In Silico Functional Analysis of Expansin-Gene Responsible for Fruit Ripening and Softening

Hojat Rezaei, Ali Salehi Sardoei* and Mohammad Hassan Bagheri
PhD student Horticultural Science, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources, Iran.
**Corresponding Author: alisalehisardoei@gau.ac.ir*

ABSTRACT

The recent advances in expansin studies were reviewed. Besides producing the strength that is needed by the plants, cell walls define cell shape, cell size and cell function. Expansins are cell wall proteins which consist of four sub families; a-expansin, b-expansin, expansin-like A and expansin-like B. These proteins mediate cell wall loosening and they are present in all plants and in some microbial organisms and other organisms like snails. The results of expansin 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 experiment the genome sequencing of peach plant, from the Rosaceae (*Prunus persica*), has been carried out, so more precise application can be designated by analysis of their regulatory areas. In the next step, the proper genes were transferred into the early or late flowering plants, and consequently their yield and resistance against biotic and abiotic stress increased.

Keywords: Cell wall, Expansin, Plant growth, In Silico.

INTRODUCTION

The cell wall plays crucial roles in various cell activities such as differentiation, transport and communication, senescence, abscission, plant-pathogen interactions and ultimately plant growth. It provides both the mechanical strength needed by the plant and the plasticity that is necessary for the development of plant tissues and organs. Since plant growth can be generalized as a function of cell size and cell number, plant growth and development therefore requires modulation of cell size and shape, which is accomplished by regulated changes in cell wall plasticity. This makes expansins very important since they are actively involved in this area (Cosgrove, 2015). Although the expansin's biochemical working mechanism is not completely understood, it is generally agreed that the action of expansin on the cell wall brings about this much needed plasticity (Cosgrove, 2000). Biomechanical analysis by creep tests showed Expansins comprise a large gene super-family which codes for small (225–300 amino acid residues) cell wall proteins (Fukuda, 2014). According to Kende et al. (2004) they can be divided into four sub families; a-expansin or expansin A (hereinafter referred to as ''EXPA''), b-expansin or expansin B (hereinafter referred to as ''EXPB''), expansin-like A (hereinafter referred to as ''EXPLA'') and expansin-like B (hereinafter referred to as ''EXPLB''). Choi et al. (2008) concurred with this classification but went on to add expansin-like X (hereinafter referred to as ''EXLX'') as another group of expansins which are remotely related to expansin genes and found both inside and outside the plant kingdom. The classification of expansin and expansin-like genes is based on their phylogenetic relationship and this has been extensively reviewed (Kende et al. 2004).

The identification of plant promoters may provide fundamental information in understanding the regulation of gene expression. Most promoter elements regulating TSS selection are localized in the proximal promoter. Many plant promoter databases have been developed based on cis-regulatory elements including PlantCARE, http://www.bioinformatics.psb.ugent.be/ webtools /plantcare/html/], PLACE, http://www.dna.affrc.go.jp/ PLACE/] or TRANSFAC, http://www.gene-regulation.com/pub/ databases.html] and ppdb, http://www.ppdb.gene.nagoya-u.ac.jp] and PlantPan, http://plantpan2.itps.ncku.edu.tw.

The objectives of the present work were to recognize the cis-elements modules and their organization in the regulatory promoter rejoin of expansin genes in peach. This was done in order to generate a comprehensive understanding of the regulation of gene expression. Furthermore, this research endeavored to identify the common motifs expansin proteins. Their functions through different data banks were studied and, finally, the proteins that interacted with expansin were recognized. The proteins were hypothesized to be involved in the acceleration or fruit ripening and softening.

Materials and Methods

Promoter analysis of expansin gene

The genomic DNA of expansin gene in *P. persica* (NC_034009) was accessed through the National Center for Biotechnology Information (NCBI, http://www. ncbi.nlm.nih.gov/) web server. It was applied as a platform in order to recognize the promoter region of the expansin genes by using the BLAST search through the Phytozome database (http://www. phytozome.net/). These were used for the purposes of investigation in this

IrHC2019

study. After identifying the genes on the chromosome using the BLAST-N algorithm, the region around the 1500 bp upstream of the start codon (ATG) in expansin gene of *P. persica* were taken as a promoter. The upstream region of the expansin in Arabidopsis and their corresponding orthologs in *P. persica* were analyzed by using the PLANT CARE and PlantPan database. For this purpose, upstream region sequences of expansin in Arabidopsis and their corresponding orthologs were applied to predict their key cis acting regulatory elements and the precise location of these elements.

Motifs identification and their functional analysis

Referring to the expansin proteins obtained from the NCBI, these sequences were aligned by the MEME web server (http://meme. sdsc.edu/ meme4_6_1/cgi-bin/meme.cgi) so as to identify their common motifs. The predictions of probable functions of conserved domains of expansin within the protein sequences was performed by the ELM program (http://elm.eu.org) and SMART (http://smart. embl-heidelberg.de). The UniProtKB (http://www.uniprot.org/) was used for the identification of some gene ontology characteristics of expansin. Primary sequence analysis was done by ProtParam (http:// expasy.org/cgi-bin/protparam). Moreover, similarity was assessed by using different programs at NCBI, such as the BLAST-P and PSI-BLAST (http://BLAST.ncbi.nlm.nih.gov/BLAST.cgi). Multiple sequence alignments were performed by using the Vector NTI Suit 9.

Protein-protein interaction networks

A well-defined protein–protein interaction network in Arabidopsis gives good reason for the use of expansin (XP_007226005.1) as a query. STRING 9.0 (http://string-db.org) was used to predict all the proteins that interact with the expansin proteins.

RESULTS

In this experiment the genome sequencing of peach plant, from the Rosaceae (*Prunus persica*), has been carried out, so more precise application can be designated by analysis of their regulatory areas. In the next step, the proper 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 plant to 94% in *Herrania umbratica* plant. Similarity in the sequence might be a proper 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 are combined together and created modern scientific system. The objective is the exploration of new biological prospects and creation of general view in which it is possible to distinguish the biological principles details (Vassilev et al., 2005). Bioinformatics is significantly important in the support of biology science for gathering, interpretation, and management of abundant 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 (Dagostino et al., 2005).

The results of expansin 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.

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

Expansin name	Sub-family	Mode of expression	Observed phenotype	References
AtEXPA1	a-Expansin	Expression analysis Silenced	Increased rate of light-induced stomatal opening and reduced sensitivity of stomata to the stimuli, respectively	Wei et al. (2011a, b)
AtEXPA2	a-Expansin	Overexpression and suppression	Over expressors germinated faster than wild type plants while germination was delayed in mutant lines	Yan et al. (2014)
AtEXP3	a-Expansin	Over expression	Enhanced growth and larger leaves under normal growth conditions	Kwon et al. (2008)
AtEXPA4	a-Expansin	Expression profile analyses	Thought to soften the cell wall of the stigma	Mollet et al. (2013)
AtEXPA7	a-Expansin	Over expression	Influenced root hair initiation and root growth	Cho and Cosgrove (2002)
AtEXPA10	a-Expansin	Over expression	Large plant cells, larger leaves and longer stems	Kuluev et al. (2012)
AtEXPA17	a-Expansin	Overexpression and knock down	Enhanced and reduced lateral root formation, respectively	Lee and Kim (2013)
AtEXPA18	a-Expansin	Over expression	Influenced root hair initiation and root growth	Cho and

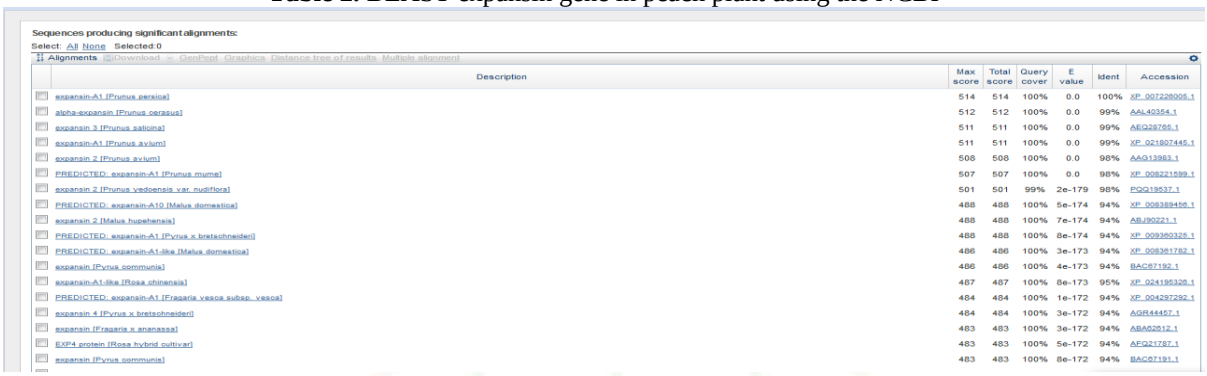
IrHC2019

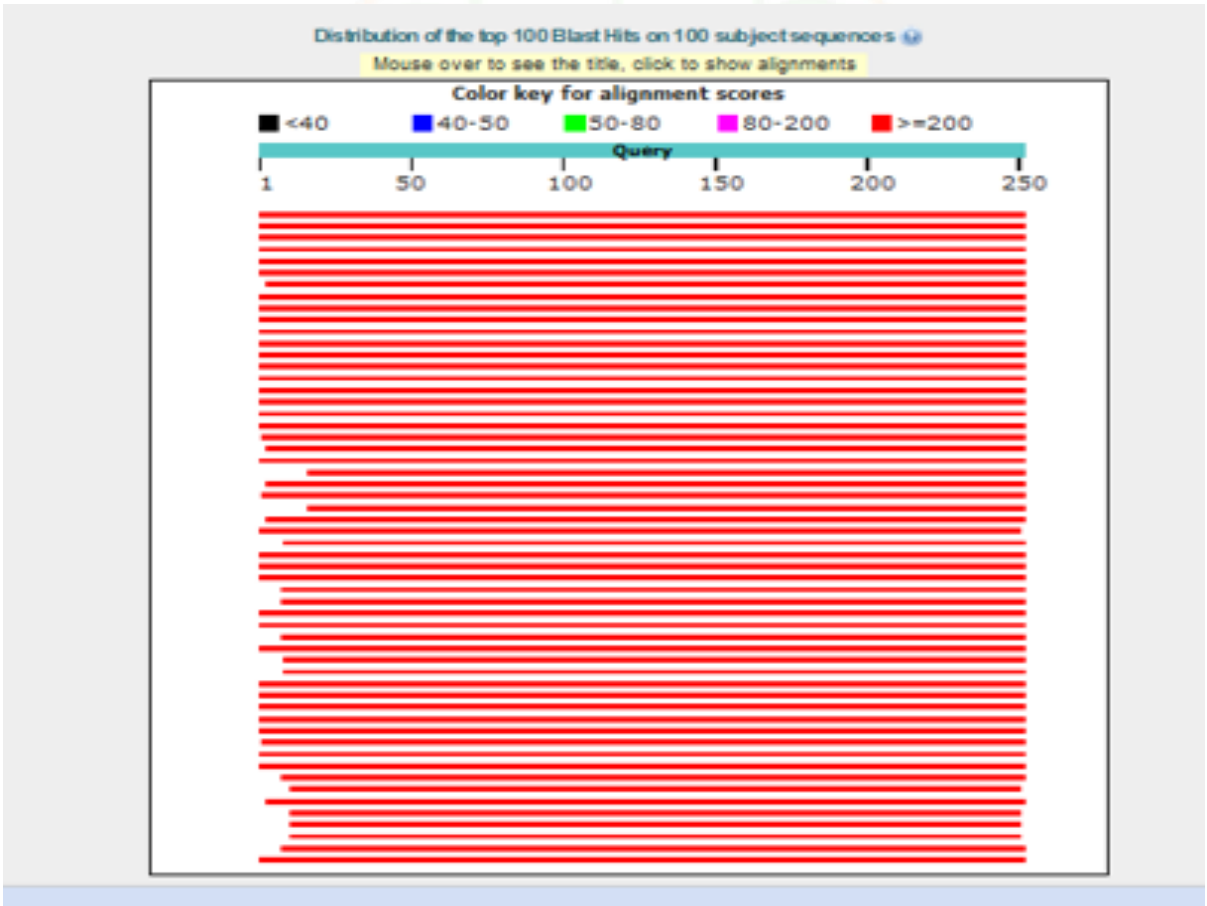
				Cosgrove (2002)
LeEXPA1	a-Expansin	Expression analysis	Proposed to be involved in fruit softening	Rose et al. (1997, 2000)
LeEXP1	a-Expansin	Overexpression and Suppression	Overexpression of the gene resulted in softer fruits while its suppression produced firmer fruits in transgenic tomatoes	Brummell et al. (1999)
LeEXPA8	a-Expansin	mRNA expression analysis	Thought to influence germination since it is expressed in germinating seeds only and appears to be involved during the initial elongation of the radicle	Chen et al. (2001)
LeEXPA10	a-Expansin	mRNA expression analysis	Thought to influence germination as well as seed development	Chen et al. (2001)
SlExp1	a-Expansin	Knockout	Increased fruit firmness	Minoia et al. (2015)
OsEXPA1	a-Expansin	Expression analysis	Thought to influence coleoptile and internode development	Cho and Kende (1997b)
OsEXPA4	a-Expansin	Over expression Antisense (RNAi)	Pleiotropic phenotypes in plant height, leaf number, flowering time and seed set as well as enhanced coleoptile growth Shorter plants, decreased coleoptile and mesocotyl lengths	Choi et al. (2003) Zou et al. (2015)
OsEXPA8	a-Expansin	Over expression	Increased root mass, number and size of leaves as well as plant height	Ma et al. (2013)
OsEXPA17	a-Expansin	Over expression	Influenced rice root development	Yu et al. (2011)
DzEXP1	a-Expansin	Expression analysis	Thought to be involved in fruit/pulp softening and peel dehiscence	Palapol et al. (2015)
NtEXPA5	a-Expansin	Over expression	Increased organ size especially the leaves and the stem	Kuluev et al. (2013)
DzEXP2	a-Expansin	Expression analysis	Thought to be involved in fruit/pulp softening as well as peel dehiscence	Palapol et al. 2015)
FaExp2	a-Expansin	Expression analysis	Thought to take part in cell wall polymer disassembly during fruit ripening	Civello et al. (1999)
MaExp1	a-Expansin	Over expression	Thought to affect banana ripening	Asif et al. (2014)
PpEXP1	a-Expansin	Over expression	Enhanced germination and abiotic stresses tolerance	Xu et al. (2014)
RhEXPA4	a-Expansin	Overexpression Overexpression and silencing	Higher germination percentage; increased lateral root formation and modified leaves	Lu¨ et al. (2013) Dai et al. (2012)
GmEXP1	a-Expansin	Over expression	Affected expansion and dehydration tolerance of rose petals Accelerated root growth	Lee et al. (2003)
GbEXPATR	a-Expansin	Over expression	Enhanced root hair development in transgenic Arabidopsis	Li et al. (2015b)
IbEXP1	a-Expansin	Over expression	More rosette leaves	Bae et al. (2014)
PnEXPA1	a-Expansin	Over expression	Large plant cells, larger leaves and longer stems	Kuluev et al. (2012)
CsEXPA1	a-Expansin	Over expression	Initiated development of the leaf primordium	Pien et al. (2001)
AtEXPB1	b-Expansin	Over expression	significantly longer petioles under normal growth conditions	Kwon et al. (2008)
AtEXPB5	b-Expansin	Expression profile analyses	Thought to soften the cell wall of the stigma	Mollet et al. (2013)
OsEXPB2	b-Expansin	Expression analysis Silenced	Thought to influence root hair and internodes development Confirmed the earlier suggested role as shown by physiological changes including reduced root and leaf sizes	Cho and Kende (1997b) Zou et al. (2015)
OsEXPB3	b-Expansin	Expression analysis	Thought to be involved in internode elongation as well as root development	Cho and Kende (1997b; Lee and Kende (2001)
OsEXPB4	b-Expansin	Expression analysis	mRNA accumulation correlated well with internode elongation	Lee and Kende (2001)
OsEXPB6	b-Expansin	Expression analysis	mRNA accumulation correlated well with internode elongation	Lee and Kende (2001)
OsEXPB11	b-Expansin	Expression analysis	mRNA accumulation correlated well with internode elongation	Lee and Kende (2001)
GmEXPB2	b-Expansin	Over expression	Enhanced overall plant growth, higher root cell division and elongation. Enhanced phosphorus uptake	Guo et al. (2011)
GmEXPB2	b-Expansin	Over expression	Increase in phosphorus efficiency	Zhou et al. (2014)
TaEXPB23	b-Expansin	Over expression	Improved tolerance of transgenic tobacco plants to oxidative stress Overexpressors performed better under drought. They showed enhanced root growth and water stress tolerance	Han et al. (2015) Li et al. (2015a)
TaEXPB23	b-Expansin	Over expression	Longer internodes, larger leaf blades, more leaves, more roots	Xing et al. (2009)

IrHC2019

HvEXPB1	b-Expansin	Promoter deletion	Shown to influence root hair formation	Won et al. (2010)
AtEXLA2	Expansin like A	Over expression	Longer roots which were significantly longer than the wild type roots	Boron et al. (2015)
AtEXPLA2	Expansin like A	Overexpression and mutant lines	Reduced EXLA2 transcript levels enhanced resistance to necrotrophic pathogens (*Botrytis cinerea*; *Alternaria brassicicola*)	Abuqamar et al. (2013)

Table 2. BLAST expansin gene in peach plant using the NCBI





IrHC2019

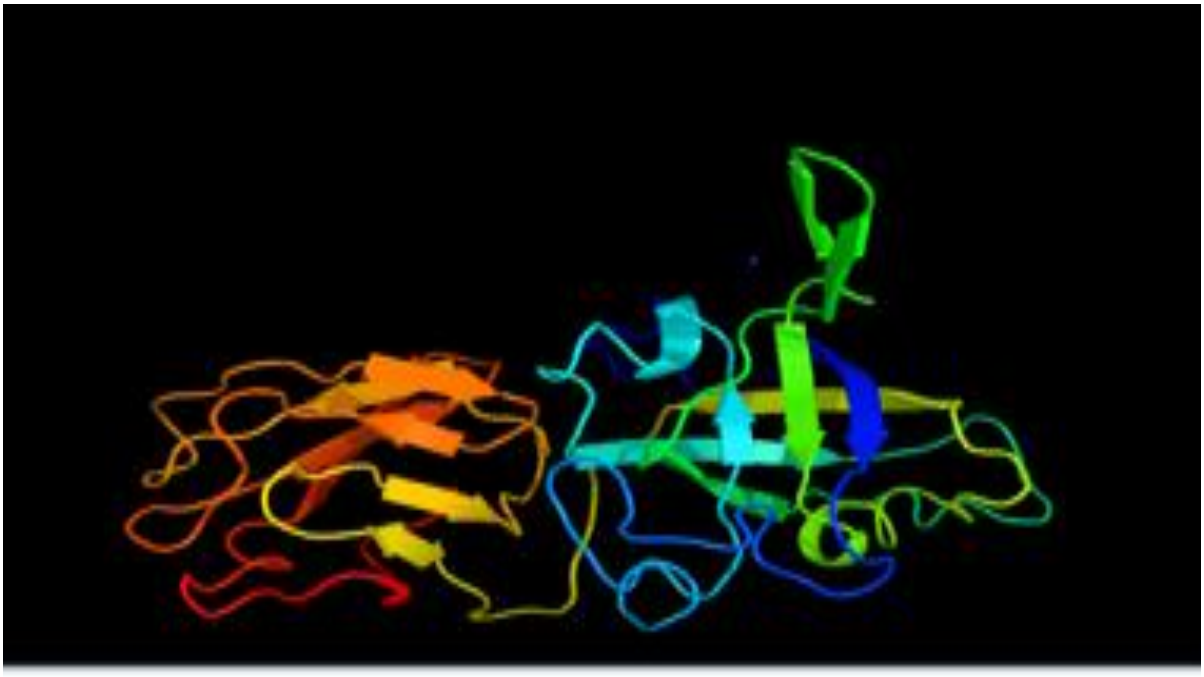


Figure 1. Protein-protein interaction network analysis of expansin using STRING 9.0 in *Arabidopsis thaliana*

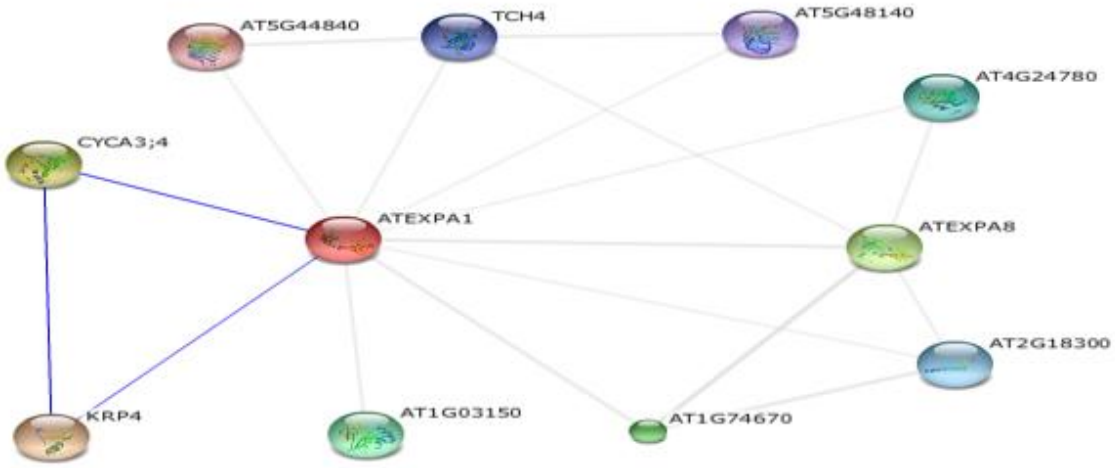


Figure 2. Protein-protein interaction network analysis of expansin using STRING 9.0 in *Arabidopsis thaliana*

Hormones and the environment play a crucial role on the growth and development of plants. Ethylene, a ripening hormone, influences the transcription level of LeEXPA1, a tomato expansin and there is a positive correlation between LeEXPA1 level and tomato fruit softening (Rose et al. 1997). It is thought that through the reported action of expansins on cell wall, this ripening-regulated expansin expression is likely to contribute to cell wall polymer disassembly which results in fruit softening by increasing access of specific cell wall polymers to hydrolase action (Rose and Bennett 1999). The role of expansins on fruit ripening has been recently endorsed by Minoia et al. (2015) who concurred with the idea that the expansins that are highly expressed during tomato fruit ripening contribute to the fruit softening. Minoia et al. (2015) demonstrated that mutations in a-expansin SlExp1 gene increased fruit firmness. They reported a 41 and 46 % fruit firmness enhancement in Slexp1-6 and Slexp1-7 mutant lines, respectively as compared to the control plants.

REFERENCES

IrHC2019

Cosgrove, D.J. 2015. Plant expansins: diversity and interactions with plant cell walls. Current opinion in plant biology, 25:162–172.

Choi. D., Kim, .J.H. and Lee, Y. 2008. Expansins in plant development. Advances in Botanical Research, 47(08):47–97.

Dagostino, D., M. Aversano, and M. L. Chiusano, 2005. ParPEST: A pipeline for EST data analysis based on parallel Computing. BMC Bioinformatics 6: 1-9.

Fukuda, H. 2014. Plant cell wall patterning and cell shape. Wiley, Hoboken.

Kende, H., Bradford, K.J., Brummell, D.a., Cho, H.T., Cosgrove, D.J., Fleming, A.J, and Voesenek, L.A.C.J. 2004. Nomenclature for members of the expansin superfamily of genes and proteins. Plant molecular biology, 55(3):311–314.

Minoia, S., Boualem, A., Marcel, F., Troadec, C., Quemener, B,, Cellini, F, and Bendahmane, A. 2015. Induced mutations in tomato SlExp1 alter cell wall metabolism and delay fruit softening. Plant science, 242:1–8

Rose, J.K.C, and Bennett, A.B. 1999. Cooperative disassembly of the cellulose-xyloglucan network of plant cell walls: parallels between cell expansion and fruit ripening. Trends in plant science, 4(5):176–183

Rose JK, Lee, H.H, Bennett, A.B. 1997. Expression of a divergent expansin gene is fruit-specific and ripening-regulated. Proceedings of the National Academy of Sciences, 94(11):5955–5960.

Vassilev, D., Leunissen, J., Atanassov, A., Nenov, A. and Dimov, G., 2005. Application of bioinformatics in plant breeding. Biotechnology & Biotechnological Equipment, 19(3): 139-152.

11th IrHC 2019
IrHC2019