analysis of GjPR10 (using MEGA4) with representative PR10 proteins from

database indicated that GjPR10 is clustered into the subgroup which consists of Major allergen Pru ar 1 (Pru) from *Theobroma cacao*, and major allergen Pru ar 1-like protein (Sol) from *Solanum tuberosum*, the amino acid identity more than 63%. GjPR10 is distant from CSBP from Mung bean (CSBPMG) and MLP from *A. thaliana* (MLPAT), the amino acid identity is 18.0% and 14.7%, respectively. The bootstrap value is given for each node (Figure .2A).

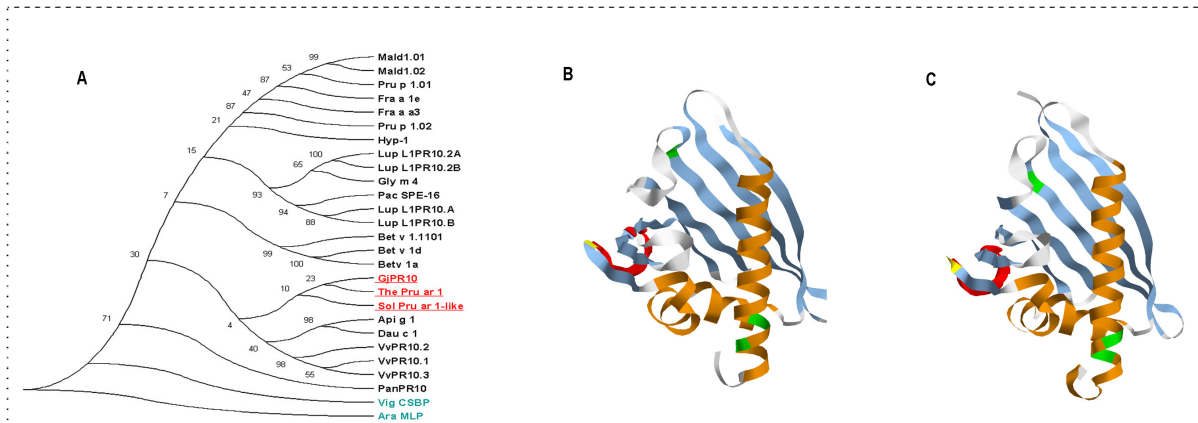


Figure 2. Phylogenetic analysis and three-dimensional structure model for GjPR10. (A): phylogenetic relationships between GjPR10 and 27 PR10 proteins from other plants. The abbreviation and Genbank accession number (or PDB code) are: GjPR10 (*Gardenia jasminoides*, AIX10939), Mal d (*Malus domestica*, AAX18288, AAX18291), Pru p (*Prunus dulcis* × *Prunus persica*, 1E09, ACE80942), Fra a (*Fragaria* × *ananassa*, 21PX, 4C94), Hyp-1 (*Hypericum perforatum*, 3IE5), Lup (*Lupinus luteus*, 1ICX, 1IFV, AAF77633, 2QIM), Gly (*Glycine max*, 2K7H), Pac (*Pachyrhizus erosus*, 1TW0), Bet v (*Betula pendula*, CAA54482, CAA54694, 1BV1), The Pru ar 1 (*Theobroma cacao*, EOY06624), Sol Pru ar 1-like (*Solanum tuberosum*, XP-006349827), Api g 1 (*Apium graveolens*, 2BK0), Dau c 1 (*Daucus carota subsp. sativus*, 2WQL), VvPR10 (*Vitis vinifera*, CAC16166, CAC16165, ACA58119), Pan (*Panax ginseng*, AII79439), Vig CSBP (*Vigna radiata var. radiata*, 2FLH), Ara MLP (*Arabidopsis Thaliana*, IVJH). (B): predicted structure of GjPR10 (Genbank accession No.), predicted by Swiss-PDB software. (C) Crystal structure of Fra a 3 (4C94). In (B and C): GjPR10 and Fra a 3 are shown in ribbon diagram,  $\alpha$ -helix are shown in orange,  $\beta$ -sheet are shown in blue, residues for RNase activity are shown in green, residues for allergenic activity are shown in yellow.

The 3D (three dimension) model structure of GjPR10 was predicted using SWISS-PDB software (Figure. 2B). The X-Ray diffraction at resolutions down to 3.0 Å of *Fragaria* × *ananassa* Strawberry Fra a 3 protein (residues from 2-161) complexes with Catechin (PDB code 4C94) was used as template [33] (Figure. 2C), the GjPR10 includes residues 1-161 was predicted. The structure was successfully built as a monomer (Figure. 3B); the fold of GjPR10 consists of antiparallel seven  $\beta$ -sheets (B<sub>1</sub>-B<sub>7</sub>) along with two consecutive short  $\alpha$ -helices (H<sub>1</sub> and H<sub>2</sub>) and a long C-terminal helix (H<sub>3</sub>). These structural elements form an extended internal hydrophobic cavity that is capable of binding a variety of physiologically relevant hydrophobic molecules, the cavity is formed between the  $\beta$ -sheet and helix  $\alpha$ 3. This conformation is in accordance with the crystal structure determined for plant PR10 proteins in strawberry, yellow lupine, *Hypericum perforatum* and birch [11, 31, 33 & 35].

This work suggests that, from structure analysis, GjPR10 belongs to PR10 proteins superfamily, and it's closely related a group of major tree allergens and food allergens. GjPR10 has RNase activity; GjPR10 may involve in response to infection by plant pathogens and under abiotic stress conditions; and the protein might responsible for allergic reactions; and also, it probably involve in binding, storage and transport of hydrophobic molecules, including phytohormones and other small molecule

ligand in plant cells. GjPR10 may have functions in developmental processes, secondary metabolism, and defence against pathogens activity.

## References

- [1] Van Loon, L.C., Rep, M., Pieterse, C.M. (2006) Significance of inducible defence-related proteins in infected plants. *Annu Rev Phytopathol.*, 44:135-162.
- [2] Lee, O.R., Pulla, R.K., Kim, Y.J. et al. (2012) Expression and stress tolerance of PR10 genes from *Panax ginseng* CA. Meyer. *Mol Biol Rep.*, 39:2365-2374.
- [3] Agarwal, P., Agarwal, P.K. (2014) Pathogenesis related-10 proteins are small, structurally similar but with diverse role in stress signaling. *Mol Biol Rep.*, 41:599-611.
- [4] Fernandes, H., Michalska, K., Sikorski, M. et al. (2012) Structural and functional aspects of PR-10 proteins. *FEBS J.*, 280:1169-1199.
- [5] Liu, J.J., Ekramoddoullah, A.K.M. (2006) The family 10 of plant pathogenesis-related proteins: their structure, regulation, and function in response to biotic and abiotic stresses. *Physiol Mol Plant Pathol.*, 68:3-13.
- [6] Pan, J., Wang, X., Li, L. et al. (2018) Purification and characterization of two pathogenesis-related class 10 protein isoforms with ribonuclease activity from the fresh *Angelica sinensis* roots. *Plant Physiol Biochem.*, 128:66-71.
- [7] Chruszcz, M., Ciardiello, M.A., Osinski, T. et al. (2013) Structural and bioinformatic analysis of the kiwifruit allergen Act d 11, a member of the family of ripening-related proteins. *Mol Immunol.*, 56:794-803.
- [8] Stemeseder, T., Klinglmayr, E., Moser, S. et al. (2017) Influence of Intrinsic and Lifestyle Factors on the Development of IgE Sensitization. *Int Arch Allergy Immunol.*, 173:99-104.
- [9] Nakamura, R., Teshima, R. (2013) Proteomics-based allergen analysis in plants. *J. Proteomics.*, 93:40-49.
- [10] Radauer, C., Lackner, P., Breiteneder, H. (2008) The Bet v 1 fold: an ancient, versatile scaffold for binnding of large, hydrophobic ligands. *BMC Evol Biol.*, 8:286.
- [11] Biesiadka, J., Bujacz, G., Sikorski, M.M. et al. (2002) Crystal structures of two homologous pathogenesis-related proteins from yellow lupine. *J Mol Biol.*, 319:1223-1234.
- [12] Sliwiak, J., Sikorski, M., Jaskolski, M. (2018) PR-10 proteins as potential mediators of melatonin-cytokinin cross-talk in plants: crystallographic studies of LIPR-10.2B isoform from yellow lupine. *FEBS J.*, 285:1907-1922.
- [13] Wangorsch, A., Jamin, A., Lidholm, J. et al. (2017) Identification and implication of an allergenic PR-10 protein from walnut in birch pollen associated walnut allergy. *Mol Nutr Food Res.*, 61.
- [14] Fontanella, S., Frainay, C., Murray, C.S. et al. (2018) Machine learning to identify pairwise interactions between specific IgE antibodies and their association with asthma: A cross-sectional analysis within a population-based birth cohort. *PLoS Med.*, 15:e1002691.
- [15] Sun, H., Kim, M.K., Pulla, R.K. et al. (2010) Isolation and expression analysis of a novel major latex-like protein (MLP151) gene from *Panax ginseng*. *Mol Biol Rep.*, 37:2215-2222.
- [16] Gao, Z.S., van de Weg, W.E., Schaart, J.G. et al. (2005) Genomic cloning and linkage mapping of the Mal d 1 (PR-10) gene family in apple (*Malus domestica*). *Theor Appl Genet.*, 111:171-183.
- [17] Schenk, M.F., Cordewener, J.H., America, A.H. et al. (2009) Characterization of PR-10 genes from eitht *Betula* species and detection of Bet v 1 isoforms in birch pollen. *BMC Plant Biol.*, 9:24.
- [18] Lebel, S., Schellenbaum, P., Walter, B. et al. (2010) Characterisation of the *Vitis vinifera* PR10 multigene family. *BMC Plant Biology.*, 10:184.
- [19] Musidłowska-Persson, A., Alm, R., Emanuelsson, C. (2007) Cloning and sequencing of the Bet v 1-homologous allergen Fra a 1 in strawberry (*Fragaria ananassa*) shows the presence of an intron and little variability in amino acid sequence. *Mol Immunol.*, 44:1245-1252.

- [20] Handschuh, L., Femiak, I., Kasperska, A. et al. (2007) Structural and functional characteristics of two novel members of pathogenesis-related multigene family of class 10 from yellow lupine. *Acta Bioch Pol.*, 54:783-796.
- [21] Kim, S.T., Yu, S., Kang, Y.H. et al. (2008) The rice pathogen-related protein 10 (JIOsPR10) is induced by abiotic and biotic stresses and exhibits ribonuclease activity. *Plant Cell Rep.*, 27: 593-603.
- [22] Karppinen, K., Derzsó, E., Jaakola, L. et al. (2016) Molecular cloning and expression analysis of hyp-1 type PR-10 family genes in *Hypericum perforatum*. *Front. Plant Sci.*, 7:526.
- [23] Chen, L., Zhang, S., Illa, E. et al. (2008) Genomic characterization of putative allergen genes in peach/almond and their synteny with apple. *BMC Genomics.*, 9:543.
- [24] Xu, P., Blancaflor, E.B., Roossinck, M.J. (2003) In spite of induced multiple defense responses, tomato plants infected with Cucumber mosaic virus and D satellite RNA succumb to systemic necrosis. *Mol Plant Microbe Interact.*, 16:467-476.
- [25] Bahramnejad, B., Goodwin, P.H., Zhang, J. et al. (2010) A comparison of two class 10 pathogenesis-related genes from alfalfa and their activation by multiple stresses and stress-related signaling molecules. *Plant Cell Rep.*, 29:1235-1250.
- [26] Agarwal, P., Dabi, M., More, P. et al. (2016) Improved shoot regeneration, salinity tolerance and reduced fungal susceptibility in transgenic tobacco constitutively expressing PR-10a gene. *Front Plant Sci.*, 7:217.
- [27] Gómez-Gómez, L., Rubio-Moraga, A., Ahrazem, O. (2011) Molecular cloning and characterisation of a pathogenesis-related protein CsPR10 from *Crocus sativus*. *Plant Biol (Stuttg.)*, 13:297-303.
- [28] Choi, D.S., Hwang, I.S., Hwang B.K. (2012) Requirement of the cytosolic interaction between PATHOGENESIS-RELATED PROTEIN10 and LEUCINE-RICH REPEAT PROTEIN1 for cell death and defense signaling in pepper. *Plant Cell.*, 24:1675-1690.
- [29] Grutsch, S., Fuchs, J.E., Freier, R. et al. (2014) Ligand binding modulates the structural dynamics and compactness of the major birch pollen allergen. *Biophysical J.*, 107:2972-2981.
- [30] Franz-Oberdorf, K., Langer, A., Strasser, R. et al. (2017) Physical interaction between the strawberry allergen Fra a 1 and an associated partner FaAP: Interaction of Fra a 1 proteins and FaAP. *Proteins.* 85:1891-1901.
- [31] Sliwiak, J., Dauter, Z., Jaskolski, M. (2016) Crystal structure of Hyp-1, a *Hypericum perforatum* PR-10 protein, in complex with melatonin. *Front Plant Sci.*, 7: 668.
- [32] Fernandes, H., Pasternak, O., Bujacz, G. et al. (2008) *Lupinus luteus* pathogenesis-related protein as a reservoir for cytokinin. *J Mol Biol.*, 378:1040-1051.
- [33] Casañal A1, Zander U, Muñoz C et al. (2013) The strawberry pathogenesis-related 10 (Pr-10) Fra a proteins control flavonoid biosynthesis by binding to metabolic intermediates. *J Biol Chem.*, 288: 35322-35332.
- [34] Bekesiova, I., J. Nap, J.P., Mlynarova, L. (1999) Isolation of high quality DNA and RNA from leaves of the carnivorous plant *Drosera rotundifolia*. *Plant Mol. Biol. Rep.*, 17:269-277.
- [35] Marković-Housley Z, Degano M, Lamba D et al. (2003) Crystal structure of a hypoallergenic isoform of the major birch pollen allergen Bet v 1 and its likely biological function as a plant steroid carrier. *J Mol Biol.*, 325: 123-133.