

Original Article

In Silico Evaluation of Expansin-Gene Function in Softening and Fruit Ripening

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ABSTRACT

The up-to-date achievements in expansin (EXP) research were evaluated in this study. We also dealt with how to strengthen the plants, how cell walls define cell function, cell shape, and cell size. Cell wall (CW) proteins that are famous as expansins consist of four subfamilies a-expansin, b-expansin, expansin-like A, and expansin-like B. These proteins alter CW extricating, and these sorts of proteins are found in plant kingdoms and a few microbial living beings, as well as other living beings like snails. The results of EXP promoter analysis on the 1.5 kb sequence upstream of the ATG (start codon) in Arabidopsis and their orthologs in *P. persica* by PLANT CARE revealed that different transcription factors (TF) are attached to specific DNA binding sites. In this assay, the genome sequencing of peach plants from the Rosaceae family (*Prunus persica*) was carried out, so more accurate applications could be designated by analyzing their regulatory areas. In the next step, the good genes were transferred into the early or late flowering plants; consequently, their yield and resistance against biotic and abiotic stress increased.

1. Introduction

The CW has vital roles in numerous cell tasks some of which are transmission and connection, differentiation, downfall, senescence, interplay of the pathogen with plants, and ultimately plant growth. Besides, mechanical firmness and plasticity are needed for the evolution of plant tissues and organs that are provided by the CWs. Plants require cell estimate and shape alteration insomuch plant development can be recognized

as cell measure, cell amount, plant development, and expansion, which is fulfilled by controlled changes in CW versatility. This drives EXPs exceptionally noteworthy for they are effectively engaged in this region [1]. The action of EXP as a general agreement on the CW brings approximately much-needed versatility; however, biochemical working mechanism in the EXP is not entirely understood [1]. The EXPs are a large gene superfamily that codes the small CW proteins that are obtained by biomechanical analysis by creep tests [2]. According to a study

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can be divided into four subfamilies, namely, expansin-like A (EXPLA) and expansin-like B (EXPLB), a-expansin or expansin A (EXPA), and b-expansin or expansin B (EXPB). Choi et al. [20] had the same opinion on this classification. Still, expansin-like X is another EXPs group with a remote link to EXP genes, which are available in both of interior and exterior plant kingdom. The EXP and EXP-like genes are classified placed on their family relationship kinship affinity, which has been broadly checked on [3].

Plant promoter's identification may supply rudimentary facts in understanding the regulation of gene transcription. Proximal promoter serves as the main location for the most promoter elements regulating TSS selection. Cis-regulatory elements are development factors for most plant promoter databases, including PlantCARE, PLACE or TRANSFAC, and ppdb, and PlantPan.

This study aimed at recognizing the cis-elements modules and their organization within the administrative promoter rejoin of EXP genes in peach. For a comprehensive understanding of the regulation of gene expression, doing this research seemed essential. In addition, researchers in this project attempted to analyze and identify the common motifs EXP proteins. Further, we considered their capacities through diverse information banks, and, at last, the proteins associated with EXP were recognized. The proteins were hypothesized to be related with natural product that plays a role in speeding up and softening of aging.

2. Materials and Methods

2.1. Promoter analysis of EXP gene

The genomic DNA of the EXP gene in *P. persica* (NC_034009) was accessed via the National Center for Biotechnology Information (NCBI, <http://www.ncbi.nlm.nih.gov/>) webserver. This information superhighway was a suitable platform to facilitate the promoter region of the EXP genes identification by utilizing the BLAST search through the Phytozome database (<http://www.phytozome.net/>). Therefore, using these applications and servers helped us to use them

as research tools in this project. After distinguishing the genes on the chromosome using the BLAST-N algorithm, the promoter was selected from the locale around the 1500 bp upstream of the start codon (ATG) in the EXP gene of *P. persica*. In this regard, the PLANT CARE and PlantPan were applied as databases to analyze the upstream part of the EXP in Arabidopsis and their corresponding orthologs in *P. persica*. To do so, upstream section sequences of EXP in Arabidopsis and their comparing orthologs were connected to anticipate their key cis-acting regulatory elements and the exact area of these components [47].

2.2. Motifs distinguishing proof and their useful examination

As primary understanding, the MEME web server aligned with the EXP proteins achieved from the NCBI to identify their common motifs. The ELM program and SMART predicted the probable functions of EXP's conserved domains within the protein sequences. The UniProtKB was utilized for the distinguishing proof of a few gene ontology characteristics of EXP. Primary sequence examination was done by Prot Param. Moreover, various programs at NCBI, such as the BLAST-P and PSI-BLAST were the main webserver to estimate the similarity. In the end, numerous sequence alignments were performed by using the Vector NTI Suit 9 [47].

2.3. Protein-protein interplay networks

A well-defined protein-protein interaction network in Arabidopsis gives a reasonable cause for utilizing EXP (XP_007226005.1) as a query. Additionally, the prediction of all the proteins that associated with the EXP proteins was facilitated by STRING 9.0.

3. Results

In this experiment, genome sequencing was carried out of peach plants from the Rosaceae family (*Prunus persica*). Thus, more precise applications can be designated by analyzing their regulatory areas. In the next step, the good genes were transferred into the early or late flowering plants, and consequently, their yield

and resistance against biotic and abiotic stress increased.

The Blast assessment revealed that α -mannosidase enzyme has the homology in 15 evaluated plants, and their similarity percentage varies from 100% in *Prunus avium* to 94% in *Herrania umbratica* plant. The similarity in the sequence might be a useful indicator of constructional and functional similarities. So, it sounds that with this interpretation, it is possible to opine about this enzyme function in other evaluated plants. Bioinformatics is a science scope in which biology, statistics, computer, and information technology sciences combine and create modern scientific systems. The objective is to explore new biological

prospects and create a general view in which it is possible to distinguish the details of the biological principles. Bioinformatics is significantly crucial in supporting biology science to gather, interpret, and manage many biological data. These data are in the forms of nucleotide and Amino acids sequences, the secondary proteins, protein structures, and the metabolic and biochemical paths genes expression methods.

The EXP promoter analysis was checked on the 1.5 kb sequence upstream of the ATG (start codon) in *Arabidopsis* and their orthologs in *P. persica* by PLANT CARE that showed different TFs attached to specific DNA binding sites.

Table 1. Selected examples of studies reporting the effects of EXPs on plant development and stress adaptation

EXP Name	Sub-Family	Mode of Expression	Observed Phenotype	References
AtEXPA1	a-EXP	Expression analysis (EXPR ANLYS) Silenced	Raised the stomatal opening rate by light-induced, & decrease stomata sensitivity to stimuli, sequentially	[6, 7]
AtEXPA2	a-EXP	Overexpression and suppression	Over expressors sprouted speedier than wild sort plants whereas deferred germination of mutant lines	[8]
AtEXP3	a-EXP	Over expression (OVR EXPR)	Growth enhancement & leaves extension in normal conditions	[9]
AtEXPA4	a-EXP	Expression profile analyses	Seemingly loosened the stigma's CW	[10]
AtEXPA7	a-EXP	OVR EXPR	Improved & decreased horizontal root arrangement, individually	[11]
AtEXPA10	a-EXP	OVR EXPR	Had the larger plant cells, leaves & stems	[12]
AtEXPA17	a-EXP	OVR EXPR and knock down	Enhanced & reduced lateral root formation, respectively	[13]
AtEXPA18	a-EXP	OVR EXPR	Induced hairy root initiation & root growth	[11]
LeEXPA1	a-EXP	EXPR ANLYS	Involved in natural products softening	[14, 15]
LeEXP1	a-EXP	OVR EXPR and Suppression	Softer fruits because of OVR EXPR, whereas its concealment created firmer natural products in transgenic tomatoes.	[16]
LeEXPA8	a-EXP	mRNA EXPR ANLYS	affected germination (communicated as it were in developed seeds) & included the starting prolongation of the radicle	[17]

LeEXPA10	a-EXP	mRNA EXPR ANALYS	Seemed to influence germination as well as seed development	[17]
SExp1	a-EXP	Knockout	Increased fruit firmness	[18]
OsEXPA1	a-EXP	EXPR ANALYS	Thought to influence coleoptile and internode development	[19]
OsEXPA4	a-EXP	OVR EXPR Antisense (RNAi)	Pleiotropic phenotypes in plant stature, leaf number, blossoming time and seed set as well as improved coleoptile development Shorter plants, diminished coleoptile and mesocotyl lengths	[20, 21]
OsEXPA8	a-EXP	OVR EXPR	Increased root mass, number and size of leaves as well as plant height	[22]
OsEXPA17	a-EXP	OVR EXPR	Influenced rice root development	[23]
DzEXP1	a-EXP	EXPR ANALYS	Thought to be involved in fruit/pulp softening and peel dehiscence	[24]
NtEXPA5	a-EXP	OVR EXPR	Increased organ size especially the leaves and the stem	[12]
DzEXP2	a-EXP	EXPR ANALYS	Thought to be involved in fruit/pulp softening as well as peel dehiscence	[24]
FaExp2	a-EXP	EXPR ANALYS	Played a role in CW polymer disassembly during fruit maturation	[25]
MaExp1	a-EXP	OVR EXPR	Affected banana ripening	[26]
PpEXP1	a-EXP	OVR EXPR	Increased sprouting and abiotic stresses tolerance	[27]
RhEXPA4	a-EXP	OVR EXPR and silencing	More prominent growing rate; expanded sidelong root arrangement and modified leaves	[28]
GmEXP1	a-EXP	OVR EXPR	Influenced extension and drying out resistance of rose petals & Accelerated root growth	[29]
GbEXPATR	a-EXP	OVR EXPR	Increased hairy root development in transgenic Arabidopsis	[30]
IbEXP1	a-EXP	OVR EXPR	More rosette leaves	[31]
PnEXPA1	a-EXP	OVR EXPR	Large plant cells, larger leaves and longer stems	[12]
CsEXPA1	a-EXP	OVR EXPR	Initiated development of the leaf primordium	[32]
AtEXPB1	b-EXP	OVR EXPR	Essentially longer petioles beneath typical development conditions	[9]
AtEXPB5	b-EXP	Expression profile analyses	Seemed to soften the stigma CW	[10]
OsEXPB2	b-EXP	EXPR ANALYS Silenced	Thought to affect root hair and internodes development, Affirmed the prior proposed part as physiological changes decreased root and leaf sizes	[33, 21]

OsEXPB3	b-EXP	EXPR ANLYS	Thought to be involved in internode elongation as well as root development	[33, 34]
OsEXPB4	b-EXP	EXPR ANLYS	mRNA accumulation correlated well with internode elongation	Lee and Kende [34]
OsEXPB6	b-EXP	EXPR ANLYS	mRNA accumulation correlated well with internode elongation	Lee and Kende [34]
OsEXPB11	b-EXP	EXPR ANLYS	mRNA accumulation correlated well with internode elongation	Lee and Kende [34]
GmEXPB2	b-EXP	OVR EXPR	Upgraded by and large plant development, higher root cell division and stretching. Improved phosphorus uptake	Guo <i>et al.</i> [35]
GmEXPB2	b-EXP	OVR EXPR	Increase in phosphorus efficiency	Zhou <i>et al.</i> [36]
TaEXPB23	b-EXP	OVR EXPR	Progressed resistance of transgenic tobacco plants to oxidative stress, Overexpression performed superior beneath drought. They improved root development and water stretch resistance	Han <i>et al.</i> [37] Li <i>et al.</i> [38]
TaEXPB23	b-EXP	OVR EXPR	Longer internodes, larger leaf blades, more leaves, more roots	Xing <i>et al.</i> [39]
HvEXPB1	b-EXP	Promoter deletion	Shown to influence root hair formation	Won <i>et al.</i> [40]
AtEXLA2	EXP like A	OVR EXPR	Longer roots which were essentially longer than the wild sort roots	Boron <i>et al.</i> [41]
AtEXPLA2	EXP like A	OVR EXPR and mutant lines	Reduced EXLA2 transcript levels, enhanced resistance to necrotrophic pathogens (<i>Botrytis cinerea</i> ; <i>Alternaria brassicicola</i>)	Abuqamar <i>et al.</i> [42]

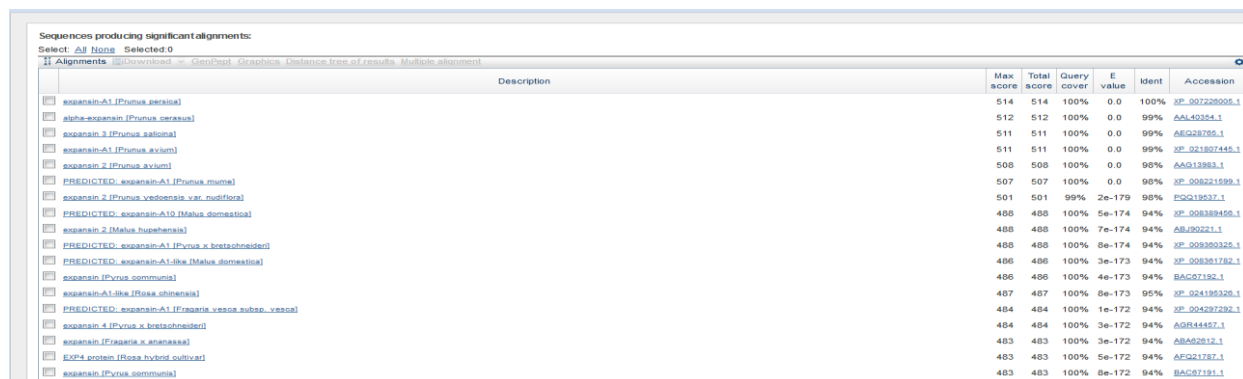


Figure 1. BLAST EXP gene in peach plant using the NCBI

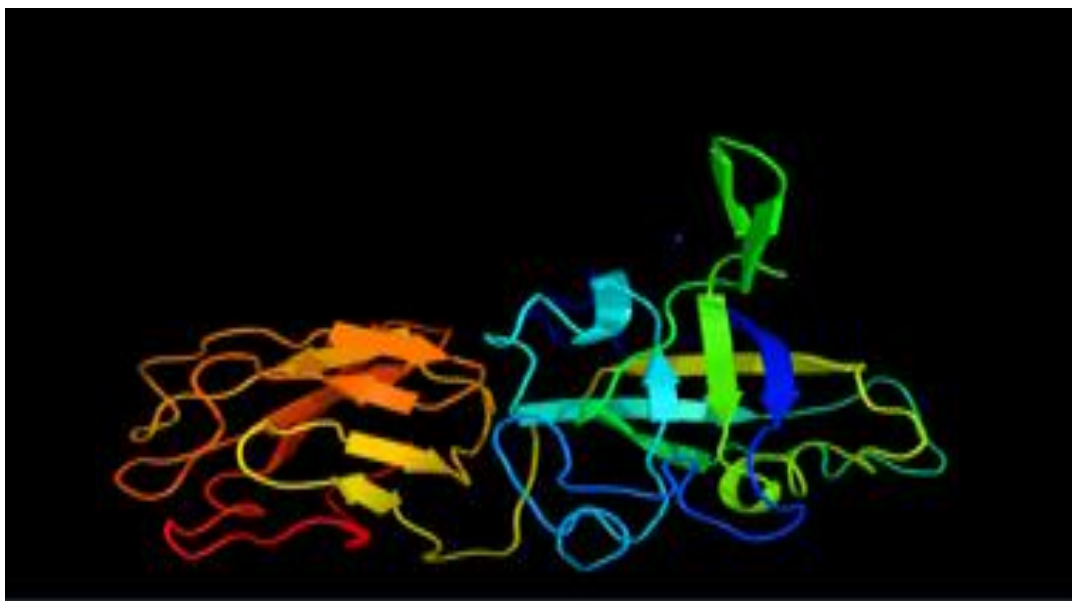
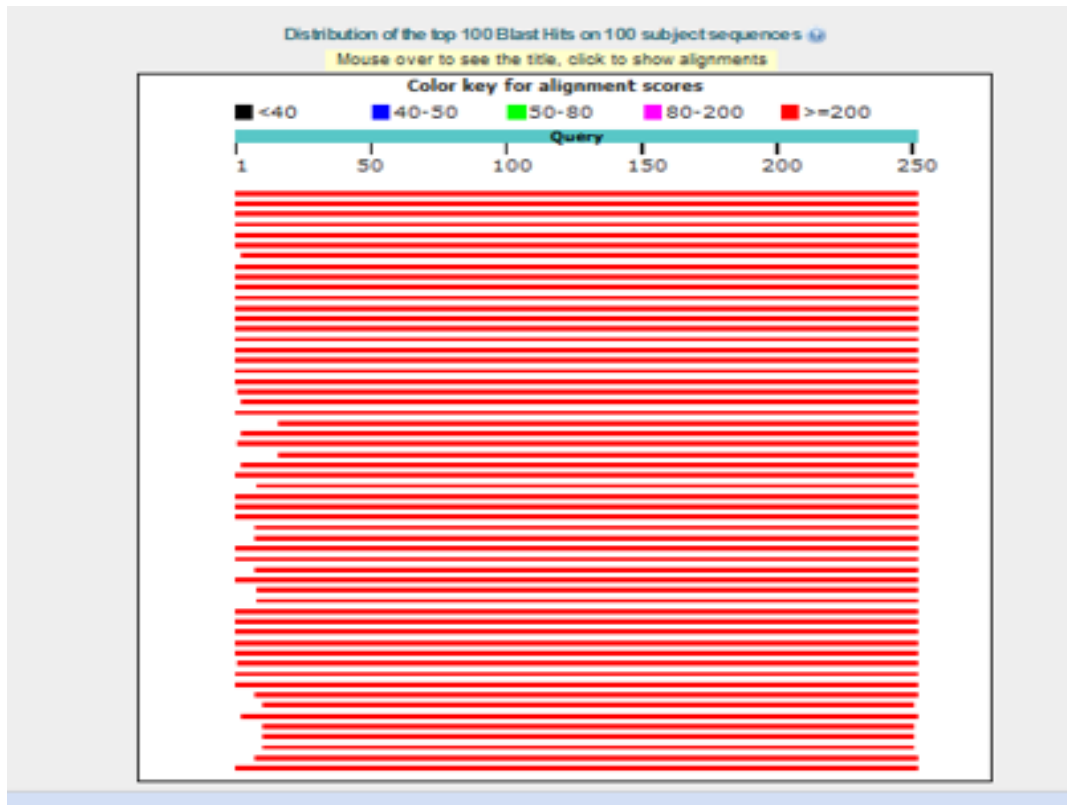


Figure 2. Protein-protein interplay network analysis of EXP using STRING 9.0 in *Arabidopsis thaliana*

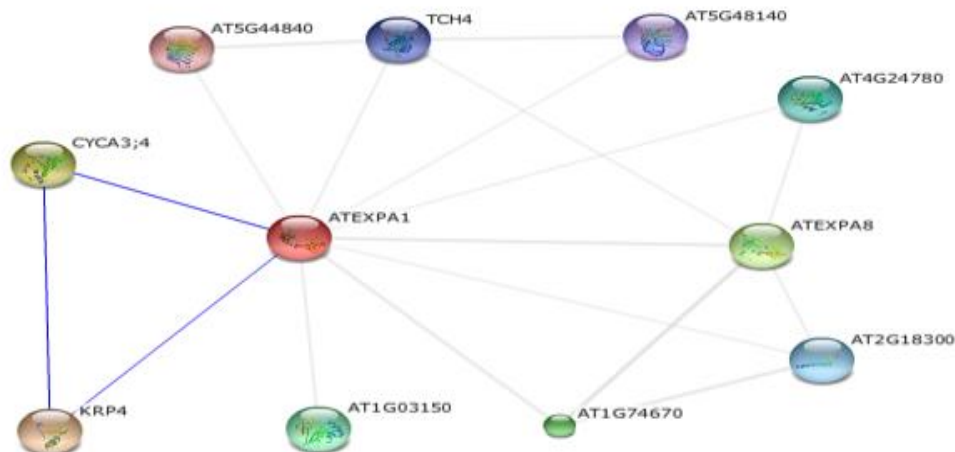


Figure 3. Protein-protein interplay network analysis of EXP using STRING 9.0 in *Arabidopsis thaliana*

Discussion

Plant growth regulators and the setting have a crucial function in plants' development and growth. Ethylene as a maturing hormone affects the translation level of LeEXPA1 and a tomato EXP, which makes a positive relationship between LeEXPA1 level and tomato natural product softening [43]. It is believed that this ripening-regulated EXP expression will soften fruits via the reported action of EXPs on the CW. In this regard, softening is due to the CW polymer disassembling by increasing access to specific CW polymers' hydrolase action [44]. Further, it was found that EXPs have a vital part in natural product maturing by Minoia et al. [45], who concurred with the thought that the EXPs that are exceedingly communicated amid tomato natural product aging contribute to the natural product softening. Minoia et al. [45] illustrated that natural product solidness is straightforwardly related to transformations within the α -EXP S1Exp1 quality. The natural product solidness was upgraded 41 and 46 % in S1exp1-6 and S1exp1-7 mutant lines, individually as compared with the control plants.

After analyzing different parts played by EXPs, it has been decided that EXPs are included in numerous morphogenetic forms, counting growing, bushy roots improvement, defoliation, natural product aging, dust tube development,

and other conceivable forms, which are however to be found [12]. It may be a common conviction that analysts are required to consider obtaining these extensions since a few EXPs like RhEXPA4 have a negative impact on plant advancement at exceptionally tall levels. As spotlighted in this paper, the incorporation of EXPs presents a potential tool to improve crops significantly in various aspects of crop improvement programs. The EXPs integration with other instruments can be accommodating in controlling numerous plant physiological viewpoints; in spite of the fact that, numerous edit characteristics are quantitative and are controlled by different qualities to resist biotic and abiotic stretch, which is turning into concern owing to current issues related to worldwide warming and climate alter. As pointed out in this paper, EXPs can help floriculturists control products' size through OVR EXP. Expansion can upgrade plants' resistance to non-living and living stresses and modify/reduce the chemical materials within the plants' proficiency beneath supplement or salt push. At the same time, its application can diminish post-harvest misfortunes, which right now floats over 50 % for fruits and vegetables [46]. In brief, combining the control EXPs with other breeding instruments can be a valuable approach to progress our crops. Be that as it may, the esteem

of this data stands in its utilization in crop improvement.

5. Conclusion

Bioinformatics is a science in which biology, statistics, computer, and information technology sciences combine and create modern scientific systems. The Blast assessment revealed that α -mannosidase enzyme has the homology in 15 evaluated plants, and their similarity percentage varies from 100% in *Prunus avium* to 94% in *Herrania umbratica* plant. The similarity in the sequence might be a useful indicator of constructional and functional similarities. So, it sounds that with this interpretation, it is possible to opine about this enzyme function in other evaluated plants.

Authors' contributions

In this study, the first author presented the design, supervised, carried out the formal and statistical analysis and wrote the manuscript; the second author carried out the experiment, and the third author helped to write the original draft.

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Ethics approval and consent to participate

No human or animals were used in the present research.

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