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EXPRESSION PATTERNS OF THE EXPANSIN GENE FAMILY IN BARLEY UNDER DROUGHT STRESS

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ABSTRACT

EXPRESSION PATTERNS OF THE EXPANSIN GENE FAMILY IN BARLEY UNDER DROUGHT STRESS

Plant cell wall expansion is essential to many physiological processes of plants including growth and stress response. The expansin family proteins have a core and unique role in this process by their non-catalytic cell wall relaxing function which allows expansion of the plant cell walls. Discovery of these proteins is relatively recent and have a great potential for better understanding the cell wall expansion mechanism in plants and drought tolerant plant breeding studies. Literature on the barley expansins by their identification and their roles in drought response mechanisms are insufficient, especially when the economic importance of barley is considered.

In this study the aim was, identification of expansin genes in barley genome and investigation of their relationship with drought response mechanism in root tissues. Investigation of expansin family genes in barley genome with using homologies of known expansin sequences in closely related plants and conserved domain search have resulted in identification of 78 barley expansin genes on barley genome, which were classified under subgroups of expansin α (39 genes), expansin β (33 genes) and Expansin like-α (six genes) with phylogenetic analysis. Expression analysis of the identified genes on different tissues and conditions by analysis of whole expression sequencing (WES) data from several studies and differential expression analysis of selected five expansin genes on 6th and 12th days of drought stress treatment were done in order to determine relationship between the expression of expansins and droughts stress. WES data showed that barley expansins are expressed in many different tissues and their expression in root were significantly affected by stresses including drought, salinity and water deficiency with a significant variance between the genes and subgroups. Differential expression analysis showed the selected five genes were up regulated by drought stress. Combined with the findings of WES data and differential expression analysis concludes that the expression of barley expansins is regulated by drought response mechanism and barley expansins are involved with the drought response in barley.

ÖZET

ARPADA KURAKLIK STRESİ ALTINDA EXPANSİN GEN AİLESİNİN EKSPRESYON PATTERNİ

Bitkilerde hücre duvarı genişlemesi bitki büyümesi ve streslere karşı tepki dahi olmak üzere birçok fizyolojik süreç için vazgeçilmez bir öneme sahiptir. Expansin ailesine ait proteinler katalitik olmayan hücre duvarı gevşetme fonksiyonları ile hücre duvarı genişlemesinde temel bir role sahiptirler. Expansin proteinlerinin nispeten yakın bir zamanda gerçekleşen keşfi, bitki hücre duvarı genişlemesi mekanizmasının aydınlatılması ve kuraklık stresine dirençli bitki ıslahı konularında için büyük bir potansiyel yaratmıştır. Ekonomik önemi göze alındığında , arpa expansinleri üzerine literatür mevcut arpa expansinlerinin belirlenmesi ve expansinlerin arpada kuraklık tepki mekanizmasındaki rolleri hakkındaki oldukça yetersizdir.

Bu çalışmada hedef, arpa expansin genlerinin tespiti ve arpa kök dokularında kuraklık stresi ile olan ilişkilerinin saptanmasıydı. Arpa genomu üzerinde benzer bitkilerde daha önce tespit edilmiş expansin genlerinin homolojileri ve expansin genlerinin evrimsel olarak korunmuş sekans alanları kullanılarak yapılan incelemede 78 expansin geni tespit edildi. Bulunan genler expansin gen ailesinin alt gurupları olan expansin α (39 gen), expansin β (33 gen) ve expansin benzeri-α (altı gen) altında filogenetik analiz ile sınıflandırıldı. Tespit edilen genlerinin farklı doku ve koşullarda ekspresyonu literatürden elde edilen birkaç tüm ekspresyon sekanslama (TES) analizi verisi ile analiz edildi ve tespit edilen genlerin arasından seçilen beş genin arpa köklerinde kuraklık stresi uygulamasının 6. ve 12. günlerinde ekspresyonlarının değişiminin analizi yapıldı. TES verileri arpa expansin genlerinin birçok dokuda ekspresyon edildiği ve kök dokusunda kuraklık, tuz ve düşük su potansiyeli stresleri altında genler ve alt guruplar arasında büyük bir değişkenlik ile ekspresyonlarının etkilendiği gözlendi. Kuraklık altında seçilen beş genin ekspresyon analizi sonuçları ise seçilen genlerin ekspresyonunun kuraklık stresi altında artığını gösterdi. TES verileri ve ekspresyon değişim analizi sonuçları, arpada expansin genlerinin ekspresyonlarının kuraklık tepki mekanizması tarafından kontrol edildiğini gösterdi.

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LIST OF ABBREVIATIONS

aa	Amino acid
BLAST	Basic local alignment search tool
bp	Base pair
C domain	Cellulose binding domain
C Domain	C-terminus polysaccharide-binding domain
CAR15	Bracts removed grain 15 days post anthesis days post anthesis
CAR5	Bracts removed grain 5 days post anthesis
cDNA	Complementary DNA
CESA	Cellulose synthase
Cq	Cycle of quantitification
CSC	Cellulose synthase complex
CSL	Cellulose synthesize-like
DF	Degree of freedom
DPA	Days post anthesis
DPBB	Double-psi beta-barrel
EMB	Embryos at 4 day dissected from germinating grains
EPI	Epidermis at 4 weeks
ETI	Etiolated 10 day old seedling
EXLA	β -Expansin
EXLB	Expansin-like B
EXPA	α-Expansin
EXPB	Expansin-like A
F	Forward strand
FPKM	Fragments per kilobase of transcript per million mapped reads
GaLA	α-14-D-Galacturonic acids
GAX	Glucoarabinoxylan
GH	Glycoside hydrolases
GH45	Glycoside hydrolase family 45
GTs	Glycosyl transferases
HG	Homogalactoronans
INF1	Young inflorescences at 5mm

INF2	Inflorescences between 1-1.5cm
KOR	Korrigan
LEA	Shoot from the seedlings at 10cm stage
LEM	Lemma 6 weeks post anthesis
LOD	Lodicule 6 weeks post anthesis post anthesis
M	Million
ME	Mixed element
N	Number of replicates
nM	Nano mol
NOD	Developing tillers at six leaf stage
PA	Post anthesis
PAL	Palea at 6 weeks post anthesis
PEG	Polyethylene glycol
PhD	Doctor of philosophy
PME	Pectin methyl transferases
PRRs	Pattern recognition receptors
qPCR	Quantitative polymerase chain reaction
R	Reverse strand
RAC	Rachis at 5 weeks post anthesis
RG-I	Rhamnogalacturonans I
RG-II	Rhamnogalacturonans II
RNA-seq	RNA sequence
ROO	Root at 4 weeks
ROO2	Roots from the seedlings at 10cm shoot stage
ROS	Reactive oxygen species
RWC	Relative water content
SD	Standard deviation
SEN	Senescing leaf at 2 months
Tm	Melting temperature
WES	Whole expression sequencing
XTHs	Endotransglycosylase/hydrolases
XyG	Xyloglucan

1. INTRODUCTION

1.1. Barley

Hordeum vulgare L or barley is a monocot that is a member of *Poaceae* (grasses) family as well as wheat and rye [1]. Domestication of the wild barley species is being estimated to be achieved as early as 8000 BC in the region called the Fertile Crescent that includes the borders of today’s trans Jordanian Basin, Syria, Iraq, South-eastern Türkiye and south-western Iran [2]. Barley has a high number of wild and domesticated variants which are cultivated around the world with different ploidies. Barley is a very important crop species that is mainly used as animal feed and for brewing. The typical seeding of the barley for the northern hemisphere is mid-October to mid-November and the harvest is usually done around early spring [3].

Barley is one of the most important crops for the world and Türkiye by having the 4th rank among cereals after wheat, rice and maize by production quantity. With its high adaptability against temperature and drought stress, is cultivated in many regions of Türkiye especially in drought belt regions [4]. Global production of barley was 151,6 M tons, of which Türkiye produced around 5,7% of it with 7,4 M tons during the 2022/2023 season [5]. Despite Türkiye being a major producer by having 7,6 % of the global barley production by area in 2023 [5], seasonal variations on precipitation and temperature have impacted the production significantly [6] and the expected increase of the impact of global warming elevates the risks on production yields [7]. Geopolitical risks on imports of barley and agricultural inputs caused by conflicts such as the Russia-Ukraine War which both nations are important exporters of barley for Türkiye and sources for critical agricultural inputs like energy and fertilizers [8] also possesses significant risks on barley prices and availability nationally. Increasing national consumption trend [6] and mentioned risks on the productivity requires incentives on increasing production yields. Genomic studies and modern breeding techniques are important for achieving this aim [9] and supporting projects for improving yield of barley production especially against a major restraint factor drought stress is economically and strategically important.

1.1.1. Barley and Drought Stress

Drought stress is a major factor limiting the production yields of the crop species, especially the crops that are grown usually without watering such as barley. Many studies have shown that the physiological impact of drought stress on barley results in significantly lowered seed quality (by lower germination rates and lower dry weight on average) [10], lowered photosynthesis rate, total dry weight [11], extended grain filling period, and lowered grain yield [12]. These impacts of drought stress on barley plants results in significant economic damages on barley production globally. For example the average production yield of barley was lowered approximately 30% in Spain in years which had below 20% or more rainfall than average. Compared to the loss of production yields being significantly higher in Spain than Germany which had more soil water availability even in same lower rainfall. These findings indicate in semi-arid areas which the barley is more commonly produced and usually food security is under more risk the drought stress and its impact on food production is severe [13].

1.2. Drought Stress

Growth, biomass accumulation, reproduction and other essential functions of plants are effected by environmental factors throughout their life cycle. Biotic agents, including pathogens (bacteria, fungi, viruses and nematodes) and pests, beside the abiotic elements including temperature, water availability, salinity, toxicity and excessive light, have major impact on plant physiology and crop yields. Both the biotic and abiotic stresses are usually impacting plants in different combinations in nature and plants have obtained various physiological and biochemical response mechanisms through evolution to increase their tolerance against these stresses [14].

Abiotic stresses are a major cause of agricultural losses globally and the stress factor with the biggest global impact is drought. The negative impact caused by drought stress has been elevated and expected to be increased in greater scales because of climate change. In order to provide the global community with food security which has increasing demand for agricultural products, increasing population and changing consumption habits, there is an important and urgent need for solutions for reducing the impact of drought stress on agricultural production [15]. This is especially important for cereal production as they are humanity’s most important calorie sources [16] and are significantly impacted by the effects of global climate change [17–19]. Considering the economic and societal importance of cereals, understanding the drought resistance mechanisms in cereals and breeding crops that have better tolerance against drought stress is an important task for plant scientists and breeders.

Plants inability to absorb enough water for their survival by reasons like low precipitation or high soil salinity can be defined as drought. The stress that drought exerts on plants has multiple physiological impacts that result in limiting biomass growth and reproduction capability, and finally lead to death of the organism if plants are not able to absorb water by a period that their drought tolerance cannot mitigate the stress. Loss of water potential by the unavailability of water results in lower photosynthesis capability by decreased stomata conductance, decreased enzyme activity, decreased nutrient absorption from roots and loss of turgor pressure which is required for cellular growth. Plants have adopted various response mechanisms for drought tolerance including adjustment of their life cycles by staying dormant through seasons with low precipitation, accumulation of solubles in the cytoplasm for preserving turgor pressure, increasing their antioxidant activity and prioritizing root development for accessing water in the lower soil. These adoptions create variability across plant species by their tolerance to drought stress [20].

1.3. Plant Cell Wall

1.3.1. Structure of the Plant Cell Wall

Plant cell walls are constituted primarily by multi-layer matrices of cellulose microfibrils and non-cellulosic polysaccharides beside structural glycoproteins that provide plant cell walls and the overall organism with a durable and flexible skeleton-like support. Plant cell walls by type are divided into two classes, which are primary cell walls and secondary plant cell walls. Even though plant cell walls are classified into the two mentioned types, plant cell walls vary extensively between the specialization of the cell and the species, and primary and secondary cell wall definition in reality represents a spectrum for plant cell walls by being the radical ends of the spectrum [21]. Primary and secondary cell walls have the same matrix structure of cellulose microfibrils and non-cellulosic polysaccharides with different compositions of components according to their difference in their functions [22]. By weight, ~90% of the plant cell walls are made up of polysaccharides and ~10% proteins. Plant cell walls are approximately 50% of the plants biomasses even though that ratio could vary among species and tissues [23].

Even though plant cell walls are discovered in the seventeenth century with some of the first uses of microscopy, understanding of their definite composition and structure has been shaped rather recently. With new findings of microscopy and chemical analyses, the current accepted model of gel like matrices of cellulose microfibrils that are cross linked by a cellulose–xyloglucan network has been shaped around the last decade of the 20th century [22] as can be observed on Figure 1.1. .

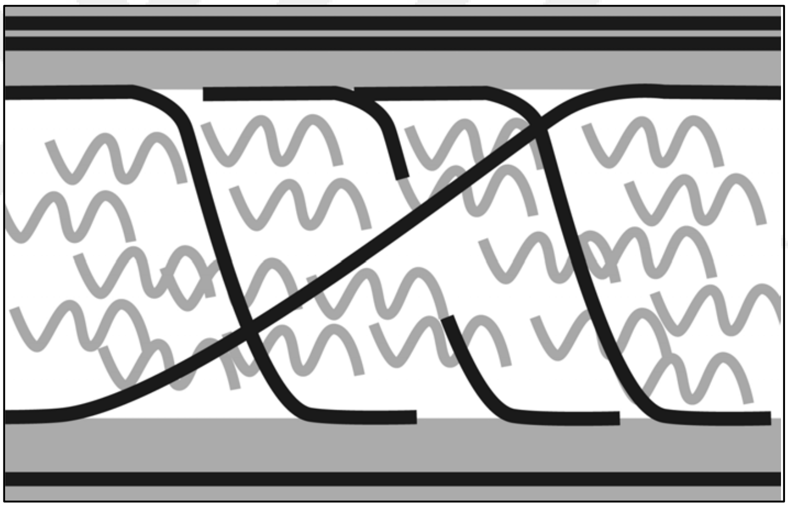


Figure 1.1. Representation of general structural model for the non-lignified plant cell walls. Xyloglucans (shown in solid black lines) cross-linked to the cellulose microfibrils (shown in grey lines or pectic polysaccharides (shown in grey wavy lines) as shown in the figure [22].

More recent studies have resulted in a change of the commonly accepted networking model for the cell wall to the “biomechanical hotspot” where the strongest force binding the cell wall is cellulose-cellulose binding with xyloglucans as the mediated amalgams that binding the cellulose fibrils to each other. The “hotspots” indicated by the model is the amalgam of cellulose-xyloglucan-cellulose as can be observed in Figure 1.2. These hotspots regions provide sturdy connections that are relatively resistant against catalytic degradation by the hydrophobicity of the amalgam, which allows them to be the main load bearing unit of connection in the cell walls. This model is supported by findings such as requirement of endoglucanases activity aiming both cellulose and xyloglucans for relaxation of cell walls and homogenously parallel orientation of cellulose microfibrils caused by lacking of xyloglucan synthesis. Also the hotspots of the celluloses connection between different microfibrils allows a non-homogenously parallel cellulose network that is important for cell growth direction regulation. Even though the model prioritizes cellulose-xyloglucan interaction, pectins are also taking role in both providing interfibril links between cellulose microfibrils and establishing the gel like matrix by their pectin-pectin interactions that is important for the cell wall structure [24].

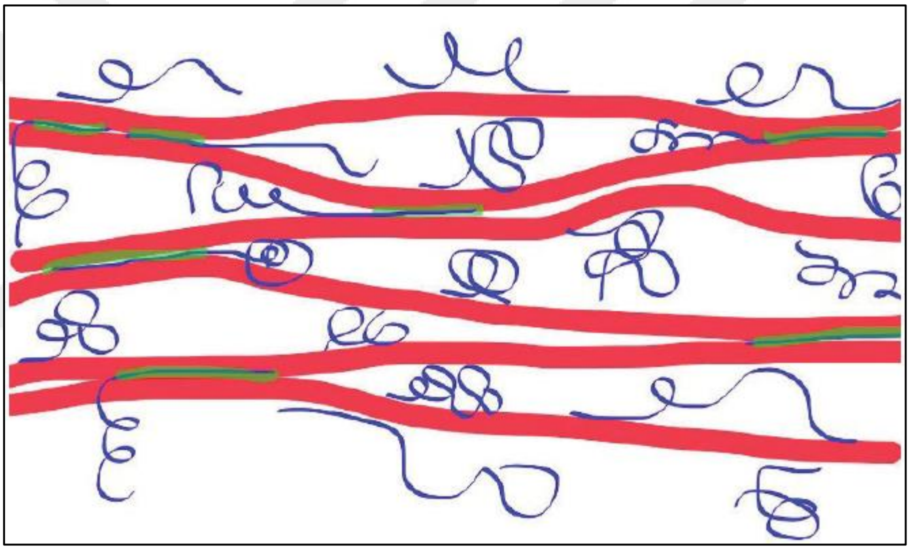


Figure 1.2. Representation of the biomechanical hotspots model for cell wall structure where the red lines represent cellulose microfibrils, green lines represent amalgams of xyloglucan-cellulose binding the cellulose microfibrils and the blue lines represent pectins [24].

Plant cell walls have major importance for mechanical strength (by providing resistance against mechanical factors impacting plants in the environment like gravity and wind), plant cellular growth, cell differentiation [21], resistance to pathogens and pests and regulation of plant/microbe interaction [25] beside many other. Plant cell walls are also a very important source for many industries including textiles, paper manufacture, and energy (as biofuel source and timber for fuel) and construction. Also, they have a big impact on plant sourced human food and animal feed by affecting their texture, processing and nutritional value [23].

Plant cell walls are an important research area of biotechnology studies because of their importance to the mentioned sectors and their major role in plant physiology.

Meristematic cells which are the undifferentiated plant cells that the other specialized plant cells are derived from, are surrounded by a non-lignified cell wall that is usually around 0.1-1 μm thick. This type of cell wall that is present in all plant cells is sufficient to provide enough resistance to the turgor pressure that is required in the growing tissues of the plants, is a major element of the apoplast with the pectic rich middle lamella which allows cells the exchange of air and nutrients is called the primary cell wall. In higher plants, the primary walls are constituted of macromolecules which are by dry weight up to ~90% polysaccharides, 2-10% glycoproteins, less than 2% phenolic esters, 1-5% bound minerals beside associated plant cell wall enzymes. These components are present in a gel like matrix which can have up to 70% water as volume. Composition, thickness and extensibility of the primary cell wall can vary between the species of plants, specialization of the cells and growth stage [26].

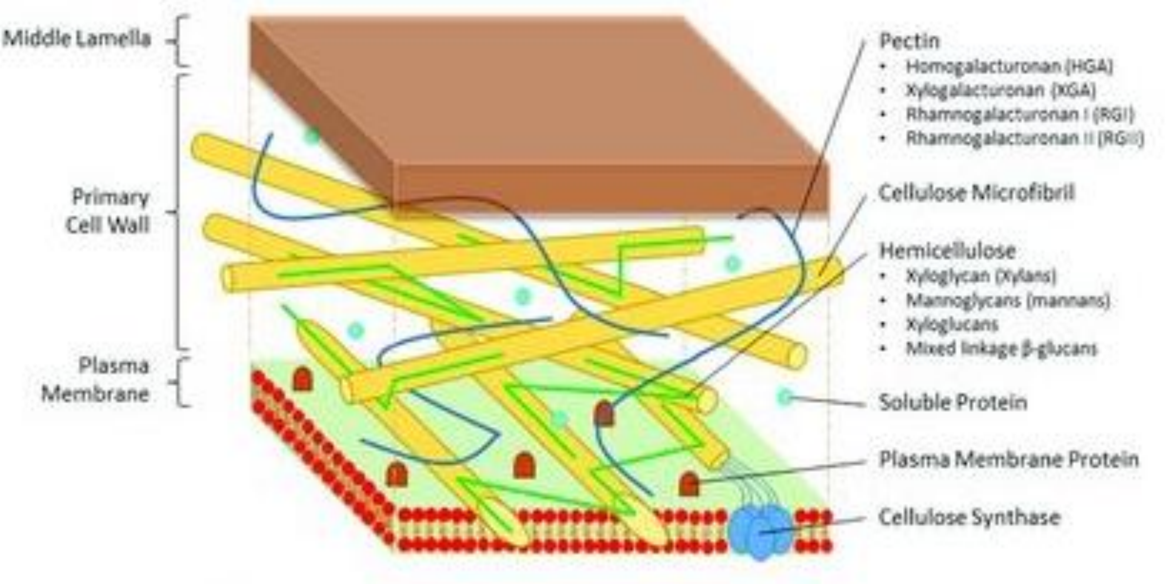


Figure 1.3. Representation of the primary cell wall with the polysaccharides and adjacent elements has been shown on the figure [27].

Primary cell walls are mainly separated into two sub groups of Type I and Type II, even though this classification is not covering the type of plant primary cell walls. Type I primary cell walls which are found in dicots, non-commelinid monocots and gymnosperms, have a cellulosic matrix that is mainly constituted by xyloglucans, galactomanans, and other non-cellulosic polysaccharides including pectin. Type II cell walls are observed in commelinid monocots which includes the *Poaceae*. They have a similar composition with Type I but with the glucoarabinoxylans (GAXs) and 1,3 and 1,4 β-d glucan as the prevalent hemicelluloses [23,26]. Barley, as with other members of *Poaceae*, has Type II primary cell walls [28].

Secondary cell walls in plants are formed after the expansion of the primary cell wall by cellular growth is completed, and functions as a sturdier layer that provides the cells and the tissues that are required for higher physical resistance or structure required for water transport in xylem. Not all plant cells have secondary cell walls and there are different types of secondary cell walls that varies among tissues and species of plants. Secondary cell walls are positioned between the primary cell wall and the plasma membrane as shown at Figure 1.4. and mainly divided into two groups by being lignified or not [22,23].

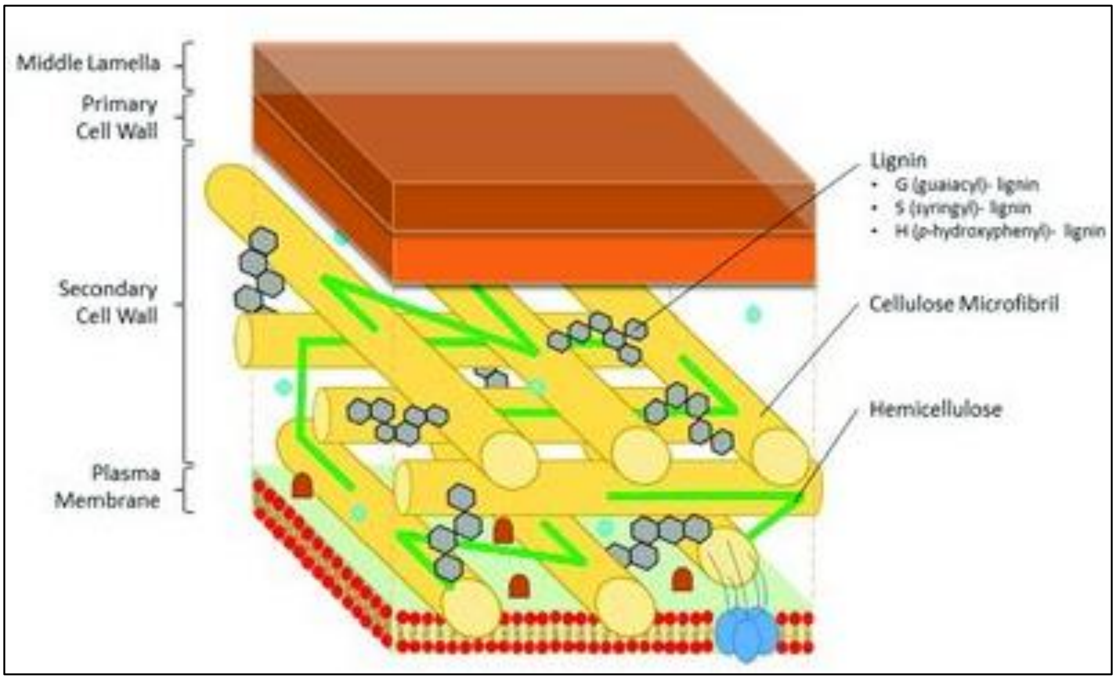


Figure 1.4. Representation of the plant secondary cell wall with the polysaccharides and adjacent elements has been shown on the figure [27].

Thick non-lignified secondary plant cell walls have been observed in different species of plants and their role as nutrient storage in endosperms of some species also been discovered. Lignified secondary cell walls are similar to non-lignified secondary cell walls and primary cell walls, where that are composed of a matrix of cellulose fibres, non-cellulosic polysaccharides and proteins but have lignin deposited in the cell wall matrix. Because of their overall ratio compared to the non-lignified secondary cell walls by prevalence and the extensive role of lignified secondary cell walls in plant dry mass and functions of important plant tissues (sclerenchyma and xylem), the type of secondary cell wall that is generally taken as the model is the lignified secondary cell walls [22]. Lignification of the secondary cell walls by deposition of phenolic lignin in highly ordered non-cellulosic polysaccharides and the dehydration of the cell wall matrix by highly hydrophobic lignin polymers results in a very resilient structure that offers extensive physical resistance and resistance against enzymatic digestion especially considering that the thickness of this type of cell wall can be up to several microns [23].

Cellulose is the polysaccharide of β (1→4) bound glucoses that is the essential polysaccharide of the plant cell walls. The chain of cellulose that is homogenously β (1→4) linked glucoses that has the 180˚ lowest energy conformation can get up to 15.000 nm in length. Hydrogen bonds are very important for celluloses by determining their interaction strength with other cellulose chains and intramolecular structure. The type I celluloses which have hydrogen bonds between the 6th C of a glucose of the chain and 3rd C of the neighbour has less densely stacked compared to type II celluloses which forms hydrogen bonds to other cellulose chains by their 6th C rather than their neighbour glucose which makes them less reactive. Type I celluloses by alkalization can be irreversibly turned into the less reactive type II celluloses crystalline form which provides great stability to the polysaccharides by limiting enzyme interactions of non-surface areas of the cellulose crystals [29].

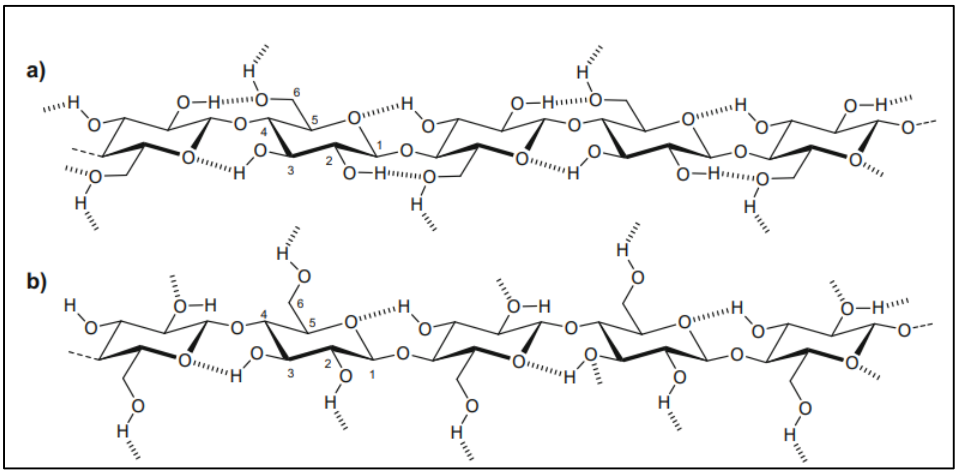


Figure 1.5. Representation of (a) cellulose I and (b) cellulose II chains [29].

In plant cell walls the crystalized celluloses are formed as approximately 100 nm long 1.5-3.5 nm elementary fibrils, which are again bundled into microfibrils during their biosynthesis and are coated with amorphous celluloses that are hemicellulose covered that are required for the network formation of the cellulose microfibrils [29].

Cellulose synthase (CESA) enzymes are responsible with the synthesis of (1,4)-β-D-glucan chain, which is the main component of the cellulose. CESA form hexameric rosette subunits which are again organized hexameric rosette cellulose synthase complex (CSC) as can be seen at Figure 1.6. [30].

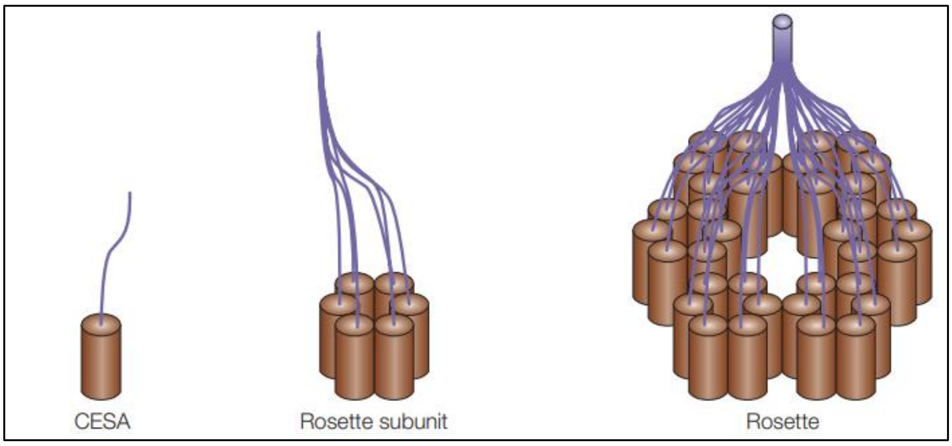


Figure 1.6. Representation of the cellulose synthesis elements which are shown by the given order of Cellulose synthase (CESA) enzyme, rosette subunit and the cellulose synthase complex (CSC) [30].

There are many types of CESA and the CESA configuration of the subunits of CSC varies between the species, tissues and types of cell walls (primary and secondary). CSC is located on the cellular membrane and is attached to microtubules that are docked to the inner side of the membrane. With using sterol glucosides which are lipids in the membrane as the initial acceptor of the chain of (1,4)-β-D-glucan, CESA in the CSC synthesize (1,4)-β-D-glucan chains in to the extracellular space. These (1,4)-β-D-glucan chains crystalize into cellulose microfibrils which consist of 12-36 (1,4)-β-D-glucan chains. The polymerization of the cellulose microfibrils moves the CSC which its direction of the movement is regulated by the microtubules inside the cells. Korrigan (KOR) endoglucanases are also important enzymes for the cellulose synthesis. By cutting the sterol glucoside to glucose link and trimming non correctly bound glucoses, they allow correct formation of the final cellulose microfibrils [30,31].

The term “hemicelluloses” defines a group of heterogeneous non-cellulosic polysaccharides in the plant cell wall structures that are non-solubilized by chelators or hot water. Members of this polysaccharide group has β (1→4) glycan (glucose, mannose or xylose) backbones with equatorial configuration. The term ‘hemicelluloses’ is commonly in use for denomination of this group, rather than ‘non-cellulosic β (1→4) glycans’ even though etymologically it is not accurate. Members of this polysaccharide group includes xyloglucans, xylans, mannans, and glucomannans. They act as a matrix formation agent by creating various bonds between cellulose fibers, pectins and with each other in plant cell walls [32].

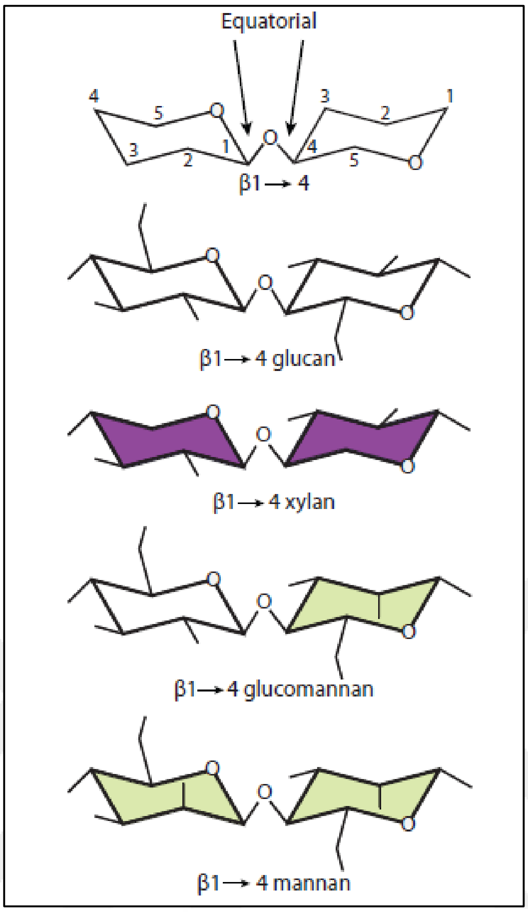


Figure 1.7. Representation of equatorial orientation of the hemicelluloses and the backbones of includes xyloglucans, xylans, glucomannans and mannans in given order [32].

Xyloglucans (XyGs) has been discovered in the plant cell walls of all plants and they are the most abundant hemicellulose type in primary cell walls of higher plants by having up to 20% of the plant cell wall biomass of meristematic cells primary cell walls except for grasses (which GAXs are). XyGs are very important polysaccharides for cell walls by their interactions with celluloses as the major joint that constitutes the cellulose network in cell walls and their interactions other cell wall polysaccharides. XyGs have repetitions of non-substituted glucans that are denoted as “G” and xylosyls which are denoted as “X” by residues that are attached to 2-O positions of backbones that are consisting β (1→4) bound of glucoses. The xylosyl are also can be substituted by galactose, arabinose and galactose-arabinoses that are respectively denoted as “L”, “S” and “F”. Most common repetitive units of XyGs are XXGG and XXXG in the plants even though there are high variations between species. Less substituted patterns that the XyGs have, their hydrophobicity increases. By using this difference of solubility caused by XyGs hydrophobicity, plant cell walls are tuned into required biophysical conditions required for the specific species and tissues at given time regarding to the cell wall elasticity and enzyme activity on the cell walls [32].

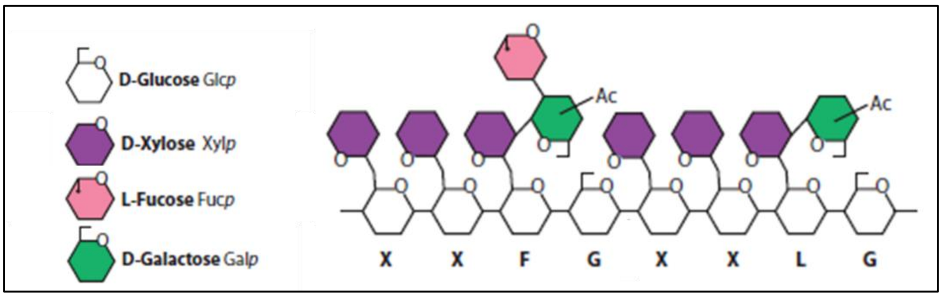


Figure 1.8. Representation of a xyloglucan chain ([32] with modifications).

Xylans are non-cellulosic β-(1→4) glucans, which have xylans in their backbones. Main type of xylans are glucuronxylans and GAXs. Glucuronxylans which are the dominant hemicellulose in dicot secondary cell walls and GAXs which are found in all plant cell walls except dicot secondary cell walls and are the most prevalent non-cellulosic polysaccharide in grass cell walls (both primary and secondary). Glucuronxylans have common substitutions of 4-O-methyl glucuronosyl residues and α-(1→2)-linked glucuronosyls and the substitutions are not paternal and are highly variable. In GAXs there are arabinose (mostly O-3 in meristemetic tissue) substitutions with commonly higher amounts of glucoronic acid substitutions which these substitutions are similarly to glucuronxylans are non-repetitive. Xylans are commonly acetylated by O-2 and O-3 positions of the xylan backbones and the acetylation rate is higher in dicot. Ferulate ester substitutions to O-5 of arabinose residues are also an important property of xylans, where they are more commonly associated with grass cell walls GAX but found also in other plants glucuronxylans [32]. Ability of dimerization between each other and ester linking to both xylans and lignins allow ferulation to be a method of constructing sturdy networks between xylans, and allows lignin to join the this network as incorporation to the secondary cell walls [33].

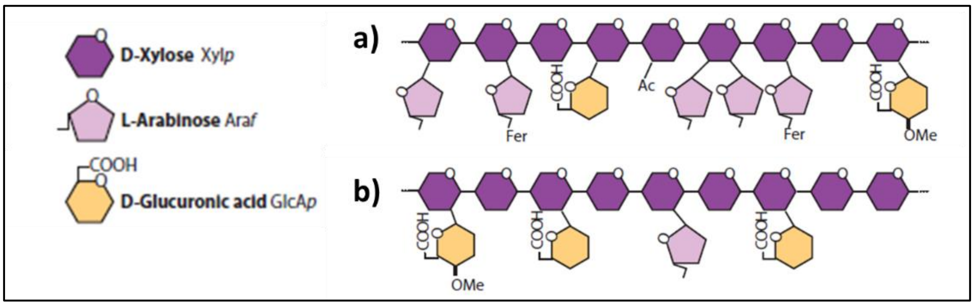


Figure 1.8. Representations of (a) glucoarabinoxylan (GAX) and (b) glucuronxylan chains ([32] with modifications).

Mannans are another group of β-(1→4) glycans in cell walls that contain mannoses in their backbones. If the backbones are homogenously constituted by mannoses the mannan polysaccharides are called homomannans and if they have glucoses beside mannoses they are called glucomannans. If the backbones of mannans have 6-O attached galactose side chains they are called either galactomanans or glucogalactomanans as could be seen in Figure. [34]. Mannans by their occurrence in plant cell walls by their types and amounts varies between species where they can be the major hemicellulose by prevalence as the case for galactoglucomanans in conifer secondary walls [32]. Mannans can function as a nutrient storage unit as they are in embryos of legumes beside their functions in cell walls [34].

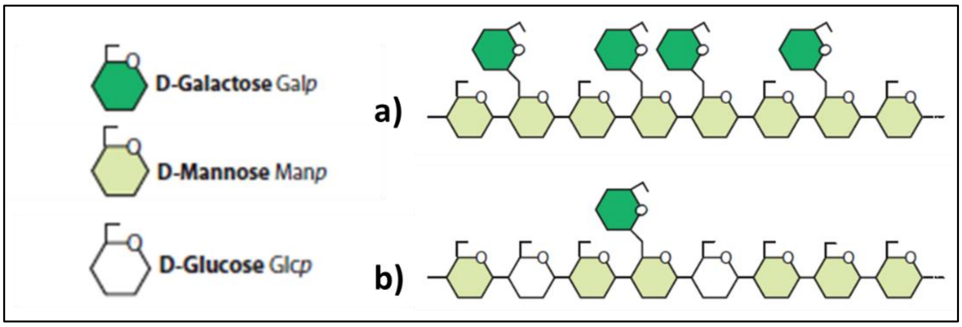


Figure 1.9. Representations of galactomannan (a) and galactoglucomannans (b) chains ([32] with modifications).

β-(1→3,1→4) glucans or the mixed lineage glucans are found in only *grasses* and are the most abundant hemicelliloses in plant cell walls of them and the their concentration is associated with the plant growth. The backbone of these polysaccharides are formed by 3 and 4 glucoses that are bound together by either β-(1→4) bond which keeps the chain linear or β-(1→3) bond which creates branching. The selection between the binding positions are not patterned and their differences result in different properties in plant cell wall solubility and viscosity [32,35].

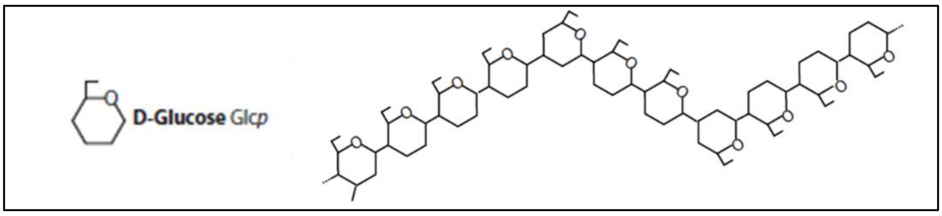


Figure 1.10. Representation of a β-(1→3,1→4) glucan chain ([32] with modifications).

Pectins are a very complex group of polysaccharides that is present in both primary and secondary plant cell walls [36]. The most complex polysaccharide group makes up around 15-25 % of the cell wall dry weight in Type I primary cell walls and have lower presence in Type II primary cell walls [26].

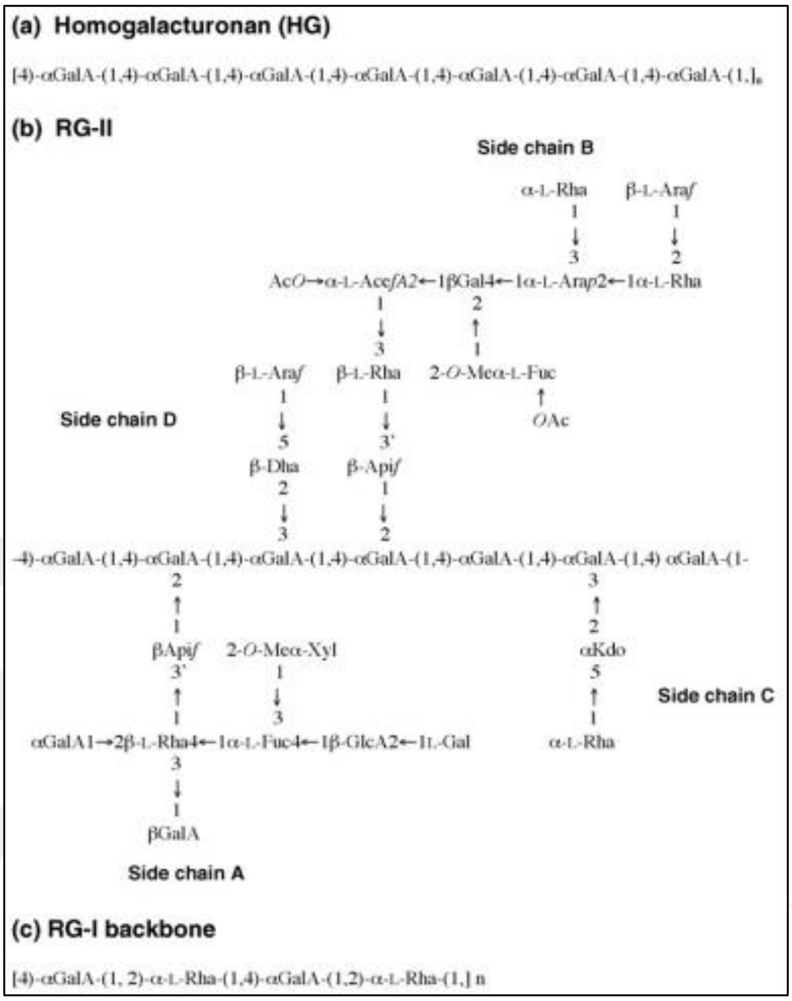


Figure 1.11. Representations of the main pectins which are (a) homogalactoronans (HG), (b) rhamnogalacturonans II (RG-II) and (c) rhamnogalacturonans II (RG-I) [36].

Main component of the pectins is galacturonic acids that are makes up most of the pectin backbones. The three main types of pectins are homogalactoronans (HG), rhamnogalacturonans I (RG-I), and rhamnogalacturonans II (RG-II). There are also xylogalacturonan and apiogalacturonan which are lesser formed pectins that are modified versions of HGs. HGs are simple polymers of α-1,4-D-galacturonic acids (GaLA) with various esterifications that are 72-100 units and are the most abundant pectins. RG-I is also a standalone chain of repeating GalA’s and α-1,2-L-rhamnoses, that are between 100-300 repetitions in length. RG-II is the branched version of HG that has side chains with great diversity that are bound to the 2nd and 3rd carbons of GalAs [37]. Pectins by their crosslinks between each other and with the other polysaccharides in the plant cell wall have an important role for creation of the polysaccharide matrix of the plant cell walls [36].

Biosynthesis of hemicelluloses and pectins is done at the Golgi and they are transferred to the cell wall within vesicles. Because of the heterogeneous nature of the non-cellulosic cell wall polysaccharides, many enzymes are involved in the complex formation mechanisms of these polysaccharides including glucan synthases that forms backbones of the polysaccharides and glycosyl transferases (GTs) like xylosyltransferase that adds xylosyl to the backbone as side chain at specific locations on XyGs [38]. CESA is a member of a bigger protein family of cellulose synthesize-like (CSL) enzymes. All members of CSL have β-glycotransferase domains but other CSL members unlike CESA, don't have zinc finger domains which gives it protein dimerization ability [39]. Different CSL members encodes different GTs that are involved with the synthesis of non-cellulosic polysaccharides and therefore regulates synthesis of vastly variable polysaccharides for the cell wall structure. Before their transfer to the cell wall these polysaccharides undergone post transcriptional modification processes and secreted to the extracellular matrix where they form their bonds with celluloses and other polysaccharides [40].

The major non-polysaccharide molecules in the plant cell walls are glycoproteins. There are many types of glycoproteins in the structure of plant cell walls that have different functions. Between these glycoproteins especially hydroxyproline-rich glycoproteins or extensins are important. These proteins have various crosslinks with the other cell wall elements and are important for their functions of positioning of the plant cells, initiation of the cell expansion, structural integrity and others [41,42].

1.3.2. Functions of the Plant Cell Wall

Mechanism of plant cellular growth is heavily integrated with the growth of the plant cellular wall. Regulation of the plant cell walls extensibility of the plant cells during cellular division is very important for cellular growth by allowing expansion of the cell into a selected direction which the sister cells will occupy after division and specialization of the cells into their mature form that is required in the tissue that they will be part of [43].

Plant cell walls also are an important element for pathogen resistance mechanisms of the plants. Besides being the first physical barrier between the pathogens and plant cells, they are involved in biochemical immunity response mechanisms against the pathogens. Alteration of the polysaccharides in plant cell walls by the pathogen induced changes in cell wall integrity with pattern recognition receptors (PRRs) on the plasma membrane act as damage indicators and promotes plant immune response [44]. Another important function of plant cell walls is their role in the symbiotic interactions between plants with microbes. The cell wall remodelling in the roots of legumes induced by the rhizobium infection allows necessary tissue modifications in the infections sites for forming nodules that allows nitrogen uptake from the soil [45].

Plant cell walls in endosperms and cotyledons of some plant species can store nutrients for the cells. Especially, mannans and galactomanans which are non-cellulosic polysaccharides present in the cell walls shown to be hydrolysed during seed germination for providing the energy needs of the cells. This process which is regulated by the extensibility of the cell wall and some plant cell wall enzymes is important for embryos to survive until adequate maturation of the organism [46].

Plant cell walls are also important for tolerance mechanisms against the abiotic stresses including drought, flooding, salt, and temperature stresses. Especially, regulation of plant cell walls extensibility provides important balancing against the changes of turgor pressure altered by the excessive or deficient water availability and allows increased tolerance of the plants against these stresses [47].

1.3.3. Plant Cellular Growth

Expansion mechanism of the cell wall could be exemplified as the controlled “creeping” of the cell wall elements caused by the turgor force of the cell that mainly generated by vacuoles and inducing synthesis and elasticity of the cell wall. This expansion of the cell wall is parallel with the expansion and shaping of the plant cells, which is especially important for their specialization into mature cells since there is no cell migration inside the organisms and the shape and size that is required for the cell to function in the specific location in the tissue is defined by expansion of its cell wall [30]. The directional growth of the plant cell is directed by the anisotropic expansion of the cell wall. Higher elasticity of specific parts of the cell wall by the preferred orientation (higher rate of horizontal prevalence perpendicular to axis of elongation) of the celluloses and increased pectin branchedness allows directed expansion of the cells in the desired direction [43].

1.3.3.1. Plant Cell Wall Loosening

Mechanical strength and resistance against outer or turgor pressure of plant cell walls can be changed by many factors including enzymes targeting cell wall elements sourced from plant cells or pathogens, oxidative agents like reactive oxygen species (ROS) or hydroxyl radicals and non-enzymatic cell wall loosening proteins which are expansins. The loosening of cell wall can be initiated by both plants themselves and pathogen for various physiological functions. The most important irreversible cell wall loosening for plants the creeping is found by studies to require involvement of expansins and is not achievable in meaningful manner by involvement of only enzymatic degradation of cell wall structure elements [24].

1.3.3.2. Enzymatic Cell Wall Loosening

There are many enzymes in plant cells that targets plant cell wall elements by hydrolysis or remodelling including glycoside hydrolases (GH) that can targets (1,4)-β-D-glucan backbones of cellulose and xyloglucan and pectin methyl transferases (PME) that targets pectins. Between many GH enzymes the GH16 or xyloglucan endotransglycosylase/hydrolases (XTHs) are one of the most important family of enzymes in cell wall loosening [24]. By xyloglucan endotransglucosylase which rearranges the xyloglucans by transfer of xyloglucan glucoses between each other and xyloglucan endohydrolase activity which hydrolyse the glycosidic links in xyloglucans, XTHs enzymes modify cell walls by changing xyloglucans in cell walls which are essential to the cell wall structure by their role as linkers of cellulose microfibrils and there for important for enzymatic cell wall loosening [48]. Another important type of enzyme for cell wall loosening is PME, which demethylates methyl-esterified galacturonic acids of pectin structure therefore modify cell wall gel like matrix. The changes on cell wall pectic gel like matrix if important for cell wall loosening [49].

1.3.3.3. The Expansin Superfamily

Expansins are a family of protein that is known for their roles in wall loosening and cell expansion in plants by their non-enzymatic mechanism of action as mentioned before [50]. By their cell wall loosening abilities, expansins are also involved in a wide range of activities in plant cell walls including softening of fruits, morphogenesis, pollination, germination, abscission, root development, leaf development, stomata regulation and biotic-abiotic plant stress response mechanisms in plants [50–52]. Beside their important role in many plant functions that involve plant cell walls, their mechanism of cell wall loosening without any catalytic action is very interesting. They are found universally in the plant kingdom and have been found in some bacteria and fungi [53]. The Expansin multi-gene family is divided phylogenetically into four sub-families; α-expansin (EXPA), β-expansin (EXPB), expansin-like A (EXLA), and expansin-like B (EXLB). The most important α-expansin (EXPA) and β-expansin (EXPB) groups are typically 250-275 amino acid long proteins have two domains which are N-terminus double-psi beta-barrel (DPBB) domain and C-terminus polysaccharide-binding domain (C domain), also with a signal peptide that is removed in mature proteins [54].

EXPAs were identified as mediators of acid-induced wall loosening, especially important for acid growth mechanism induced by auxin. EXPAs are most active in the acidic conditions of lower than pH 4 and EXPA induced cell wall loosening doesn’t lower the cell wall stiffness. EXPA activity also doesn’t result in polysaccharide fragment release from the cell wall which is an indicator of non-enzymatic activity model for EXPA with the preservation of stiffness. The other important expansin class EXPB similar to EXPA is found in genomes of all land plants. Most of the studies focused on pollen EXPB and the rest of the EXPB group is not sufficiently studied. Because of their allergenic effect on humans they are also identified as group I grass pollen allergens by medical literature. Pollen EXPB that were under the group I allergen subgroup are involved with the penetration of pollen tube through stigma by their solubilization of cell wall polysaccharides of arabinoxylan and homogalacturonan specifically in grasses. Contrary to EXPA, the pollen EXPB activity results in loosing of cell wall stiffness even though like the other expansins they lack catalytic ability. This is hypnotized to be caused by weakening of hydrogen bonds of cellulose microfibrils network and solubilization of middle lamella and cell wall matrix polysaccharides. Another important group of expansins are bacterial expansins which are found in many bacteria and are involved with colonization of bacterial pathogens on plant surfaces. These expansin are thought to have horizontally transferred to the bacterial genomes from plants and are important for studying expansins mechanism of activity since they are highly homologous to pollen EXPB and easier to isolate and preserve in in vitro studies unlike plant expansins [24].

Mechanism of expansin activity in cell wall loosening is not yet clearly established. However many studies shown even though DPBB domains conserved in expansins is homologous to the Glycoside hydrolase family 45 (GH45) family of endoglucanases the lack of critical aspartic acid in the catalytic region of those endoglucanases in DPBB homologs prevent lytic activity. These non-enzymatic proteins are thought to bind and disrupt the non-covalent bonds in polysaccharides and loosen the cell wall by enabling turgor-driven creep. For exact mechanism of sub-groups or specific expansins there are many alternative hypothesis are offered and our knowledge about expansins exact working mechanisms is not sufficient [55]. Due to their various roles in plants, the expansin gene family is an important area of research and have a significant potential for transgenic crop development studies [52].

Under drought conditions plants prioritize root elongation for accessing water from the soil [20]. This response requires directed cell expansion in root tissues and by their role in plant cell growth by cell wall regulation expansins are an important factor in drought response. Different studies showed the relationship between the expansin expression association with drought response in barley [56], potato [57], and enhanced drought tolerance by the expression of the expansin genes in tobacco [58,59], sugarcane [60], cotton [61], wheat [62].

1.4. Barley Expansins

Research on the barley expansins are expanding but is not sufficient to this date. There are several studies that investigated the HvEXB1 (HORVU.MOREX.r3.2HG0189530)[63], HvEXB7 (HORVU.MOREX.r3.3HG0318580) [56], HvEXPA1 (HORVU.MOREX.r3.2HG0104860) [64] and some other barley expansin genes [65] roles in root hair development. However, the current literature on the expansins role in root tissues, especially in aspect of drought stress and with a wide coverage of expansins by their subgroups is not sufficient. One of the most important barley expansins study was done by Maria Lombardi in her PhD thesis work in which she identified 34 barley expansins (14 EXPA, 17 EXPB and 3 EXLA) and performed transcription analysis on identified expansins [66]. Another recent study has mapped and classified 46 barley expansin genes (24 EXPA, 16 EXPB, and 6 EXLA) with using RNA-seq data [67]. However due to recent developments in barley genome sequencing like the latest released MorexV3 [68] assembly and many new RNA sequencing studies, reassessment of the knowledge barley expansins is required.

Table 1.1. Selected studies and their findings about barley expansins.

Genes	Findings	Study
HvEXPB1 (HORVU.MOREX.r3. 2HG0189530)	HvEXPB1 gene is involved in initiation of root hair formation.	[63]
HvEXPB1 (HORVU.MOREX.r3. 2HG0189530)	Promoters of HvEXPB1 and the gene is associated with barley root hair morphogenesis.	[65]
HvEXPB7 (HORVU.MOREX.r3. 3HG0318580)	HvEXPB7 was found to be significantly over expressed in barley roots under drought stress and silencing of HvEXB7 has resulted in reduced root hair formation.	[56]
HvEXPA1 HORVU.MOREX.r3.2 HG0104860)	HvEXPA1 is an Aluminum inducible expansin gene, which participates in the root cell elongation and influences the Al content through regulating root cell wall loosening in barley.	[64]

1.5. Objective of the Thesis

Expansins are an essential group of proteins and their plant cell wall relaxation ability is core for many plant physiological mechanisms including plant growth, pollination and stress tolerance. Despite their importance for plants and plant biology, the literature on them is inadequate for understanding their mechanisms and possible uses. Beside the insufficient amount of knowledge about expansins, current studies mainly focus on analysing their mechanisms of work and correlation of their expression with plant growth and stress response mostly on a single target gene of interest without an aim of more general coverage on the groups of the expansins. In addition, in vivo studies on expansins are mostly done on a narrow selection of model plants and extensively controlled conditions that were not representative of in-field conditions. And lastly, beside the economical and academic importance of barley as a model plant for grasses, the literature on barley is extensively insufficient particularly with respect to the mapping and classification of the barley expansins being inadequate and non-uniform.

In this study we aimed to contribute to the literature on barley expansins and the expansin-drought stress response relationship considering the deficiencies of the literature. In the experimental design of this study firstly we mapped and categorized the barley expansin gene familys with using basic local alignment search tool (BLAST) method to find homologs of phylogenetically closely related and extensively studied expansins of wheat, rice and *Arabidopsis thaliana* besides the previously identified barley expansin genes [66]. After identification of the barley expansin genes we selected 5 target expansin genes by considering the expression profiles of identified genes in barley roots with the data from barley RNA-seq databases and coverage of different expansin groups in barley. After the selection of our target barley expansins, we performed expression analysis on mature barley plant roots grown in greenhouse with or without drought stress on multiple time points alongside analysing the stress treated and control plants physiologically. With having differential expression data on both early and late stages of our target genes we aimed to show the relationship of expansin expression and the stage of drought response mechanisms. With the results achieved, we had drawn conclusions on the involvement of expansins on drought response mechanisms and possible target genes for further studies on barley expansins.

2. MATERIALS AND METHOD

2.1. Investigation of Barley Expansin Genes

With using the BLAST tool in https://plants.ensembl.org/ barley expansin genes has been searched by using the expressed sequences of known expansin genes from wheat, rice and *Arabidopsis thaliana* from EnsemblPlants [69] database which are closely evolutionary related to the barley that is in grass family [70] and known barley expansin genes [66]. By using the known sequences of expansins as queries for search for homologous genes in MorexV3_pseudomolecules_assembly [68] data, an initial list of sequences with high similarity has been obtained. Sequences with missing domains has been detected and sequences has been categorized under subgroups of expansins by using phylogenetic analysis with using tools of MEGA11 software [71].

2.2. Selection of Target Genes and Primer Design

Expression profiles of identified barley expansin genes from the genome sequencing data MorexGenes - Barley RNA-seq Database in root tissue samples were investigated from the data obtained by and EoRNA databases [72]. Five genes from identified barley expansins (HORVU.MOREX.r3.7HG0636890.1, HORVU.MOREX.r3.2HG0104860.1, HORVU.MOREX.r3.6HG0601160.1, HORVU.MOREX.r3.1HG0056580.1 and HORVU.MOREX.r3.4HG0409870.1) were selected as the targets for differential expression analysis. Selection was done by choosing genes with top expression in root samples and representing wider barley expansin super family and subgroups. Amount of target genes were limited by five because of financial and technical constraints. MYB-type transcription factor (HvMYB1) (HORVU.MOREX.r3.5HG0500520) as reference gene for normalization in differential expression step. Selection of MYB1 is done between other candidate reference genes out of necessities caused by problems with RNA samples that were explained in discussion part. For the determined target genes, reverse and forward primers were designed using Primer3 [73] with around 20 base pair (bp) lengths that are listed in Table 2.1. .

Table 2.1. Primers used in the differential gene expression analysis, Theoretical melting tempreatures were calculated by NetPrimer [74].

Designated Name of Gene	MorexV3 ID [68]	Chromosome	Location Left	Location Right	Product length (bp)	Direction	Primer Sequence	Theoretical Tm (˚C)
EXPA1	HORVU.MOREX.r3.7HG0636890.1	7	4841829	4841849	198	F	GCCAACCAACTCTCTGCTCT	56,7
			4841631	4841651		R	TACTGGGGAGTTAAGCCTGC	57,1
EXPA2	HORVU.MOREX.r3.2HG0104860.1	2	19058285	19058305	280	F	GCTCAACATCGGCGTCTACC	60,4
			19058565	19058585		R	TTGGGGAAGGTGATCGTCTG	60,4
EXPB1	HORVU.MOREX.r3.6HG0601160.1	6	414797506	414797526	93	F	GGTGCTCTCGCTTCTTGTGA	58,5
			414797413	414797433		R	GGTTAGTGGCGGTCTGGTTT	59,1
EXPB2	HORVU.MOREX.r3.1HG0056580.1	1	378184784	378184804	153	F	CATACGCATCACCACCGACA	60,6
			378184631	378184651		R	GGGAACCTCCAGATGACACC	58,6
EXPB3	HORVU.MOREX.r3.4HG0409870.1	4	587326862	587326882	176	F	CACGAGTGGAGGTGAAAGCA	59,1
			587327038	587327058		R	CGCTACCGCTACGAGTCATC	60,0
HvMYB1	HORVU.MOREX.r3.5HG0500520	5	504537841	504537861	93	F	ACCACGTGGACCACCATCAC	60,6
			504537768	504537788		R	TGCACAGGTCCAGGTTCAGG	61,3

2.3. Phylogenetic Analysis

Found barley expansin gene sequences were aligned with MUSCLE global alignment tool [75] via MEGA11 [71]. Using the alignment results and the homologies of the sequences with the known expansin sequences from other plants the genes were classified under subclasses by the phylogenetic analysis done with the maximum likelihood method via MEGA11 [71] and visualization of the phylogenetic analysis were done with iTOL [76].

2.4. Expression Profiling of Detected Expansin Genes with Whole Expression Sequencing Data

Detected barley expansin genes expression levels in various stress treatments and tissues from whole expression sequencing data were investigated from BarleyExpDB [77]. Fragments per kilobase of transcript per million mapped reads (FPKM) data for each sample group were normalized with barley elongation factor 1-alpha (HORVU.MOREX.r3.4HG0385270) [78]. Obtained data were analysed and visualized with Microsoft Excel [79]. Data sets used in the analyses is listed on the Table 2.2. .

Table 2.2. Whole expression sequencing (WES) data used in relative expression analysis.

SRA Project Number [80]	**Title of the Project**	**Study**
PRJEB14349	RNA-Seq of 16 developmental stages of barley	[81]
PRJNA439267	Barley seminal root zone specific gene expression in response to water deficit	[82]
PRJEB13621	A study of transcriptome response to salt treatment across three zones of the root in a barley landrace and malting cultivar	[83]
PRJNA496380	Temporal tissue-specific regulation of transcriptomes during barley (*Hordeum vulgare*) seed germination	[84]

2.5. Plant Material Growth and Stress Treatment

Barley seeds (EFES-98 variety) were steeped with 10.10.10 mixed element (ME) fertilizer solution (10% (w/w) Urea Nitrogen, 10% (w/w) Phosphorus Pentoxide and 10% (w/w) Potassium Oxide) for 24 hour at room temperature. Steeped seeds were transferred to vials for germination in peat. One day after germination, seeds with successful germination were selected and transferred to pots (25 cm diameter x 20 cm height) with soil mix (1:2 soil, 1:4 river side soil, 1:5 peat, 1:20 fertilizer) 5 plant per pot and grown in greenhouse (20-25 °C, 16/8 hour light/dark) until they reach the 5th leaf stage at approximately 3 weeks. After the barley plants reached the 5th leaf stage, plants were separated between control and drought groups. Both drought and control group plants were cut from watering in pre-treatment period until the soil moisture content of the drought group was measured as dry by ZD-07 4 in 1 soil survey instrument. After soil moisture content of the drought group was confirmed as dry stress treatment by the soil moisture measurement, stress treatment period was started. During the stress treatment control group plants were watered in every two days with 200 mL water per pot while drought group plants were not watered. Physiological indicators including chlorophyll content on leaves with SPAD-502Plus and shoot relative water content (RWC) [85] were measured during drought stress treatment. Images of plants were also recorded during treatment. Root samples (0-10 cm) of drought and control group plants were harvested and transferred in liquid nitrogen to long term storage at -80 °C by for differential expression analysis step. Stress treatment were terminated at 15th day by the assessment of terminal stage of drought plants by observation.

2.6. Differential Expression Analysis

Total RNA extraction was performed with Norgen Plant RNA purification kit (Cat: 31350) according to manufacturer’s instructions from root samples stored at -80 °C. Nucleic acid quantity and quality of the extracted total RNA samples were controlled with UV spectrometry (BioSpec-nano). From the obtained total RNA samples reverse transcription was done using Thermo RevertAid First Strand cDNA Synthesis Kit (Catalog : K1621), according to its instruction manual. Expression of target expansin genes were analyzed with qPCR by using Biorad iTaq Universal SYBR Green SupermixKit, CFX96 Touch Real-Time

PCR Detection System and ordered primers for target genes. Tm values determined by gradient qPCR results of -2 ˚C and +2 ˚C gradient of theoretical melting temperature (Tm) of primer pairs of each gene that were calculated by NetPrimer [74]. Quantitative polymerase chain reaction (qPCR) were done according to the manual of iTaq™ Universal SYBR® Green Supermix [86], all wells were loaded with 40ng Total cDNA, 0.5 uM Primer concentration (each direction) with total 20 µL load to each well by 3 technical replicates for each 3 biologic replicate, total 9 replicates. Results of qPCR were analyzed using Biorad CFX Manager Software and relative expression of sample were calculated by the obtained Cq (quantification cycle) data according to the Pfaffl method [87]. Gradient qPCR for melting temperature (Tm) determination were done with same procedure with only 12th day drought sample. And finally, one tailed and independent Welch's t-Test [88] is calculated for control and drought groups of each target gene normalized Cq results in order show statistical difference caused by drought stress on target gene expressions.

3. RESULTS

3.1. Bioinformatics Analyses

3.1.1. BLAST results

Table 3.1. List of detected barley expansin genes with BLAST® [89], genes were classified under subgroups and F indicates forward and R indicates reverse strand.

MorexV3 ID [68]	Location	Strand	Protein length (amino acid)	UniProt Accession Number [90]	GenBank Accession Number [91]	Sub Group
HORVU.MOREX.r3.2HG0104860	2H:19057608-19058899	F	316	A0A8I6WN29	XM_045113317.1	Expansin A
HORVU.MOREX.r3.2HG0185850	2H:578558771-578565001	R	259	A0A8I6WWG6	XM_045111461.1	Expansin A
HORVU.MOREX.r3.3HG0256620	3H:198247505-198249000	F	244	A0A8I6X6W8	XM_045116461.1	Expansin A
HORVU.MOREX.r3.4HG0401040	4H:554499314-554500681	F	259	F2DCQ7	XM_045126715.1	Expansin A
HORVU.MOREX.r3.5HG0519910	5H:549029431-549030398	R	260	A0A8I6Y376	XM_045093337.1	Expansin A
HORVU.MOREX.r3.7HG0722730	7H:544318755-544319787	F	279	A0A8I6YY59	XM_045104075.1	Expansin A
HORVU.MOREX.r3.4HG0401050	4H:554584822-554585796	F	259	A0A8I7BCZ3	XM_045123529.1	Expansin A
HORVU.MOREX.r3.7HG0723940	7H:548841209-548843706	R	265	M0V162	XM_045100877.1	Expansin A
HORVU.MOREX.r3.7HG0636890	7H:4840646-4841867	R	326	A0A8I6Z5R4	XM_045103671.1	Expansin A
HORVU.MOREX.r3.4HG0401290	4H:555572936-555574125	R	253	A0A8I6Y3H8	XM_045123537.1	Expansin A
HORVU.MOREX.r3.4HG0401310	4H:555614172-555615090	F	253	A0A8I6Y9J4	XM_045123538.1	Expansin A
HORVU.MOREX.r3.3HG0297700	3H:530826484-530899009	F	249	F2EDT2	XM_045121550.1	Expansin A
HORVU.MOREX.r3.7HG0704780	7H:425488959-425490571	R	258	A0A8I6YEH1	XM_045104217.1	Expansin A
HORVU.MOREX.r3.3HG0223260	3H:9706033-9707033	F	264	A0A8I6WY89	XM_045115950.1	Expansin A
HORVU.MOREX.r3.3HG0250010	3H:145511692-145512567	F	261	M0W694	XM_045116382.1	Expansin A
HORVU.MOREX.r3.3HG0250020	3H:145565696-145566637	F	261	M0W694	XM_045116384.1	Expansin A
HORVU.MOREX.r3.3HG0250040	3H:145625472-145626355	F	261	A0A8I6XCT7	XM_045116385.1	Expansin A
HORVU.MOREX.r3.3HG0250050	3H:145691256-145692071	F	239	A0A8I6XSN5	XM_045116386.1	Expansin A
HORVU.MOREX.r3.4HG0347880	4H:80621193-80622186	F	265	A0A8I6XGM6	XM_045122844.1	Expansin A

HORVU.MOREX.r3.4HG0396570	4H:534335240-534336211	R	259	A0A8I6Y180	XM_045123348.1	Expansin A
HORVU.MOREX.r3.4HG0401060	4H:554612521-554613372	F	253	A0A8I6XEL7	XM_045123531.1	Expansin A
HORVU.MOREX.r3.1HG0054170	1H:361887174-361888390	F	259	F2E8A4	XM_045103990.1	Expansin A
HORVU.MOREX.r3.1HG0054180	1H:362068330-362069269	F	280	A0A8I6WIV3	XM_045093670.1	Expansin A
HORVU.MOREX.r3.1HG0054190	1H:362150973-362151849	F	259	A0A8I6WTU5	XM_045104000.1	Expansin A
HORVU.MOREX.r3.4HG0401070	4H:554652931-554654106	F	254	A0A8I6XHK5	XM_045123532.1	Expansin A
HORVU.MOREX.r3.5HG0532120	5H:575833568-575835784	F	254	A0A8I6XV87	XM_045093891.1	Expansin A
HORVU.MOREX.r3.5HG0532140	5H:575851320-575852371	F	256	A0A8I6XVY9	XM_045089922.1	Expansin A
HORVU.MOREX.r3.5HG0532150	5H:575892617-575893487	F	254	A0A8I6YXA5	XM_045089923.1	Expansin A
HORVU.MOREX.r3.5HG0533630	5H:578267105-578268266	F	255	A0A8I6Y4U3	XM_045093923.1	Expansin A
HORVU.MOREX.r3.1HG0071050	1H:453813638-453815396	F	251	F2DVR7	XM_045121509.1	Expansin A
HORVU.MOREX.r3.6HG0615400	6H:506644209-506645783	R	287	A0A8I6Z3J6	XM_045096412.1	Expansin A
HORVU.MOREX.r3.5HG0526530	5H:563758338-563760759	R	262	A0A8I7BAB5	XM_045129820.1	Expansin A
HORVU.MOREX.r3.3HG0252190	3H:162047274-162048510	R	285	A0A8I6XL38	XM_045116417.1	Expansin A
HORVU.MOREX.r3.3HG0293420	3H:509477147-509478450	R	249	A0A8I6XGH7	XM_045116901.1	Expansin A
HORVU.MOREX.r3.3HG0293430	3H:509496134-509496984	R	249	A0A8I6XY58	XM_045116902.1	Expansin A
HORVU.MOREX.r3.3HG0293450	3H:509515974-509516823	R	249	A0A287LRJ4	XM_045116903.1	Expansin A
HORVU.MOREX.r3.3HG0293480	3H:509594791-509605128	R	246	A0A8I6XR91	XM_045116904.1	Expansin A
HORVU.MOREX.r3.3HG0293490	3H:509627774-509628622	R	249	A0A8I7B5W9	XM_045116905.1	Expansin A
HORVU.MOREX.r3.3HG0252250	3H:162663035-162664293	R	255	A0A8I6WQZ1	XM_045116420.1	Expansin A
HORVU.MOREX.r3.6HG0601180	6H:415185549-415186770	F	287	M0UM32	XM_045096108.1	Expansin B
HORVU.MOREX.r3.6HG0601160	6H:414796014-414797669	R	289	F2E1I9	XM_045095723.1	Expansin B
HORVU.MOREX.r3.5HG0508200	5H:521113153-521116958	R	415	F2ED17	XM_045128695.1	Expansin B
HORVU.MOREX.r3.2HG0107600	2H:25684571-25685662	F	254	A0A8I6W9W2	XM_045108210.1	Expansin B
HORVU.MOREX.r3.2HG0214300	2H:656231361-656232338	F	252	A0A8I6X2I7	XM_045113025.1	Expansin B
HORVU.MOREX.r3.2HG0107610	2H:25730775-25731730	F	254	A0A8I6WGH0	XM_045113399.1	Expansin B
HORVU.MOREX.r3.2HG0189540	2H:595640396-595641747	R	262	A0A8I6XB64	XM_045114537.1	Expansin B
HORVU.MOREX.r3.6HG0601090	6H:414549292-414550493	F	265	M0YQS0	XM_045096125.1	Expansin B
HORVU.MOREX.r3.6HG0598380	6H:392809583-392824811	F	267	A0A8I7BC35	XM_045095540.1	Expansin B
HORVU.MOREX.r3.2HG0183770	2H:567009683-567013964	F	282	A0A8I6XE38	XM_045111329.1	Expansin B
HORVU.MOREX.r3.2HG0107640	2H:25741953-25742679	F	202	A0A8I7B241	XM_045113401.1	Expansin B
HORVU.MOREX.r3.1HG0056580	1H:378184391-378186016	R	271	F2DVX9	XM_045104498.1	Expansin B

HORVU.MOREX.r3.1HG0056600	1H:378383800-378385466	R	273	F2E8V0	XM_045104509.1	Expansin B
HORVU.MOREX.r3.3HG0293090	3H:508370550-508371449	R	199	A0A8I6Y1S8	XM_045096852.1	Expansin B
HORVU.MOREX.r3.6HG0543550	6H:13198787-13199770	F	272	A0A8I7BAW6	XM_045096852.1	Expansin B
HORVU.MOREX.r3.1HG0056620	1H: 378,522,806-378,524,551	R	317	A0A8I7B3A3	XM_045115792.1	Expansin B
HORVU.MOREX.r3.5HG0439640	5H:73747655-73751084	R	353	A0A8I6Y5X2	XR_006609950.1	Expansin B
HORVU.MOREX.r3.1HG0056630	1H: 378,714,726-378,716,942	F	277	A0A8I6WJN4	XM_045115793.1	Expansin B
HORVU.MOREX.r3.4HG0415870	4H:603436113-603438740	F	294	A0A287Q147	XM_045127366.1	Expansin B
HORVU.MOREX.r3.2HG0189470	2H:595598531-595599901	R	261	A0A8I6XNZ2	XM_045114534.1	Expansin B
HORVU.MOREX.r3.2HG0189530	2H:595630065-595631547	R	282	F2DJK8	XM_045114536.1	Expansin B
HORVU.MOREX.r3.1HG0056570	1H: 378,086,356-378,087,902	R	265	F2DDY1	XM_045115791.1	Expansin B
HORVU.MOREX.r3.6HG0543540	6H:13190918-13191939	R	274	A0A8I6YFZ5	XM_045096886.1	Expansin B
HORVU.MOREX.r3.3HG0318580	3H:592827799-592829147	F	306	M0YZK5	KR732966.1	Expansin B
HORVU.MOREX.r3.3HG0219910	3H:3657844-3659085	F	277	A0A8I6WVN7	XM_045115886.1	Expansin B
HORVU.MOREX.r3.3HG0318560	3H:592818991-592820493	F	302	A0A8I6X8R9	XM_045122429.1	Expansin B
HORVU.MOREX.r3.3HG0318870	3H:593707506-593708521	R	307	A0A8I6Y233	XM_045117454.1	Expansin B
HORVU.MOREX.r3.3HG0318880	3H:593718367-593719404	R	308	A0A8I6WWE7	XM_045117455.1	Expansin B
HORVU.MOREX.r3.4HG0415820	4H:603127012-603129060	R	361	A0A8I6XLP1	XM_045127365.1	Expansin B
HORVU.MOREX.r3.6HG0592630	6H:344004326-344004811	R	121	A0A8I6Z1T1	XM_045096367.1	Expansin B
HORVU.MOREX.r3.6HG0592640	6H:344006734-344008161	R	297	A0A8I6YBB5	XM_045096366.1	Expansin B
HORVU.MOREX.r3.1HG0055780	1H:372138710-372139971	R	266	F2EI89	XM_045104333.1	Expansin B
HORVU.MOREX.r3.2HG0195810	2H:614308017-614309178	R	265	F2E1K9	XM_045114648.1	Expansin B
HORVU.MOREX.r3.4HG0409710	4H:587071772-587072914	R	274	A0A8I6XKZ3	XM_045127093.1	Expansin Like A
HORVU.MOREX.r3.4HG0409760	4H:587107159-587108139	R	271	A0A8I7B7Q4	XM_045123900.1	Expansin Like A
HORVU.MOREX.r3.4HG0409870	4H:587325235-587327404	F	280	M0WQL2	XM_045127097.1	Expansin Like A
HORVU.MOREX.r3.1HG0054870	1H:366556372-366557912	F	275	A0A8I6WIY2	XM_045104155.1	Expansin Like A
HORVU.MOREX.r3.7HG0749090	7H:622566299-622567817	F	309	A0A8I6YT26	XM_045106005.1	Expansin Like A
HORVU.MOREX.r3.7HG0749130	7H:622597775-622598731	F	318	A0A8I6YI60	XM_045103173.1	Expansin Like A

With the BLAST® [89] results and analysis of annotated barley expansins from [90] to date, the final list of barley expansins if formed as Table 3.1. The new total number of found barley expansin genes is 78. Of the mentioned 78 genes, 39 belong to the EXPA subgroup, 33 belong to the EXPB subgroup and 6 to the EXLA subgroup. No member of expansin like-B subgroup were identified. Found barley expansin genes distribution among the barley chromosomes are; 11 genes on 1st chromosome, 11 genes on 2nd chromosome, 20 genes on 3rd chromosome, 13 genes on 4th chromosome, 8 genes on 5th chromosome, 9 genes on 6th chromosome and finally 6 genes on 7th chromosome.

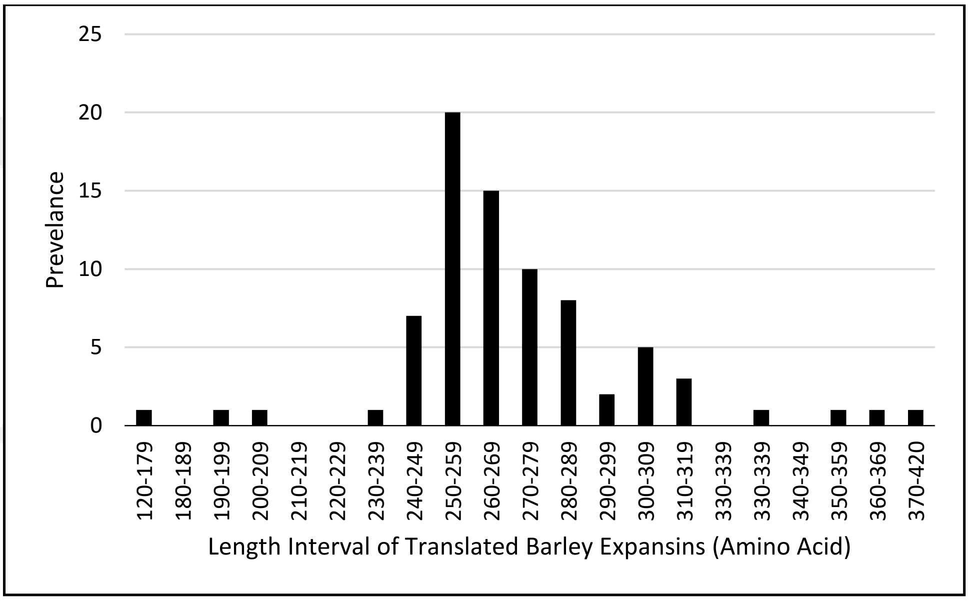


Figure 3.1. Distribution of lengths of translated barley expansin proteins.

Translated protein sequences of found barley expansin sequences by amino acid length varied between 121 to 415 amino acids, which the bulk of these proteins having the lengths between 240 and 280 as it can be observed at the Figure 3.1. .

3.1.2. Phylogenetic Analysis

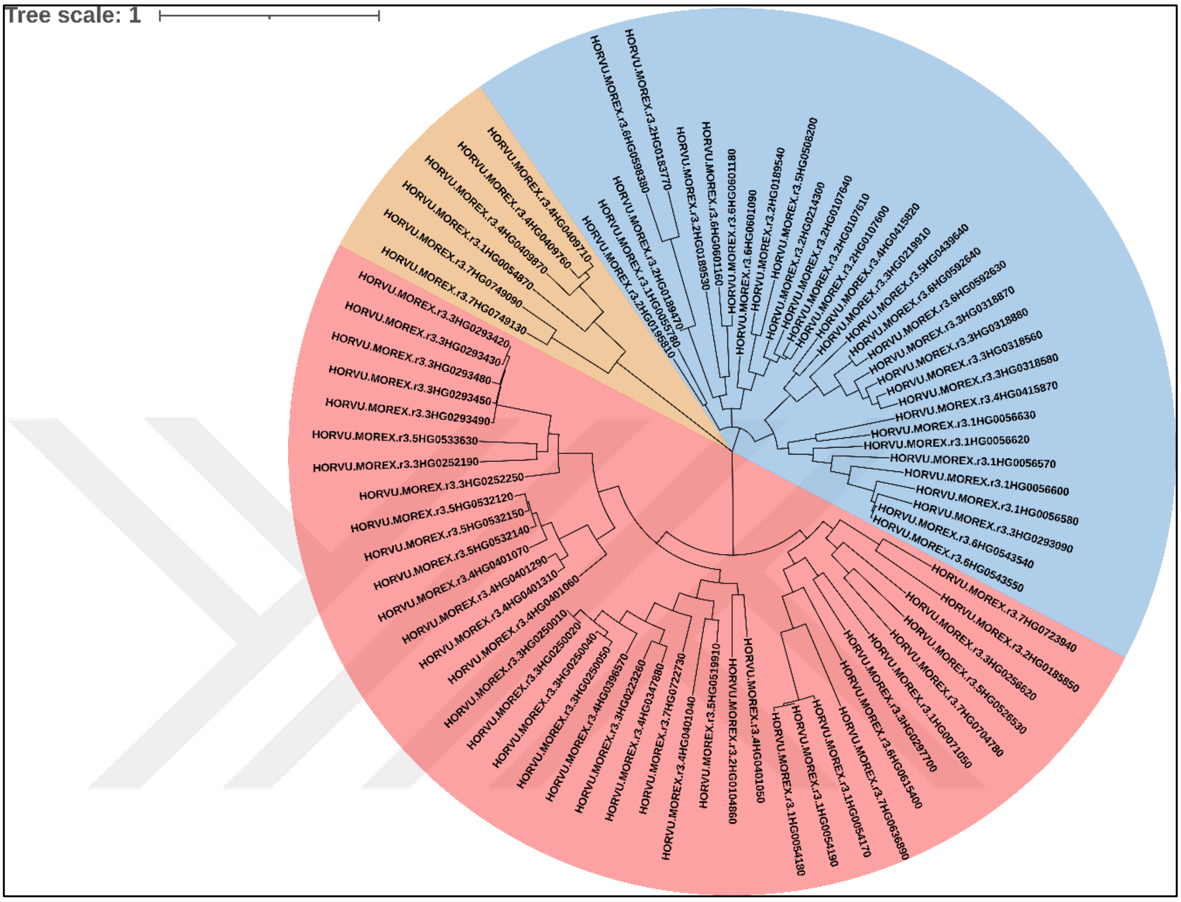


Figure 3.2. Phylogenetic tree of found barley expansin genes genomic sequences by global alignment via MUSCLE tool [75], by maximum likelihood method tree generation via MEGA11 [71] and visualized by Itol [76] where classification of subgroups of Expansin A (red), Expansin B (blue) and Expansin Like A (orange) groups were done by using homologies of found sequences to rice barley sequences.

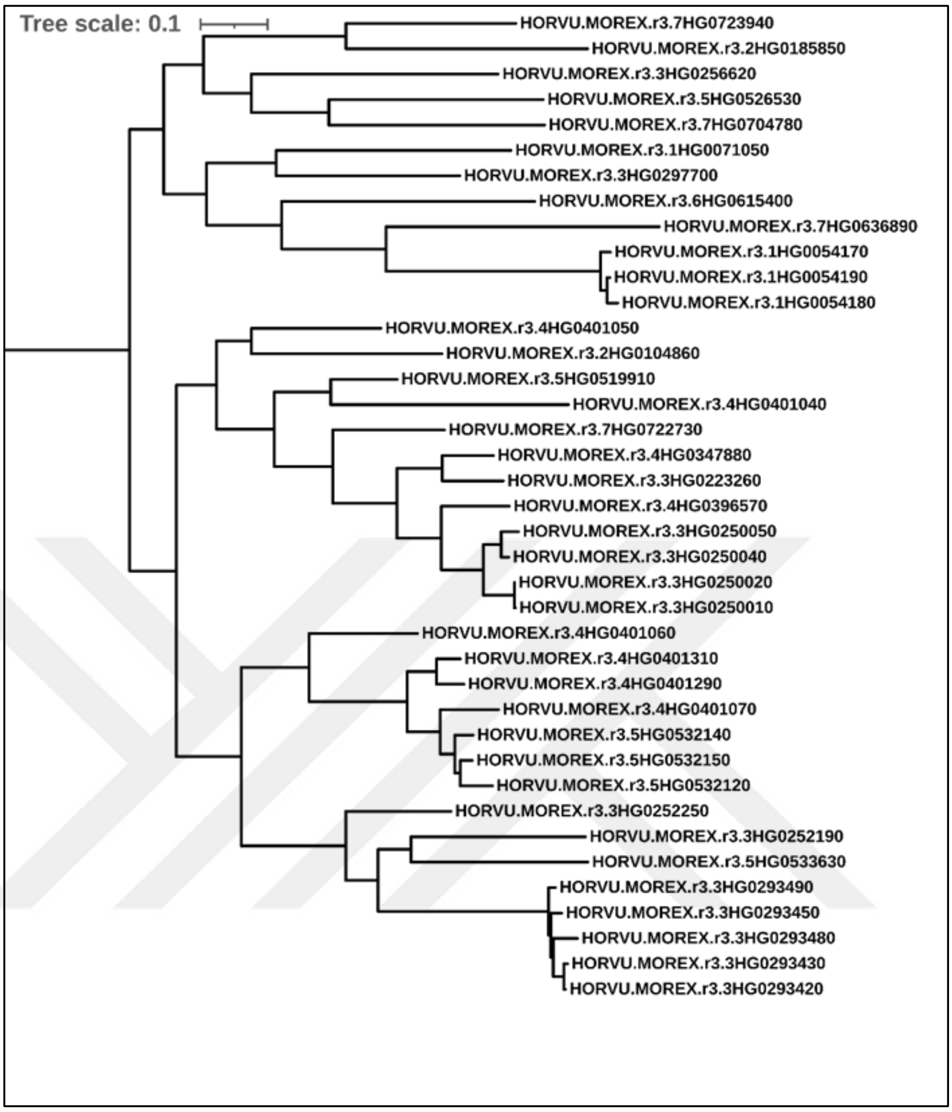


Figure 3.3. Phylogenetic tree of barley expansin A subgroup genes that were aligned by MUSCLE global alignment method [75], phylogenic tree constructed by maximum likelihood method via MEGA11 [71] and visualization is done via Itol [76].

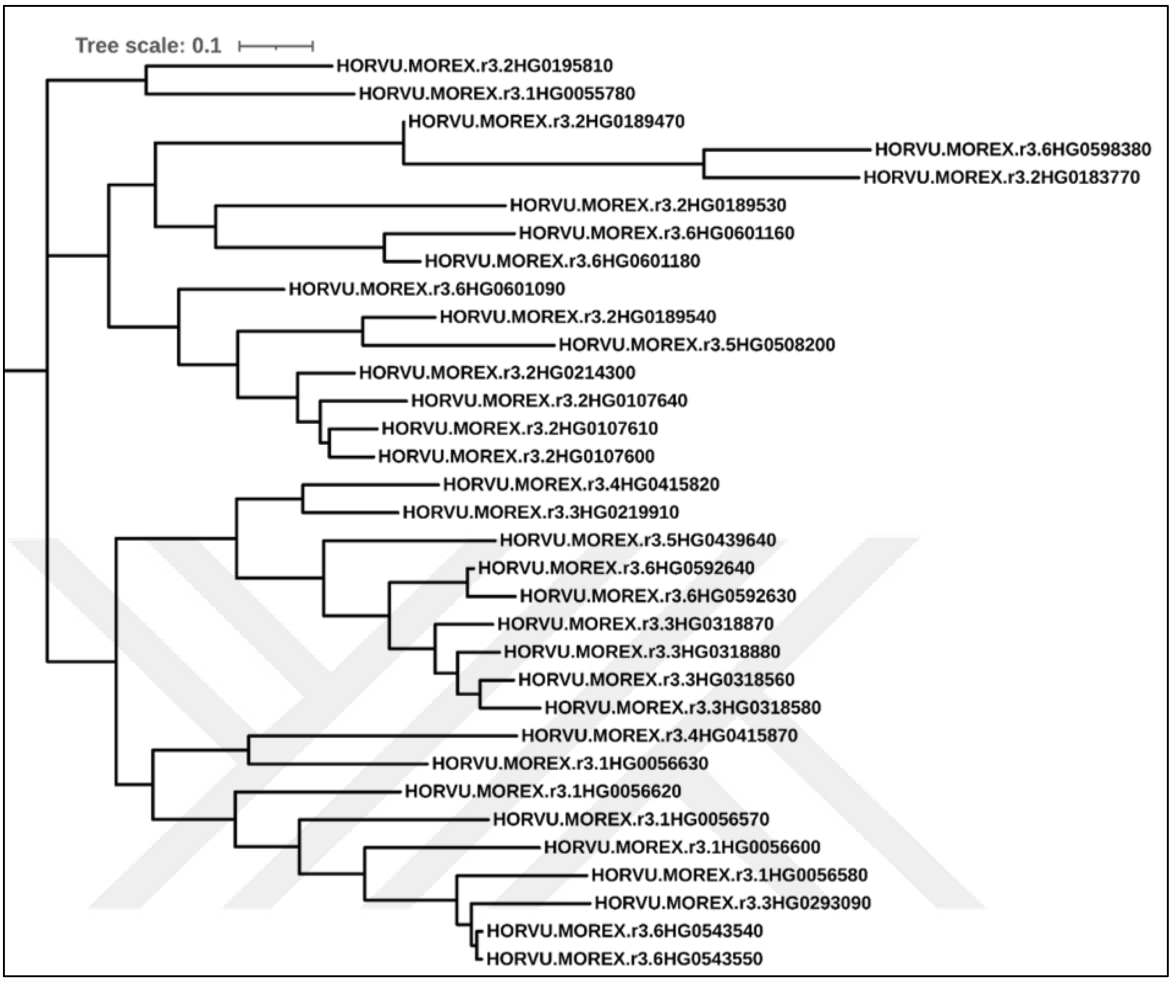


Figure 3.4. Phylogenetic tree of barley expansin B subgroup genes that were aligned by MUSCLE global alignment method [75], phylogenic tree constructed by maximum likelihood method via MEGA11 [71] and visualization is done via Itol [76].

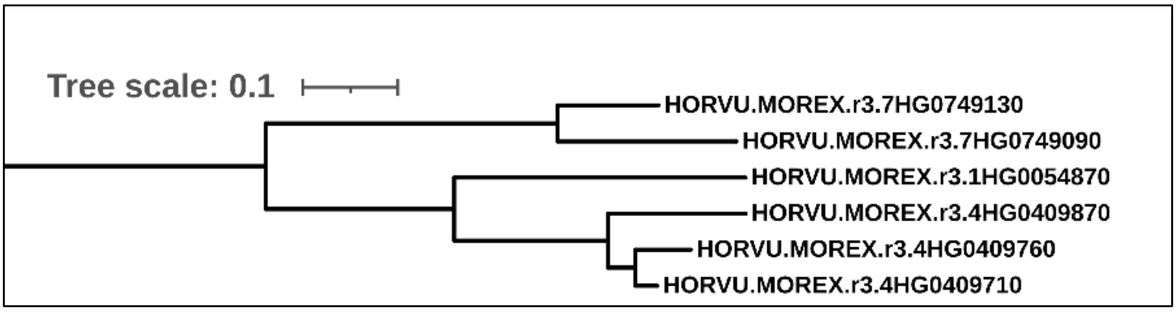


Figure 3.5. Phylogenetic tree of barley expansin like-A subgroup genes that were aligned by MUSCLE global alignment method [75], phylogenic tree constructed by maximum likelihood method via MEGA11[71] and visualization is done via Itol [76].

3.1.3. Mapping of Expansins on Barley Genome

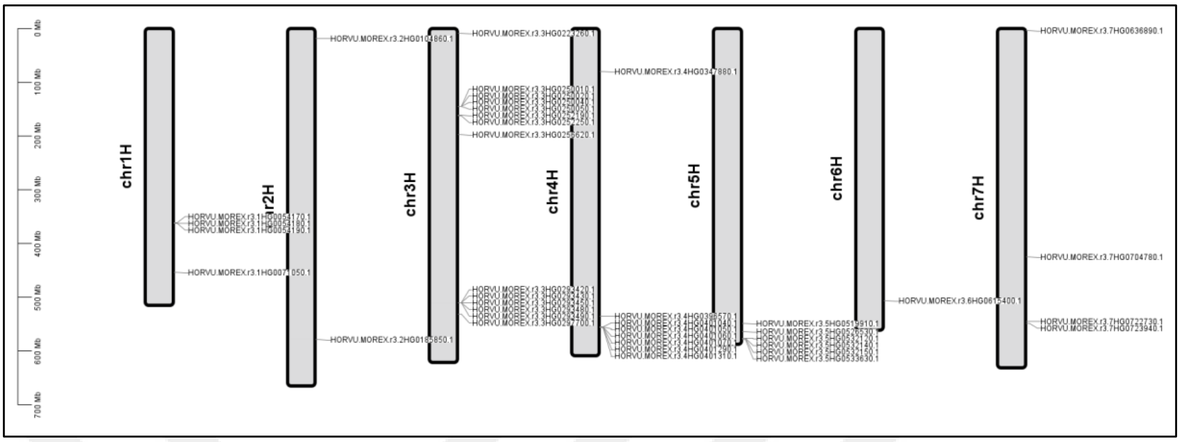


Figure 3.6. Mapping of Expansin A group genes on barley genome MorexV3 assembly [68], visualization done with TBtools-II [92].

Found Barley EXPA genes distribution among the barley chromosomes are; 4 gene on 1st chromosome, 2 gene on 2nd chromosome, 14 gene on 3rd chromosome, 8 gene on 4th chromosome, 6 gene on 5th chromosome, 1 gene on 6th chromosome and finally 4 gene on 7th chromosome.

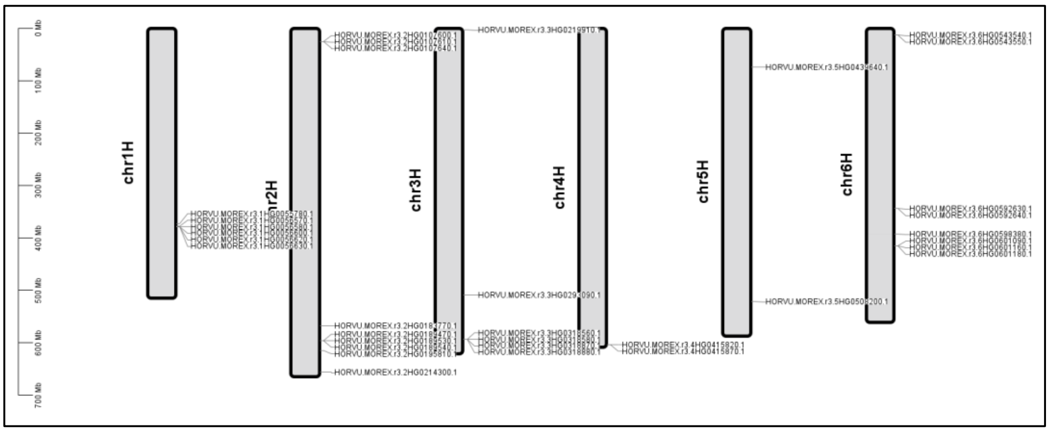


Figure 3.7. Mapping of Expansin B group genes on barley genome Morex.V3 assembly [68], visualization done with TBtools-II [92].

Found Barley EXPB genes distribution among the barley chromosomes are; 6 gene on 1st chromosome, 9 gene on 2nd chromosome, 6 gene on 3rd chromosome, 2 gene on 4th chromosome, 2 gene on 5th chromosome and 8 gene on 6th chromosome

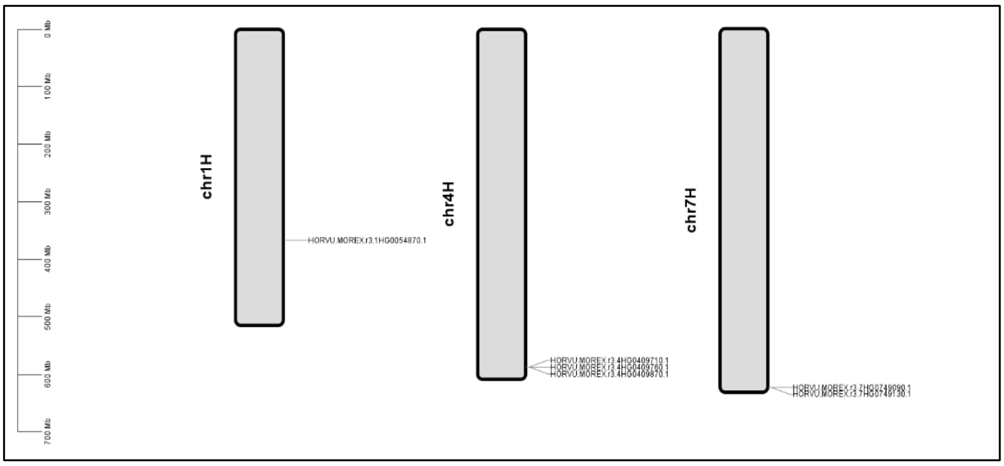


Figure 3.8. Mapping of Expansin-like A group genes on barley genome Morex.V3 assembly [68], visualization done with TBtools-II [92].

Found Barley EXPB genes distribution among the barley chromosomes are; 1 gene on 1st chromosome, 3 gene on 4th chromosome and 2 gene on 7th chromosome.

3.1.4. Conserved Domain Analysis

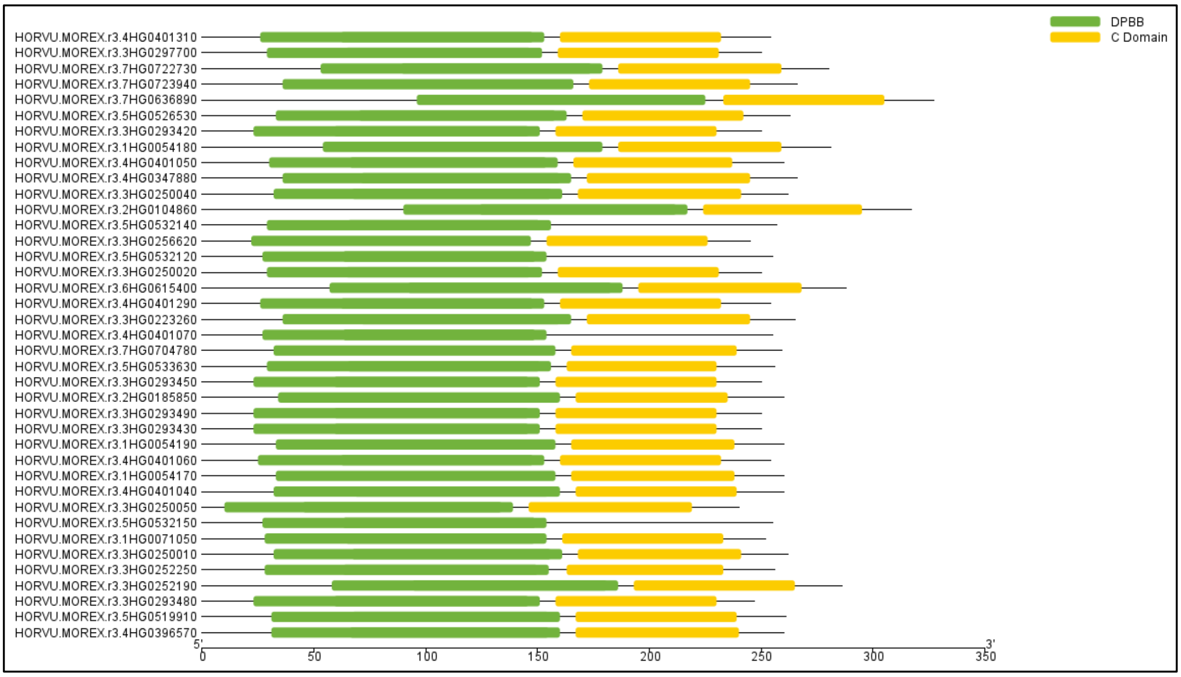


Figure 3.9. Conserved domains on EXPA protein sequences from the data obtained CDD [93] and visualized with TBtools-II [92].

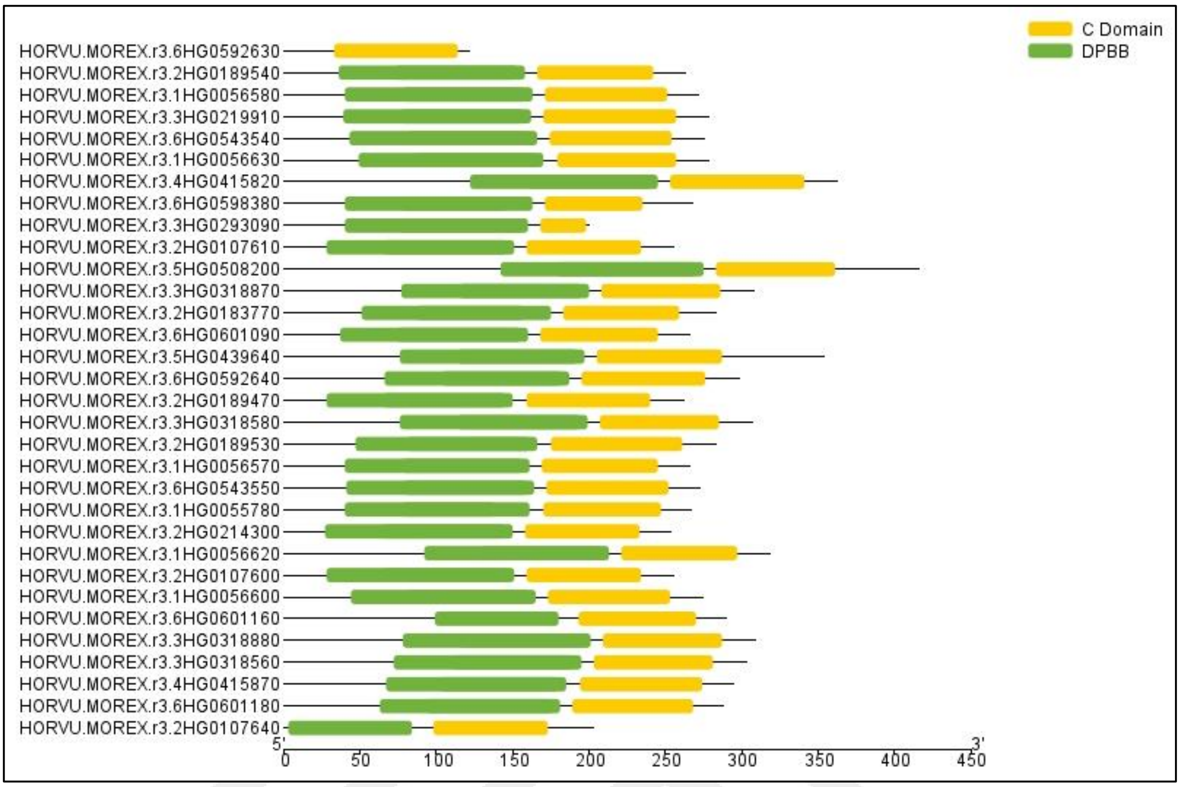


Figure 3.10. Conserved domains on EXPB protein sequences from the data obtained CDD [93] and visualized with TBtools-II [92].

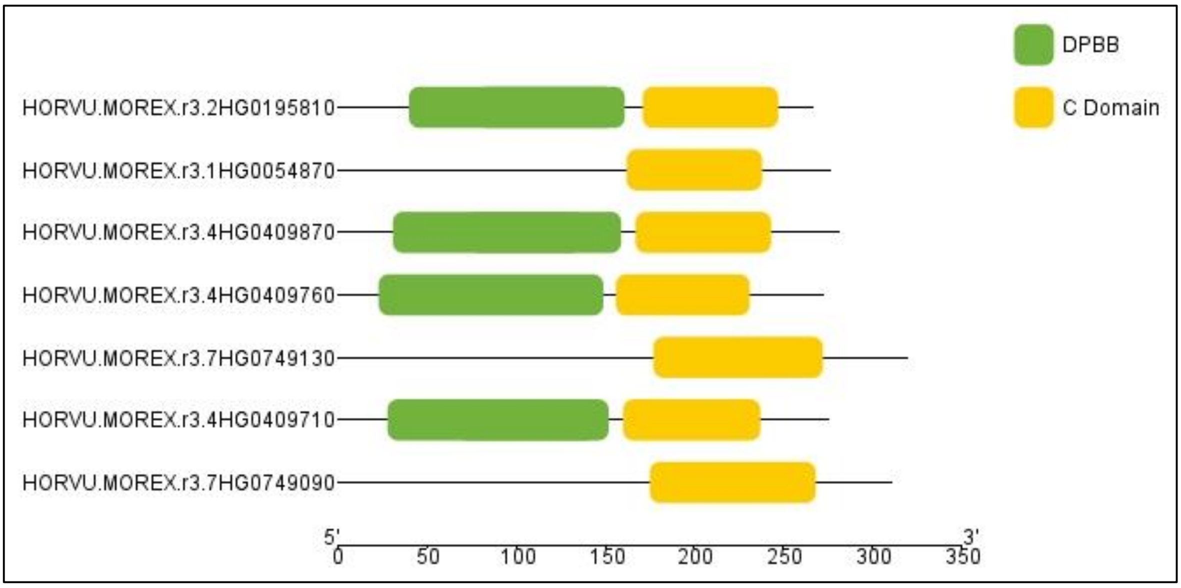


Figure 3.11. Conserved domains on EXLA protein sequences from the data obtained CDD [93] and visualized with TBtools-II [92].

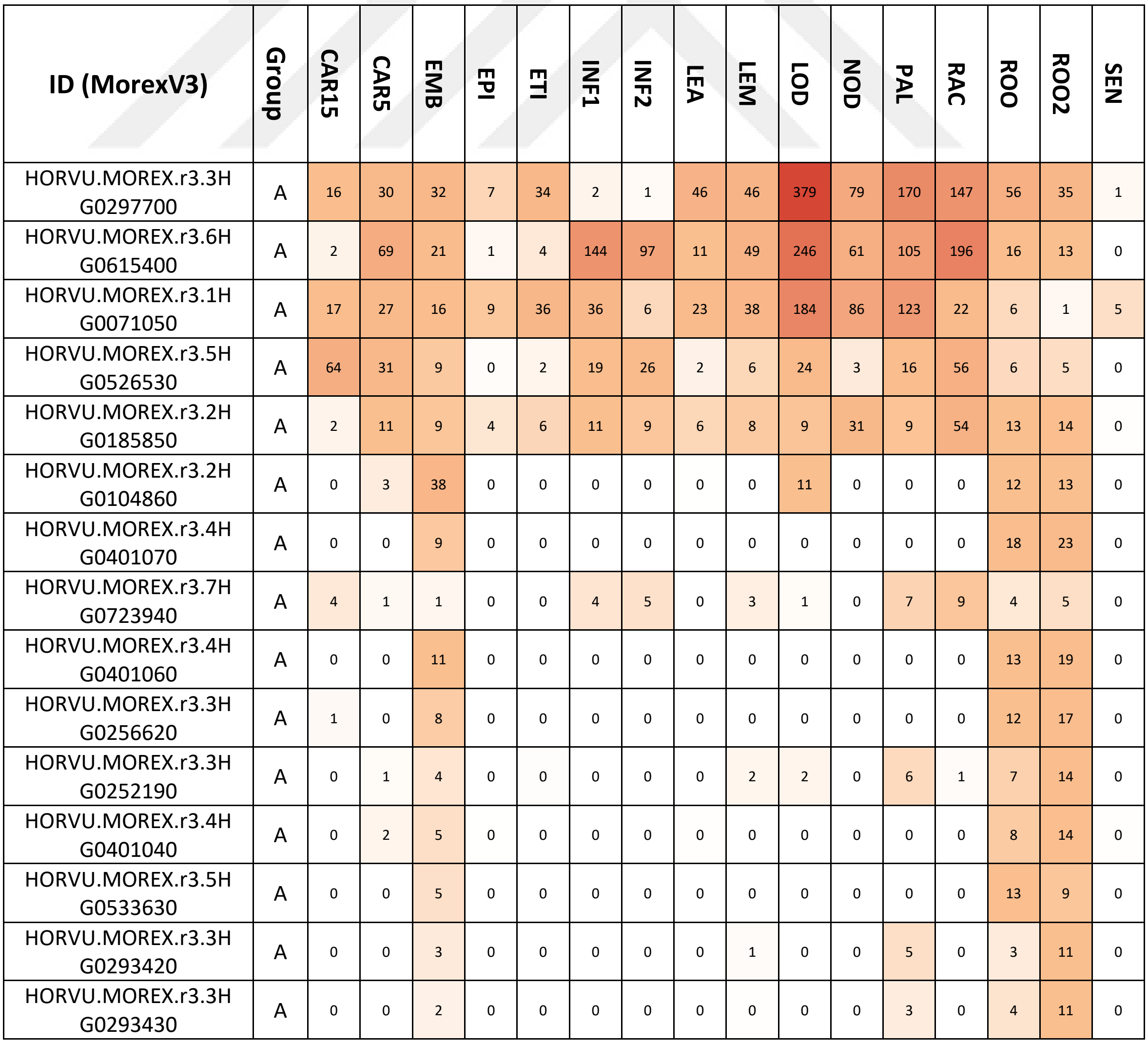
Table 3.2. Sequences with non-complete specific hit conserved domains from the data obtained via CDD [93].

Gene ID	Group	Missing domain
HORVU.MOREX.r3.5HG0532140	EXPA	C Domain
HORVU.MOREX.r3.5HG0532120	EXPA	C Domain
HORVU.MOREX.r3.4HG0401070	EXPA	C Domain
HORVU.MOREX.r3.5HG0532150	EXPA	C Domain
HORVU.MOREX.r3.3HG0293090	EXPB	Sequences missing at C terminus of the C Domain
HORVU.MOREX.r3.6HG0592630	EXPB	DPBB
HORVU.MOREX.r3.1HG0054870	EXLA	DPBB
HORVU.MOREX.r3.7HG0749130	EXLA	DPBB
HORVU.MOREX.r3.7HG0749090	EXLA	DPBB

Conserved domain analysis of the found barley expansin genes showed despite some of the genes as can be observed on Table 3.2. , all had DPBB domains at 5’ terminus and C domains at 3’ terminus.

3.1.5. WES Data Expression Analysis

Table 3.3. Expression of barley expansin genes with detected expression in 16 different tissues obtained from PRJEB14349 [81] WES data via BarleyExpDB [77] from in FPKM values that were colored according to the expression level. Definitions of abbreviations are CAR15 (bracts removed grain 15 days post anthesis (DPA)), CAR5 (bracts removed grain 5 DPA), EMB (embryos at 4 day dissected from germinating grains), EPI (epidermis at 4 weeks), ETI (etiolated 10 day old seedling), INF1 (young inflorescences at 5mm), INF2 (inflorescences between 1-1.5cm), LEA (shoot from the seedlings at 10cm stage), LEM (lemma 6 weeks PA), LOD (lodicule 6 weeks post anthesis (PA)), NOD (developing tillers at six leaf stage), PAL (palea at 6 weeks PA), RAC (rachis at 5 weeks PA), ROO (root at 4 weeks), ROO2 (roots from the seedlings at10cm shoot stage) and SEN (senescing leaf at 2 months).



ID (MorexV3)	Group	CAR15	CAR5	EMB	EPI	ETI	INF1	INF2	LEA	LEM	LOD	NOD	PAL	RAC	ROO	ROO2	SEN
HORVU.MOREX.r3.3HG0297700	A	16	30	32	7	34	2	1	46	46	379	79	170	147	56	35	1
HORVU.MOREX.r3.6HG0615400	A	2	69	21	1	4	144	97	11	49	246	61	105	196	16	13	0
HORVU.MOREX.r3.1HG0071050	A	17	27	16	9	36	36	6	23	38	184	86	123	22	6	1	5
HORVU.MOREX.r3.5HG0526530	A	64	31	9	0	2	19	26	2	6	24	3	16	56	6	5	0
HORVU.MOREX.r3.2HG0185850	A	2	11	9	4	6	11	9	6	8	9	31	9	54	13	14	0
HORVU.MOREX.r3.2HG0104860	A	0	3	38	0	0	0	0	0	0	11	0	0	0	12	13	0
HORVU.MOREX.r3.4HG0401070	A	0	0	9	0	0	0	0	0	0	0	0	0	0	18	23	0
HORVU.MOREX.r3.7HG0723940	A	4	1	1	0	0	4	5	0	3	1	0	7	9	4	5	0
HORVU.MOREX.r3.4HG0401060	A	0	0	11	0	0	0	0	0	0	0	0	0	0	13	19	0
HORVU.MOREX.r3.3HG0256620	A	1	0	8	0	0	0	0	0	0	0	0	0	0	12	17	0
HORVU.MOREX.r3.3HG0252190	A	0	1	4	0	0	0	0	0	2	2	0	6	1	7	14	0
HORVU.MOREX.r3.4HG0401040	A	0	2	5	0	0	0	0	0	0	0	0	0	0	8	14	0
HORVU.MOREX.r3.5HG0533630	A	0	0	5	0	0	0	0	0	0	0	0	0	0	13	9	0
HORVU.MOREX.r3.3HG0293420	A	0	0	3	0	0	0	0	0	1	0	0	5	0	3	11	0
HORVU.MOREX.r3.3HG0293430	A	0	0	2	0	0	0	0	0	0	0	0	3	0	4	11	0

HORVU.MOREX.r3.4HG0396570	A	17	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
HORVU.MOREX.r3.3HG0293450	A	0	0	1	0	0	0	0	0	0	0	0	0	0	3	13	0
HORVU.MOREX.r3.7HG0636890	A	0	0	13	0	0	0	0	0	0	0	0	0	0	3	1	0
HORVU.MOREX.r3.3HG0293490	A	0	0	2	0	0	0	0	0	0	0	0	0	0	3	8	0
HORVU.MOREX.r3.3HG0250020	A	8	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
HORVU.MOREX.r3.7HG0704780	A	1	9	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HORVU.MOREX.r3.3HG0250010	A	6	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
HORVU.MOREX.r3.5HG0532150	A	0	0	0	0	0	0	0	0	0	0	0	0	0	4	3	0
HORVU.MOREX.r3.5HG0532120	A	0	0	1	0	0	0	0	0	0	0	0	0	0	3	3	0
HORVU.MOREX.r3.5HG0532140	A	0	0	0	0	0	0	0	0	0	0	0	0	0	2	3	0
HORVU.MOREX.r3.3HG0223260	A	0	0	2	0	0	0	0	0	0	0	0	0	0	1	1	0
HORVU.MOREX.r3.1HG0054170	A	0	0	2	0	0	0	0	0	0	0	0	0	0	1	1	0
HORVU.MOREX.r3.1HG0054190	A	0	0	2	0	0	0	0	0	0	0	0	0	0	1	1	0
HORVU.MOREX.r3.5HG0519910	A	0	2	0	0	0	0	0	0	0	0	0	1	0	0	1	0
HORVU.MOREX.r3.3HG0250040	A	0	0	2	0	0	0	0	0	0	0	0	0	0	0	1	0
HORVU.MOREX.r3.3HG0252250	A	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
HORVU.MOREX.r3.4HG0347880	A	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
HORVU.MOREX.r3.1HG0054180	A	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
HORVU.MOREX.r3.1HG0056620	B	60	131	42	9	23	4	2	33	150	41	477	590	343	78	41	0
HORVU.MOREX.r3.1HG0056630	B	26	214	47	1	19	79	91	27	64	568	56	170	302	52	53	0
HORVU.MOREX.r3.1HG0056570	B	4	1	97	21	78	0	0	41	3	3	333	4	9	132	156	0
HORVU.MOREX.r3.4HG0415870	B	3	56	83	0	0	99	120	2	0	21	5	1	55	91	75	0
HORVU.MOREX.r3.6HG0601090	B	288	60	0	0	0	0	0	0	0	0	0	0	0	9	10	0
HORVU.MOREX.r3.6HG0601160	B	6	25	35	5	8	2	2	15	3	4	46	8	53	37	50	0
HORVU.MOREX.r3.3HG0219910	B	2	214	0	1	0	0	0	0	0	0	2	0	0	0	0	0
HORVU.MOREX.r3.2HG0183770	B	1	3	9	1	0	13	17	2	1	28	1	2	26	5	4	0
HORVU.MOREX.r3.6HG0601180	B	1	2	21	0	0	1	1	8	0	0	26	2	13	22	15	0
HORVU.MOREX.r3.1HG0056580	B	0	0	16	0	0	0	0	0	0	0	0	1	0	25	51	0
HORVU.MOREX.r3.1HG0056600	B	0	1	0	0	2	0	0	14	1	0	28	1	2	22	13	0
HORVU.MOREX.r3.4HG0415820	B	15	12	4	4	1	6	5	6	0	1	0	2	6	10	3	0

HORVU.MOREX.r3.5HG0439640	B	1	5	4	1	3	11	13	2	4	4	5	4	4	2	1	5
HORVU.MOREX.r3.3HG0318580	B	0	0	0	0	0	0	0	1	0	0	0	0	0	53	5	0
HORVU.MOREX.r3.5HG0508200	B	6	5	2	0	1	8	9	0	1	5	0	1	3	1	1	0
HORVU.MOREX.r3.6HG0543550	B	0	0	0	0	0	0	0	0	0	0	0	0	0	16	21	0
HORVU.MOREX.r3.3HG0318880	B	0	0	0	0	0	0	0	0	0	0	0	0	0	32	4	0
HORVU.MOREX.r3.6HG0543540	B	0	0	0	0	0	0	0	0	0	0	0	0	0	13	21	0
HORVU.MOREX.r3.6HG0592640	B	1	2	2	0	0	2	0	1	0	0	0	0	1	20	4	0
HORVU.MOREX.r3.3HG0318560	B	0	0	0	0	0	0	0	2	0	0	0	0	0	29	0	0
HORVU.MOREX.r3.6HG0598380	B	0	4	2	0	0	0	0	2	0	1	0	1	9	1	1	0
HORVU.MOREX.r3.2HG0189470	B	0	0	0	0	0	0	0	0	5	0	0	13	0	0	0	0
HORVU.MOREX.r3.2HG0189530	B	0	0	8	0	0	0	0	0	0	0	0	0	0	2	2	0
HORVU.MOREX.r3.3HG0318870	B	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1	0
HORVU.MOREX.r3.2HG0195810	B	0	0	2	0	0	0	0	0	0	0	0	0	0	2	1	0
HORVU.MOREX.r3.2HG0107640	B	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
HORVU.MOREX.r3.2HG0189540	B	0	0	2	0	0	0	0	0	0	0	0	0	0	1	0	0
HORVU.MOREX.r3.4HG0409870	LA	3	13	12	21	7	6	3	11	4	7	6	3	2	24	5	4
HORVU.MOREX.r3.1HG0054870	LA	0	2	1	4	3	0	0	1	13	4	9	6	6	1	9	0
HORVU.MOREX.r3.4HG0409760	LA	0	39	0	0	0	0	0	0	0	0	0	1	0	0	0	0
HORVU.MOREX.r3.4HG0409710	LA	1	2	3	6	3	0	0	5	2	1	3	1	1	5	1	4
HORVU.MOREX.r3.7HG0749090	LA	0	1	1	1	8	0	0	1	0	0	3	0	1	0	0	0

The WES data from PRJEB14349 [81] that shows expression of barley genes on 16 different barley tissues showed only 65 of the 78 found barley expansins at Table 3.3. had detectible levels of expression in any given tissue. Beside the 14 expansin gene (7 EXPA, 10 EXPB and 1 EXLA) that were unexpressed across all tissues, many other barley expansins had very low expression levels that were commonly only restricted to one or two tissues such as HORVU.MOREX.r3.2HG0189540. Expression profiles of the barley expansins has varied significantly between tissues where tissue from roots had the most prevalent occurrence of detectible expression of barley expansins across all expansin groups.

Table 3.4. Total expression of barley expansin at 16 different tissues from PRJEB14349 [81] data given in the order of higher to lower total expression with coloring.

Name of the Tissue Samples	**Total Expression of Barley Expansins on all Given Tissue Samples (FPKM)**
Lodicule (6 weeks PA)	1544,12
Rachis (5 weeks PA)	1320,55
Developing tillers (six leaf stage)	1260,64
Palea (6 weeks PA)	1260,35
Grain, bracts removed (5DPA)	982,3
Root (4 weeks)	902,47
Roots from the seedlings (10cm shoot stage)	789,52
Embryos (4d dissected from germinating grains)	603,83
Bracts removed grain 15 days post anthesis	554,79
Young Inflorescences (5mm)	448,69
Inflorescences (1-1.5cm)	408,88
Lemma (6 weeks PA)	404,09
Shoot from the seedlings (10cm shoot stage)	262,92
Etiolated (10 day old seedling)	240,13
Epidermis (4 weeks)	95,71
Senescing leaf (2 months)	21,05

As it can be observed at Table 3.4. , the tissue with the highest total barley expression detected is 6 weeks post anthesis lodicule and lowest is 10 day old etiolated seedlings.

Table 3.5. Expression of barley expansin genes with detected expression in radicle tissue during different stages of seed germination. WES data is obtained from PRJNA496380 [84] via BarleyExpDB [77] from in FPKM values that were colored according to the expression level.

ID (MorexV3)	**Group**	**8 Hour**	**16 Hour**	**24 Hour**	**32 Hour**	**40 Hour**	**48 Hour**
HORVU.MOREX.r3.3HG0297700	A	198	189	90	97	42	86
HORVU.MOREX.r3.6HG0615400	A	21	15	47	31	6	7
HORVU.MOREX.r3.1HG0071050	A	6	20	16	24	14	26
HORVU.MOREX.r3.5HG0526530	A	0	2	4	46	8	30
HORVU.MOREX.r3.2HG0185850	A	9	10	13	23	14	20
HORVU.MOREX.r3.3HG0256620	A	19	2	7	2	20	5
HORVU.MOREX.r3.7HG0723940	A	1	2	4	4	0	2
HORVU.MOREX.r3.2HG0104860	A	0	0	1	10	1	1
HORVU.MOREX.r3.3HG0252190	A	0	0	0	8	0	2
HORVU.MOREX.r3.4HG0401040	A	2	0	0	0	1	0
HORVU.MOREX.r3.3HG0250010	A	0	0	0	0	0	0
HORVU.MOREX.r3.4HG0415870	B	7	57	377	502	170	248
HORVU.MOREX.r3.1HG0056630	B	0	5	10	119	12	75
HORVU.MOREX.r3.1HG0056570	B	0	1	6	113	24	46
HORVU.MOREX.r3.1HG0056600	B	0	0	0	69	4	20
HORVU.MOREX.r3.5HG0508200	B	3	2	3	5	5	5
HORVU.MOREX.r3.4HG0415820	B	0	0	0	5	3	5
HORVU.MOREX.r3.5HG0439640	B	2	2	3	1	2	1
HORVU.MOREX.r3.1HG0056620	B	0	0	0	5	0	0
HORVU.MOREX.r3.2HG0183770	B	0	0	0	1	0	1
HORVU.MOREX.r3.1HG0056580	B	0	0	0	1	0	0
HORVU.MOREX.r3.6HG0598380	B	0	0	0	0	0	1
HORVU.MOREX.r3.4HG0409870	LA	23	18	21	46	8	15
HORVU.MOREX.r3.1HG0054870	LA	12	7	2	1	0	0

The WES data from PRJNA496380 [84] that shows expression of barley expansin genes with detected expression in radicle tissue during different stages of seed germination showed only 24 of the 78 found barley expansins at Table 3.5. had detectible levels of expression at any given period of germination. Beside the 54 expansin gene (28 EXPA, 22 EXPB and 4 EXLA) that were unexpressed across different stages of germination in radicle tissue, many expansins across all groups had insignificant expression such as HORVU.MOREX.r3.2HG0183770. Expression profiles of the barley expansins has varied significantly between germination stages and between the genes.

Table 3.6. Total expression of barley expansin genes in radicle tissue during different stages of seed germination as FPKM from PRJNA496380 [84] WES data.

Time After Germination Begun	8 Hour	16 Hour	24 Hour	32 Hour	40 Hour	48 Hour
Total Expression of Barley Expansins	302	330	606	1112	335	596

Total expression results of the barley expansin genes in radicle tissue is shows the variance of expression between the genes and stages of germination as it is observed on Table 3.6. Total expression increases between 8th and 32nd hours of germination where it peaks, after the peak total expression gets significantly decreased at 40th hour to rise again at 48th hour.

Table 3.7. Expression of barley expansin genes with detected expression in different root parts of Clipper variant barley 3 days after germination, where control plants were treated with 0 mM NaCl nutrient medium and salt group were treated with 100 mM NaCl nutrient medium. WES data is obtained from PRJEB13621 [83] via BarleyExpDB [77] in FPKM values which were colored according to their expression levels and the changes of expression (expression of salt/control) were given.

ID (MorexV3)	**Group**	**Meristematic Zone (Control)**	**Meristematic Zone (Salt)**	**Change**	**Elongation Zone (Control)**	**Elongation Zone (Salt)**	**Change**	**Maturation Zone (Control)**	**Maturation Zone (Salt)**	**Change**
HORVU.MOREX.r3.7HG0636890	**A**	7	9	31%	112	111	-1%	35	35	1%
HORVU.MOREX.r3.3HG0297700	**A**	85	104	22%	53	76	43%	32	15	-52%
HORVU.MOREX.r3.2HG0104860	**A**	89	103	16%	34	43	29%	25	1	-96%

HORVU.MOREX.r3.4HG0401070	**A**	31	49	59%	45	78	73%	11	3	-73%
HORVU.MOREX.r3.4HG0401040	**A**	52	68	29%	0	5	N/A	13	0	-97%
HORVU.MOREX.r3.4HG0401060	**A**	43	55	27%	19	27	39%	13	1	-96%
HORVU.MOREX.r3.3HG0223260	**A**	41	47	13%	15	9	-39%	22	8	-64%
HORVU.MOREX.r3.3HG0256620	**A**	39	54	39%	0	6	1087%	10	0	-96%
HORVU.MOREX.r3.2HG0185850	**A**	16	16	-1%	12	12	-3%	15	23	60%
HORVU.MOREX.r3.3HG0293430	**A**	5	12	130%	16	61	278%	3	3	0%
HORVU.MOREX.r3.3HG0293420	**A**	4	11	158%	16	58	252%	2	3	32%
HORVU.MOREX.r3.5HG0533630	**A**	19	25	31%	1	3	384%	4	0	-100%
HORVU.MOREX.r3.3HG0293450	**A**	4	9	116%	10	53	415%	2	3	60%
HORVU.MOREX.r3.4HG0396570	**A**	5	6	16%	3	3	-6%	5	4	-26%
HORVU.MOREX.r3.3HG0293490	**A**	5	9	70%	13	34	163%	2	2	-21%
HORVU.MOREX.r3.3HG0252190	**A**	5	9	68%	15	23	54%	2	1	-43%
HORVU.MOREX.r3.7HG0723940	**A**	1	2	46%	10	9	-10%	8	11	36%
HORVU.MOREX.r3.6HG0615400	**A**	10	11	12%	3	4	25%	10	9	-9%
HORVU.MOREX.r3.4HG0347880	**A**	7	7	6%	3	2	-29%	5	4	-22%
HORVU.MOREX.r3.1HG0054190	**A**	2	3	36%	7	18	144%	1	0	-66%
HORVU.MOREX.r3.5HG0532120	**A**	2	4	118%	14	27	92%	1	1	75%
HORVU.MOREX.r3.5HG0526530	**A**	1	1	21%	7	5	-28%	10	14	32%
HORVU.MOREX.r3.4HG0401310	**A**	0	1	265%	15	27	72%	1	2	24%
HORVU.MOREX.r3.1HG0054170	**A**	2	3	13%	7	18	158%	2	0	-68%
HORVU.MOREX.r3.5HG0532140	**A**	1	3	190%	9	21	122%	1	1	32%
HORVU.MOREX.r3.5HG0532150	**A**	0	2	1283%	3	15	478%	0	1	N/A
HORVU.MOREX.r3.3HG0250020	**A**	5	4	-17%	2	1	-59%	3	0	-85%
HORVU.MOREX.r3.3HG0250040	**A**	4	4	-1%	1	2	63%	4	3	-14%
HORVU.MOREX.r3.1HG0054180	**A**	1	1	46%	3	6	119%	0	0	-63%
HORVU.MOREX.r3.3HG0252250	**A**	2	3	60%	8	12	43%	1	1	-38%
HORVU.MOREX.r3.3HG0250010	**A**	5	4	-12%	2	1	-60%	3	0	-83%
HORVU.MOREX.r3.1HG0071050	**A**	1	2	49%	4	3	-37%	2	3	64%
HORVU.MOREX.r3.7HG0722730	**A**	1	1	66%	1	1	-16%	0	0	-45%
HORVU.MOREX.r3.4HG0401290	**A**	0	0	N/A	4	8	88%	0	0	667%
HORVU.MOREX.r3.5HG0519910	**A**	3	3	0%	0	0	N/A	1	0	-100%
HORVU.MOREX.r3.1HG0056570	**B**	222	264	19%	623	636	2%	118	51	-57%
HORVU.MOREX.r3.4HG0415870	**B**	183	203	11%	57	106	86%	52	6	-89%
HORVU.MOREX.r3.6HG0601160	**B**	78	83	5%	159	184	15%	63	47	-26%
HORVU.MOREX.r3.1HG0056620	**B**	10	13	29%	111	116	5%	29	25	-12%
HORVU.MOREX.r3.2HG0189530	**B**	7	8	9%	91	89	-3%	32	31	-3%
HORVU.MOREX.r3.6HG0601180	**B**	41	48	17%	113	150	33%	33	25	-24%
HORVU.MOREX.r3.1HG0056580	**B**	84	115	37%	56	114	102%	25	5	-81%
HORVU.MOREX.r3.1HG0056630	**B**	42	52	24%	17	25	48%	38	37	-1%
HORVU.MOREX.r3.2HG0195810	**B**	25	28	13%	9	5	-42%	11	3	-74%
HORVU.MOREX.r3.6HG0592640	**B**	1	5	234%	4	12	175%	1	3	296%
HORVU.MOREX.r3.2HG0189540	**B**	7	8	6%	3	2	-47%	4	3	-31%
HORVU.MOREX.r3.3HG0318580	**B**	0	0	N/A	1	1	78%	1	6	367%

HORVU.MOREX.r3.6HG0543550	**B**	3	5	54%	9	17	81%	2	1	-69%
HORVU.MOREX.r3.6HG0543540	**B**	3	5	65%	9	16	80%	2	1	-71%
HORVU.MOREX.r3.4HG0415820	**B**	0	2	251%	2	2	-6%	2	6	244%
HORVU.MOREX.r3.2HG0183770	**B**	4	4	1%	4	4	11%	4	4	-3%
HORVU.MOREX.r3.3HG0318560	**B**	0	0	N/A	0	0	70%	1	4	423%
HORVU.MOREX.r3.5HG0439640	**B**	2	2	20%	0	0	N/A	1	0	-87%
HORVU.MOREX.r3.3HG0318870	**B**	0	0	N/A	0	0	N/A	0	0	N/A
HORVU.MOREX.r3.3HG0318880	**B**	0	0	N/A	0	0	N/A	0	0	N/A
HORVU.MOREX.r3.5HG0508200	**B**	2	2	27%	0	0	N/A	0	0	-100%
HORVU.MOREX.r3.6HG0601090	**B**	0	0	N/A	0	0	N/A	6	2	-71%
HORVU.MOREX.r3.1HG0056600	**B**	1	1	74%	1	1	-21%	1	0	-55%
HORVU.MOREX.r3.6HG0598380	**B**	0	1	65%	0	0	N/A	0	0	-100%
HORVU.MOREX.r3.4HG0409870	**LA**	1	2	86%	17	13	-21%	17	33	98%
HORVU.MOREX.r3.4HG0409710	**LA**	0	0	-100%	12	16	37%	16	22	39%
HORVU.MOREX.r3.1HG0054870	**LA**	0	0	-100%	7	5	-36%	8	14	83%
HORVU.MOREX.r3.7HG0749090	**LA**	0	0	33%	3	4	10%	1	0	-73%

As can be observed on Table 3.7. , 63 of 78 barley expansin genes had detectible expression in any different root parts of Clipper variant barley 3 days after germination obtained from PRJEB13621 [83] which 15 genes without expression were 4 EXPA, 9 EXPB and 2 EXLA. Change of expression from control plants were treated with 0 mM NaCl nutrient medium and salt group were treated with 100 mM NaCl nutrient solutions had significant variance between the parts of sample roots. Results on Table 3.5. shows that of the 63 barley expansin genes 51 were up regulated and 4 were down regulated in meristematic part of the barley root, 38 were up regulated and 18 were down regulated in elongation part of barley the root and 18 were up regulated and 48 were down regulated in maturation part of the barley root.

Table 3.8. Expression of barley expansin genes with detected expression in different parts of seminal roots where water deficient group barleys were treated with PEG-8000 in their nutrient medium compared to control group which were untreated. Parts of the roots that samples were taken are A (0-12.5% of the total seminal root length), B (25-37.5% of the total seminal root length) and C (50-62.5% of the total seminal root length). WES data is obtained from PRJNA439267 [94] via BarleyExpDB [77] in FPKM values which were colored according to their expression levels and the changes of expression (expression of water deficient/control) were given.

ID (MorexV3)	**Group**	**Control A**	**Water Deficient A**	**Change**	**Control-B**	**Water Deficient B**	**Change**	**Control C**	**Water Deficient C**	**Change**
HORVU.MOREX.r3.4HG0401070	**A**	41	80	94%	14	5	-62%	34	56	64%
HORVU.MOREX.r3.3HG0297700	**A**	25	80	220%	10	15	48%	13	41	201%
HORVU.MOREX.r3.4HG0401060	**A**	45	65	44%	9	3	-66%	22	38	74%
HORVU.MOREX.r3.4HG0401040	**A**	29	45	55%	16	7	-53%	21	47	120%
HORVU.MOREX.r3.3HG0256620	**A**	23	42	83%	17	8	-53%	20	41	104%
HORVU.MOREX.r3.5HG0532150	**A**	17	43	146%	5	3	-41%	14	25	79%
HORVU.MOREX.r3.5HG0533630	**A**	13	36	173%	4	5	14%	11	28	161%
HORVU.MOREX.r3.5HG0532120	**A**	15	23	53%	7	2	-78%	17	19	14%
HORVU.MOREX.r3.2HG0185850	**A**	11	16	49%	10	12	21%	9	14	56%
HORVU.MOREX.r3.3HG0252190	**A**	8	14	63%	9	3	-70%	13	23	68%
HORVU.MOREX.r3.7HG0636890	**A**	8	11	41%	4	2	-41%	15	13	-16%
HORVU.MOREX.r3.3HG0293490	**A**	4	18	325%	3	2	-30%	5	18	251%
HORVU.MOREX.r3.3HG0293430	**A**	4	12	221%	5	2	-48%	7	19	173%
HORVU.MOREX.r3.3HG0223260	**A**	9	16	77%	2	2	13%	4	14	254%
HORVU.MOREX.r3.5HG0532140	**A**	8	16	97%	3	1	-68%	8	11	42%
HORVU.MOREX.r3.4HG0401310	**A**	10	16	61%	2	1	-73%	7	7	-4%
HORVU.MOREX.r3.3HG0293450	**A**	4	6	76%	6	2	-60%	11	15	37%
HORVU.MOREX.r3.3HG0293420	**A**	4	14	302%	2	1	-42%	4	17	332%
HORVU.MOREX.r3.5HG0526530	**A**	20	9	-57%	2	1	-23%	7	1	-84%
HORVU.MOREX.r3.7HG0723940	**A**	5	6	10%	2	4	58%	5	5	-9%
HORVU.MOREX.r3.2HG0104860	**A**	14	7	-52%	0	0	N/A	0	0	N/A
HORVU.MOREX.r3.6HG0615400	**A**	4	5	32%	2	2	-12%	3	3	18%
HORVU.MOREX.r3.1HG0054170	**A**	3	2	-41%	2	1	-74%	8	3	-64%
HORVU.MOREX.r3.4HG0347880	**A**	4	7	55%	0	1	509%	1	5	618%

HORVU.MOREX.r3.5HG0519910	**A**	4	3	-15%	2	1	-72%	3	4	34%
HORVU.MOREX.r3.3HG0250010	**A**	4	5	28%	1	1	-28%	1	5	280%
HORVU.MOREX.r3.1HG0054190	**A**	3	3	0%	1	0	-66%	6	3	-49%
HORVU.MOREX.r3.4HG0396570	**A**	2	7	190%	0	1	295%	0	4	678%
HORVU.MOREX.r3.3HG0250020	**A**	3	4	32%	1	1	-11%	1	4	243%
HORVU.MOREX.r3.4HG0401290	**A**	4	4	26%	1	0	-82%	2	2	-27%
HORVU.MOREX.r3.3HG0250040	**A**	3	3	19%	1	1	-42%	1	3	167%
HORVU.MOREX.r3.7HG0722730	**A**	1	2	115%	2	1	-46%	1	3	163%
HORVU.MOREX.r3.1HG0071050	**A**	1	2	64%	1	0	-62%	0	1	303%
HORVU.MOREX.r3.1HG0054180	**A**	1	1	8%	0	0	-60%	2	1	-47%
HORVU.MOREX.r3.4HG0401050	**A**	0	2	408%	0	0	N/A	0	0	N/A
HORVU.MOREX.r3.1HG0056580	**B**	62	93	51%	40	11	-73%	58	79	34%
HORVU.MOREX.r3.1HG0056570	**B**	83	119	44%	27	8	-69%	41	43	6%
HORVU.MOREX.r3.4HG0415870	**B**	65	89	38%	18	6	-68%	37	38	4%
HORVU.MOREX.r3.1HG0056630	**B**	23	42	83%	10	8	-25%	10	10	-6%
HORVU.MOREX.r3.6HG0543540	**B**	11	15	38%	11	3	-71%	24	26	8%
HORVU.MOREX.r3.6HG0543550	**B**	10	13	31%	10	3	-74%	22	24	9%
HORVU.MOREX.r3.1HG0056620	**B**	14	14	0%	24	19	-21%	6	6	7%
HORVU.MOREX.r3.6HG0601160	**B**	13	29	117%	4	2	-41%	6	9	44%
HORVU.MOREX.r3.2HG0195810	**B**	10	16	55%	4	2	-52%	7	18	150%
HORVU.MOREX.r3.2HG0189540	**B**	5	28	497%	0	1	232%	0	4	N/A
HORVU.MOREX.r3.2HG0189530	**B**	7	5	-28%	3	1	-65%	13	7	-44%
HORVU.MOREX.r3.4HG0415820	**B**	3	8	177%	7	7	2%	3	6	130%
HORVU.MOREX.r3.3HG0318580	**B**	1	22	3338%	1	0	-66%	1	8	1124%
HORVU.MOREX.r3.6HG0601180	**B**	5	10	111%	1	1	-23%	2	3	103%
HORVU.MOREX.r3.6HG0592640	**B**	2	14	820%	0	0	78%	0	3	3388%
HORVU.MOREX.r3.2HG0183770	**B**	4	3	-27%	2	3	103%	2	2	15%
HORVU.MOREX.r3.5HG0439640	**B**	2	4	54%	2	2	-18%	2	3	32%
HORVU.MOREX.r3.3HG0318560	**B**	0	9	9956%	0	0	-64%	0	2	N/A
HORVU.MOREX.r3.6HG0601090	**B**	2	4	79%	2	2	-9%	0	0	27%
HORVU.MOREX.r3.1HG0056600	**B**	2	5	166%	1	0	-50%	0	1	N/A
HORVU.MOREX.r3.3HG0318880	**B**	0	4	1725%	1	0	-100%	0	2	1143%
HORVU.MOREX.r3.5HG0508200	**B**	1	2	23%	1	0	-82%	1	2	70%
HORVU.MOREX.r3.4HG0409870	**LA**	10	25	157%	9	14	60%	10	13	26%
HORVU.MOREX.r3.4HG0409710	**LA**	3	8	199%	8	14	71%	12	7	-42%
HORVU.MOREX.r3.1HG0054870	**LA**	3	8	146%	5	5	3%	9	5	-44%
HORVU.MOREX.r3.7HG0749090	**LA**	0	0	344%	0	0	N/A	1	0	-100%

Expression of barley expansin genes with detected expression in different parts of seminal roots medium which were A (0-12.5% of the total seminal root length), B (25-37.5% of the total seminal root length) and C (50-62.5% of the total seminal root length) from WES data is obtained from PRJEB13621 [94] 61 of 78 had detectible expression of barley expansins in any tissues.4 EXPA, 11 EXPB and 2 EXLA constituted the 17 gene that had no expression on any tissue. Change of expression between water deficient groups barleys that were treated with PEG-8000 in their nutrient compared to control groups which were untreated varied significantly between the parts of roots taken. Between barley expansins 52 up regulated 7 down regulated in 0-12.5% part of seminal root, 14 up regulated and 38 down regulated in 25-37.5% part of seminal root, 43 up regulated and 13 down regulated in 50-62.5% part of seminal root.

3.2. Physiological Analyses of Drought Treatment

3.2.1. Images of Barley Plants During Stress Treatment

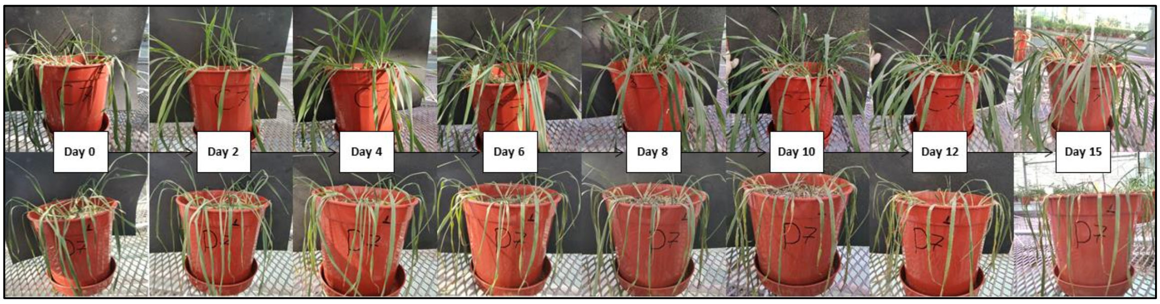


Figure 3.12. Images of control (upper row) and drought (lower row) plants from day 0 to day 15 during drought stress application.

Figure 3.12. shows the difference that the drought stress treatment created on observable physiologies of barley plants between control group plants that were watered and drought group plants that were cut from watering. Between day 0 and 15 it is observable that the leaves of drought plants had lost their mechanical strength and bowed down where control plants maintained their turgor pressure and kept their leaves tensile against gravity.

3.2.2. Relative Water Content Analysis

$$Relative\ Water\ Content\ (\%) = \left(\frac{Fresh\ Weight - Dry\ Weight}{Turgid\ Weight - Dry\ Weight}\right) \times 100$$ [85] (3.1)

$$Standard\ deviation\ (SD) = \sqrt{\frac{\sum(xi - Mean\ x)^2}{N-1}}$$ [95] (3.2)

N= Number of replicates

Table 3.9. Leaf relative water content of the barley during stress treatment.

Day	Relative Water Content (%) (Equation 3.1)		SD (Equation 3.2)	
	Control	Drought	Control	Drought
0	61,52	60,30	2,55	1,77
2	59,44	58,68	5,20	2,07
4	78,15	65,54	2,78	4,18
6	80,08	56,86	3,61	3,55
8	79,99	68,56	0,87	0,75
10	76,79	59,46	0,09	5,17
12	78,22	52,46	1,09	6,57
15	74,54	41,56	2,26	3,19

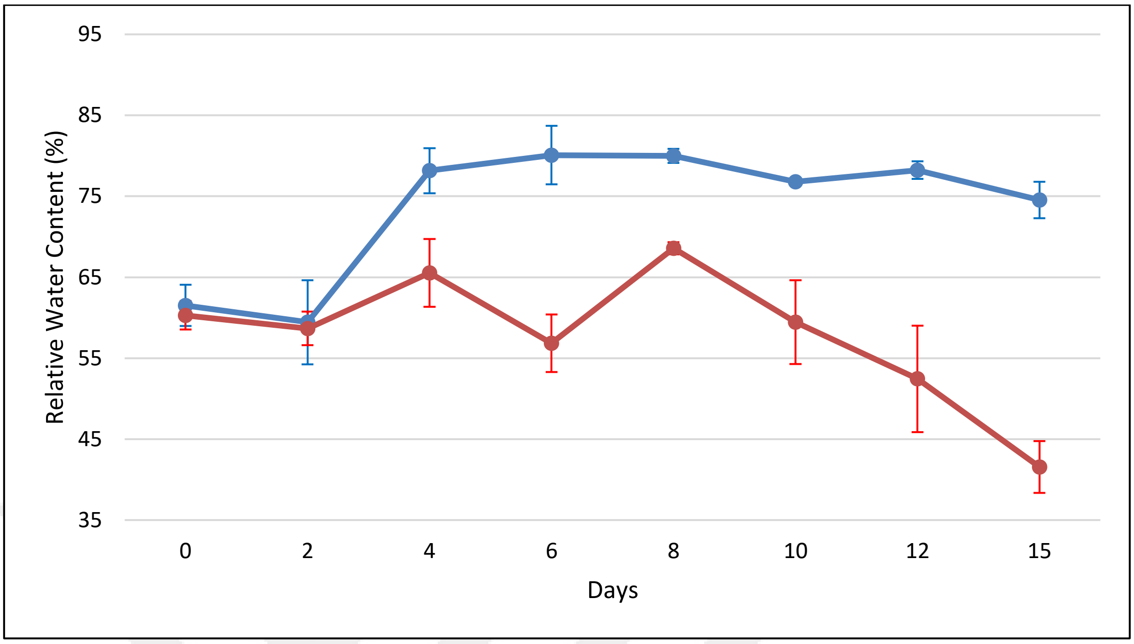


Figure 3.13. Mean relative water content (RWC) of the control (blue) and drought (red) group barley leaves during the 15 day stress treatment period, error bars were set by normal distribution of each samples SD.

Mean relative water content during the drought stress treatment as observed on Figure 3.13. deviated after 2nd day and the difference between control and drought groups increased to %29.98 by the end of drought treatment as can be observed at Table 3.9. at 15th day where drought group plants lost significant relative water content.

3.2.3. Leaf Chlorophyll Content Analysis

Table 3.10. Chlorophyll contents of control and drought plants during stress treatment with their standard deviations.

Day	Chlorophyll content (nM/cm2)		SD (Equation 3.2)	
	Control	Drought	Control	Drought
0	50,46	51,02	4,79	4,47
2	49,06	49,98	4,18	4,95
4	49,86	50,50	8,95	3,95
6	50,04	49,87	4,46	4,38
8	51,61	49,27	7,02	4,57

10	54,41	50,42	6,10	5,37
12	56,24	47,94	6,34	4,93
15	56,78	44,00	6,58	8,33

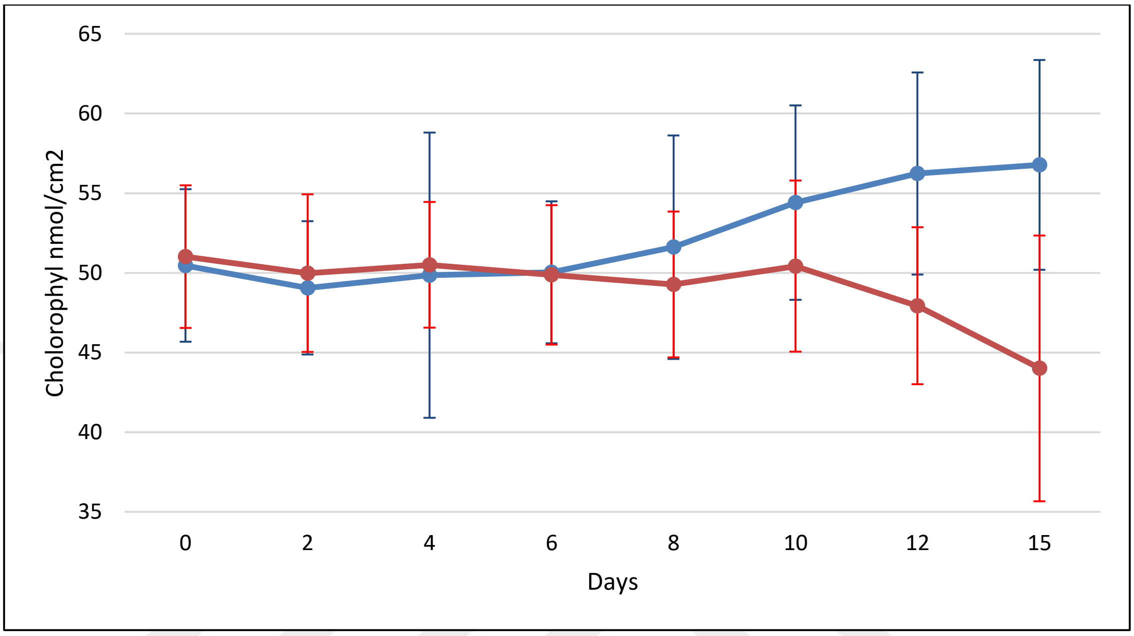


Figure 3.14. Mean chlorophyll content change between 0th and 15th days of drought stress treatment in leaves of control (blue) and drought (red) barley plants, error bars were set by normal distribution of each samples SD.

Difference between chlorophyll content of control and drought group barley leaves started to increase at 6th day of stress treatment as it could be observed on Figure 3.14. By the 15th day mean chlorophyll content difference between control and drought groups increased to 12.78 nM/cm2 as it can be observed on Table 3.10. where the chlorophyll content of drought decrease and control plants increased.

3.3. Differential Expression Analysis

3.3.1. Selection of Primer Pair Melting Temperature Values

Table 3.11. List of mean cycle of quantity (Cq) of target genes from gradient qPCR done in Tm range of approximently -2 ˚C and +2 ˚C of mean of theoretical Tm values of primer pairs of selected genes which the chosen Tm for the qPCR is highlighted in bold and N/A is non available.

EXPA1		**EXPA2**		**EXPB1**		**EXPB2**		**EXPB3**		**HvMYB1**	
Tm Temperature ˚C	Mean Cq	Tm Temperature ˚C	Mean Cq	Tm Temperature ˚C	Mean Cq	Tm Temperature ˚C	Mean Cq	Tm Temperature ˚C	Mean Cq	Tm Temperature ˚C	Mean Cq
57,5	30,84	58	N/A	58	33,74	58	34,64	58	34,81	58	32,27
56,6	27,75	57,5	36,83	57,5	33,75	57,5	36,76	57,5	31,41	57,5	30,29
54,9	27,29	56,6	33,03	56,6	31,21	56,6	31,24	56,6	29,88	56,6	29,35
52,9	**25,63**	54,9	31,13	54,9	28,41	54,9	29,45	54,9	28,04	**54,9**	**26,21**
51,2	32,09	**52,9**	**27,20**	52,9	27,81	52,9	26,31	52,9	26,27	52,9	26,64
50,1	30,44	51,2	31,91	**51,2**	**27,10**	**51,2**	**26,10**	**51,2**	**25,48**	51,2	26,74

With selection of lowest Cq from use of different Tm values in gradient qPCR Tm values of genes that will be used in differential expression analysis were determined as; 52.9 ˚C for EXPA1, 52.9 ˚C for EXPA2, 51.2˚C for EXPB1, 51.2˚C for EXPB2, 51.2˚C for EXPB3 and 54.9 ˚C for HvMYB1 as it can be observed on Table 3.11. .

3.3.2. Assessment of Quality and Quantity of Obtained Total RNA Samples

Table 3.12. Spectrometric Analysis of Total RNA samples that were measured by nanodrop with rations of wawelength of 260 nm wawelength to 280 nm and 260 nm to 230 nm.

Sample Name	Nucleic Acid Concentration (ng/µL)	Absorbance at 260nm/280nm	Absorbance at 260nm/230nm

Day 6 Control 1	93,62	1,68	0,84
Day 6 Control 2	92,21	1,91	1,24
Day 6 Control 3	74,75	1,87	1,1
Day 6 Drought 1	449,01	2,15	1,85
Day 6 Drought 2	427,05	2,13	1,87
Day 6 Drought 3	514,88	2,13	1,89
Day 12 Control 1	259,31	1,97	1,38
Day 12 Control 2	145,87	1,94	1,2
Day 12 Control 3	122,6	1,99	1,33
Day 12 Drought 1	1008,01	2,17	2,14
Day 12 Drought 2	452,41	2,14	1,67
Day 12 Drought 3	490,33	2,16	1,95

The spectrometric analysis of isolated Total RNA samples shows significant difference between control and drought samples, where total isolated nucleic acid concentration of control groups were significantly lower than drought groups as it can be observed on Table 3.12. Also there is significant difference between the absorbance rations between control and drought group samples. Control group samples had mean rations of 1.89 at 260nm/280nm and 1.18 at 230nm/280nm where drought group samples had mean rations of 2.14 at 260nm/280nm and 1.89 at 230nm/280nm.

3.3.3. Differential Expression Analysis of Target Genes

$$Mean\ Cq = \frac{\sum Cq}{N} \qquad [95](3.3)$$

N= Number of replicates

$$Standard\ deviation\ (SD) = \sqrt{\frac{\sum(Cq_{Observation} - Mean\ Cq)^2}{N-1}}$$ [95] (3.4)

Table 3.13. Expression of target genes on different sample groups where Cq is cycle of quantitiy, N is number of replicates that are available to be used in analysis and SD is standard deviation calculated by equation 3.2.

Gene Name	Day 6 Control			Day 6 Drought			Day 12 Control			Day 12 Drought		
	Mean Cq	N	SD	Mean Cq	N	SD	Mean Cq	N	SD	Mean Cq	N	SD
MYB1	27,56	3	0,24	26,06	9	1,65	30,32	4	1,52	25,54	9	1,90
EXPA1	33,21	5	4,54	25,72	6	0,48	30,74	3	1,40	26,05	9	0,78
EXPA2	34,17	6	1,86	28,54	9	0,83	30,25	3	6,74	26,47	7	0,50
EXPB1	31,56	4	1,95	26,99	9	1,09	35,83	3	7,15	27,21	9	0,68
EXPB2	29,28	2	0,68	28,64	9	2,51	34,64	4	5,30	26,84	9	0,40
EXPB3	30,05	2	1,11	26,89	9	0,97	32,58	6	2,33	26,43	9	0,35

$$\Delta \mathrm{Cq} = \mathrm{Cq}\ (target)\ - Cq\ (reference)$$ [87] (3.5)

$$\Delta\Delta \mathrm{Cq} = \ \Delta Cq\ (drought)\ -\ \Delta Cq\ (control)$$ [87] (3.6)

$$\text{Differential Expression} =\ 2^{\ -[\Delta\Delta \mathrm{Cq}]}$$ [87] (3.7)

Table 3.14. Relative expressions of barley expansin genes and HvMYB1 where HvMYB1 used as reference gene for calculations for expansins. Days of the samples taken are indicated by the number at beginning of each sample, C is abbreviation for control and D is abbreviation of drought groups.

Name	**Mean Cq**	**Number of replicates**	**Standard Deviation (Equation 3.4)**	**ΔCq (Equation 3.5)**	**Differential Expression (Equation 3.7)**	**Fold Change Between Day 6 and 12 ($RF_{(Drought)}$/ $RF_{(Control)}$)**
6C HvMYB1	27,56	3	0,24			
6D HvMYB1	26,06	9	1,65			
12C HvMYB1	30,32	4	1,52			
12D HvMYB1	25,54	9	1,90			
6C EXPA1	33,21	5	4,54	5,65	63,96	11,06
6D EXPA1	25,72	6	0,48	-0,34		
12C EXPA1	30,74	3	1,40	5,20	707,54	
12D EXPA1	26,05	9	0,78	-4,27		
6C EXPA2	34,17	6	1,86	6,61	17,52	21,61
6D EXPA2	28,54	9	0,83	2,48		
12C EXPA2	30,25	3	6,74	4,71	378,66	
12D EXPA2	26,47	7	0,50	-3,85		
6C EXPB1	31,56	4	1,95	4,00	8,42	1287,18
6D EXPB1	26,99	9	1,09	0,93		
12C EXPB1	35,83	3	7,15	10,29	10834,41	
12D EXPB1	27,21	9	0,68	-3,11		
6C EXPB2	29,28	2	0,68	1,72	0,55	11089,79
6D EXPB2	28,64	9	2,51	2,58		
12C EXPB2	34,64	4	5,30	9,10	6124,08	
12D EXPB2	26,84	9	0,40	-3,48		
6C EXPB3	30,05	2	1,11	2,49	3,17	615,95
6D EXPB3	26,89	9	0,97	0,83		
12C EXPB3	32,58	6	2,33	7,04	1952,51	
12D EXPB3	26,43	9	0,35	-3,89		

$$\text{Relative Expression} = 2^{-[(\text{Mean }(\Delta\text{Cq (observation)}) - (\text{Mean }\Delta\text{Cq reference})]} \quad [87]\ (3.8)$$

$$\text{Minimum Relative Expression} = 2^{-[(\text{Mean }(\Delta\text{Cq (observation)}+\text{SD}) - (\text{Mean }\Delta\text{Cq reference})]} \quad [87]\ (3.9)$$

$$\text{Maximum Relative Expression} = 2^{-[(\text{Mean }(\text{Cq (observation)}-\text{SD}) - \text{Mean }\Delta\text{Cq (reference)}]} \quad [87]\ (3.10)$$

Table 3.15. Relative expression and maximum and minimum relative expression calculation by normal distribution of standard deviation of target barley expansins on different sample types.

Name	**SD (Equation 3.4)**	**Relative Expression (Equation 3.8)**	**Minimum Relative Expression (Equation 3.9)**	**Maximum Relative Expression (Equation 3.10)**
6C EXPA1	5,08	0,02	0,00	0,67
6D EXPA1	0,52	1,27	0,88	1,83
12C EXPA1	1,71	0,03	0,01	0,09
12D EXPA1	0,83	19,29	10,84	34,35
6C EXPA2	2,04	0,01	0,00	0,04
6D EXPA2	0,88	0,18	0,10	0,33
12C EXPA2	8,25	0,04	0,00	11,61
12D EXPA2	0,54	14,43	9,93	20,99
6C EXPB1	2,25	0,06	0,01	0,30
6D EXPB1	1,16	0,53	0,24	1,17
12C EXPB1	8,75	0,00	0,00	0,34
12D EXPB1	0,72	8,65	5,25	14,28
6C EXPB2	0,96	0,30	0,16	0,59
6D EXPB2	2,66	0,17	0,03	1,06
12C EXPB2	6,12	0,00	0,00	0,13
12D EXPB2	0,43	11,14	8,30	14,96
6C EXPB3	1,57	0,18	0,06	0,53
6D EXPB3	1,03	0,56	0,28	1,15
12C EXPB3	2,55	0,01	0,00	0,04
12D EXPB3	0,37	14,80	11,44	19,15

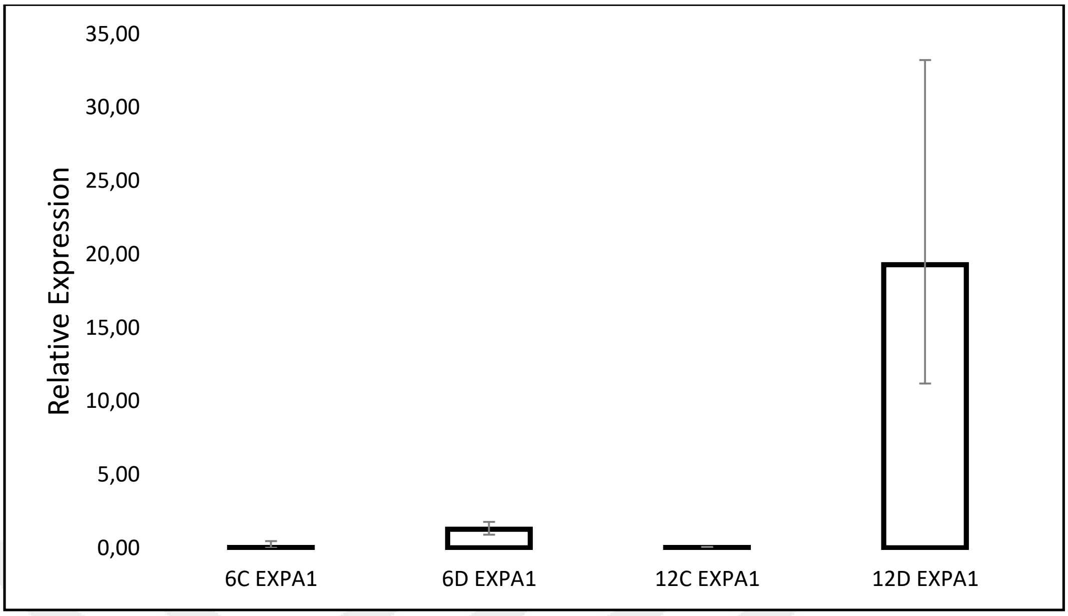


Figure 3.15. Relative expression of EXPA1 on barley root samples from control and drought groups at 6th and 12th days of drought stress treatment. Error bars were set by normal distribution of each samples SD.

Relative expression of EXPA1 gene was higher in drought groups both in 6th and 12th day and was significantly up regulated in roots treated with drought stress from 6th to 12th days. Expression of EXPA1 in control groups seems stable between 6th and 12th day samples as it can be observed on Figure 3.15. .

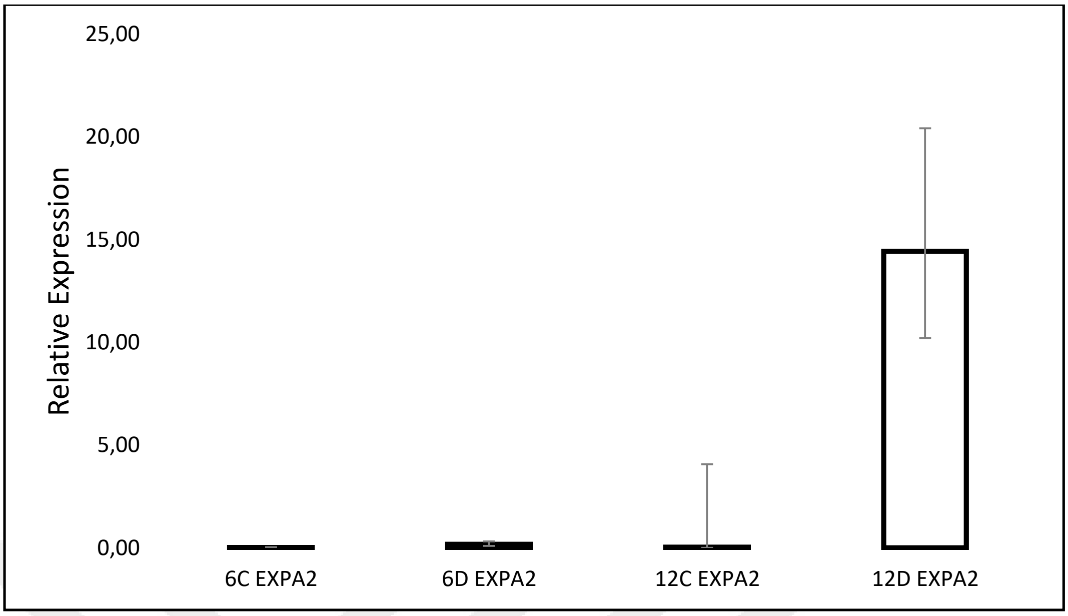


Figure 3.16. Relative expression of EXPA2 on barley root samples from control and drought groups at 6th and 12th days of drought stress treatment. Error bars were set by normal distribution of each samples SD.

Relative expression of EXPA2 was significantly higher on drought samples at 12th day to other samples as it can be observed on Figure 3.13. EXPA2 expression was very low and the variation between relative expression levels between 12th control sample and 6th day samples were very high as is can be derived from 3.16.

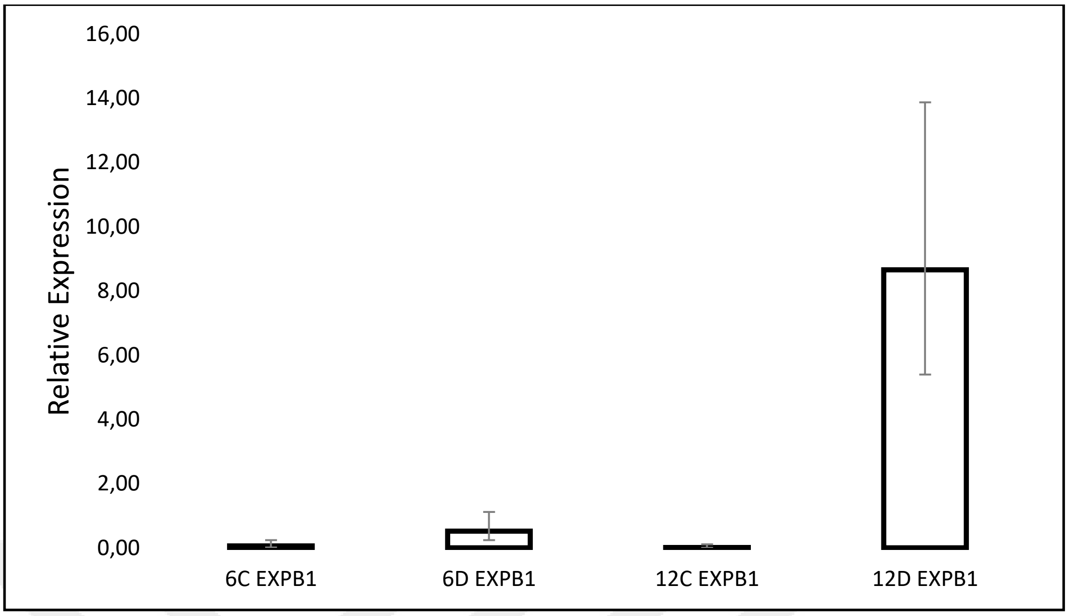


Figure 3.17. Relative expression of EXPB1 on barley root samples from control and drought groups at 6th and 12th days of drought stress treatment. Error bars were set by normal distribution of each samples SD.

Relative expression of EXPB1 gene was higher in drought groups both in 6th and 12th day compared to control groups and was significantly up regulated in roots treated with drought stress from 6th to 12th days. Expression of EXPA1 in control groups seems stable between 6th and 12th day samples as it can be observed on Figure 3.17. but the levels of relative expression is very low as it can be derived from Table 3.13. .

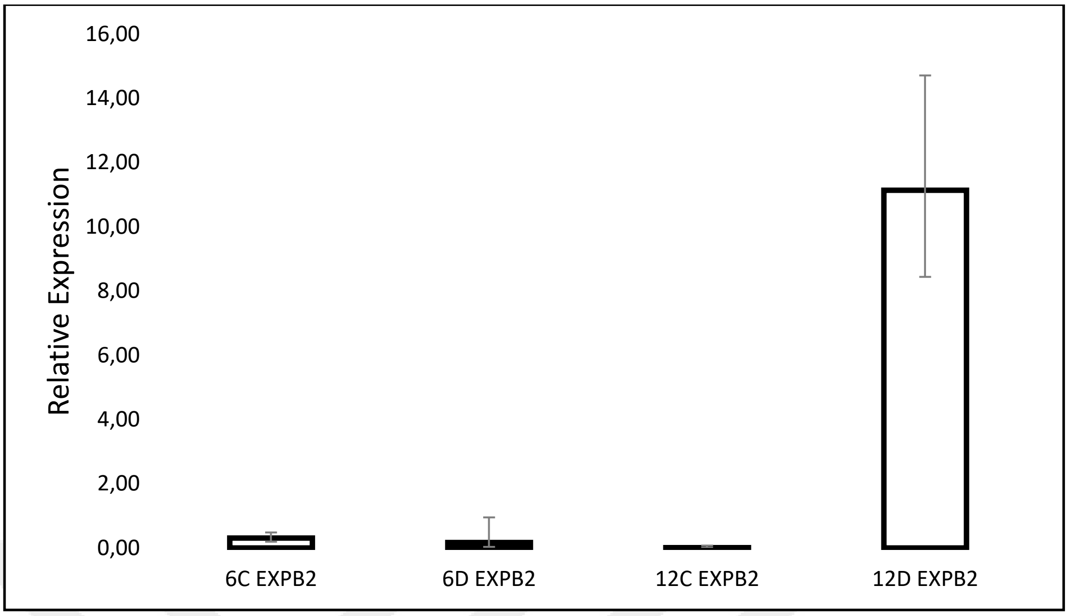


Figure 3.18. Relative expression of EXPB2 on barley root samples from control and drought groups at 6th and 12th days of drought stress treatment. Error bars were set by normal distribution of each samples SD.

Relative expression of EXPB2 was lower in drought group compared to control group on 6th day, however this claim is not very strong if we include the high SD on 6th day drought sample. Expression of EXPB2 from 6th to 12th days in drought samples was significantly up regulated in roots as it can be observed on Figure 3.18. Similarly to other relative expression results levels of control group relative expression is very low as it can be derived from Table 3.13. .

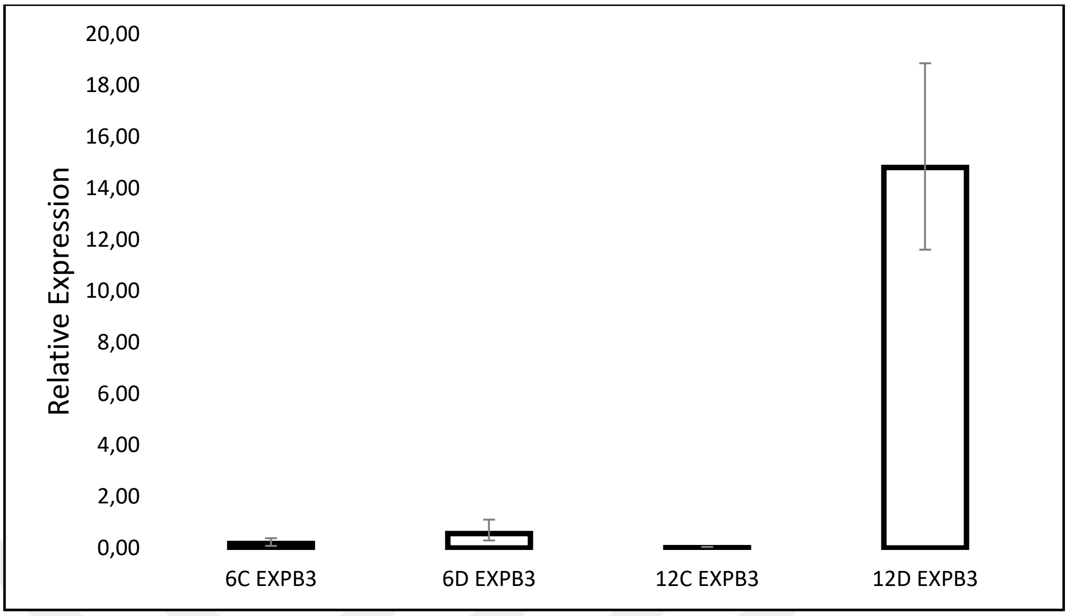


Figure 3.19. Relative expression of EXPB3 on barley root samples from control and drought groups at 6th and 12th days of drought stress treatment. Error bars were set by normal distribution of each samples SD.

Relative expression of EXPB3 gene was higher in drought groups both in 6th and 12th day and was significantly up regulated in roots treated with drought stress from 6th to 12th days. Expression of EXPB3 in control groups seems stable between 6th and 12th day samples as it can be observed on Figure 3.19. hover the relative expression in both control group samples were very low for clear assessment of the difference between control and drought groups as it can be observed on Table 3.13. .

$$\text{Expression} = 2^{-(\text{Mean Cq})} \quad [87]\ (3.11)$$

$$\text{Minimum Expression} = 2^{-(\text{Mean Cq} + \text{SD})} \quad [87]\ (3.12)$$

$$\text{Maximum Expression} = 2^{-(\text{Mean Cq} - \text{SD})} \quad [87]\ (3.13)$$

Table 3.16. Calculation of expression levels with of HvMYB1 on different samples where N is number of replicates.

Name of Sample	**N**	**Mean Cq**	**SD (Equation 3.4)**	**Expression Equation (Equation 3.11)**	**Minimum Relative Expression (Equation 3.12)**	**Maximum Relative Expression (Equation 3.13)**
HvMYB1 6C	3	27.56	0,30	5.05E-09	4,11E^{-09}	6,22E^{-09}
HvMYB1 6D	9	26.06	1,75	1.43E-08	4,26E^{-09}	4,81E^{-08}
HvMYB1 12C	4	30.32	1,76	7.46E-10	2,20E^{-10}	2,53E^{-09}
HvMYB1 12D	9	25.54	2,02	2.05-08	5,04E^{-09}	8,29E^{-08}

3.3.4. Welch’s t-Test of Relative Expression Results

$$\text{Normalized Cq} = 2^{-Cq} \qquad (3.14)$$

$$t = \frac{Mean\ Normalized\ Cq_{(control)} - Mean\ Normalized\ Cq_{(drought)}}{\sqrt{\frac{SD_{(control)}{}^{2}}{N_{(control)}} + \frac{SD_{(drought)}{}^{2}}{N_{(drought)}}}} \qquad [88]\ (3.15)$$

$$DF = \frac{\left(\frac{SD_{(control)}{}^{2}}{N_{(control)}} + \frac{SD_{(drought)}{}^{2}}{N_{(drought)}}\right)^{2}}{\frac{\left(\frac{SD_{(control)}{}^{2}}{N_{(control)}}\right)^{2}}{N_{(control)}-1} + \frac{\left(\frac{SD_{(drought)}{}^{2}}{N_{(drought)}}\right)^{2}}{N_{(drought)}-1}} \qquad [88]\ (3.16)$$

SD = Standard deviation

N = Number of samples

DF = Degrees of freedom

Null Hypothesis is, between control and drought group samples there is no significant (p=0.05) difference.

Table 3.17. Normalized Cq values of qPCR results.

Sample Group	Gene					Sample Group	Gene				
	EXPA1	EXPA2	EXPB1	EXPB2	EXPB3		EXPA1	EXPA2	EXPB1	EXPB2	EXPB3
Day 6 Control	2,34E-10	1,63E-10	6,41E-10	2,46E-09	1,94E-09	Day 12 Control	3,84E-10	1,07E-12	9,01E-11	2,16E-10	1,39E-11
	9,91E-10	1,9E-10	5,51E-11	9,58E-10	4,17E-10		2,11E-09	1,63E-08	1,94E-14	1,62E-09	4,44E-11
	5,77E-09	1,38E-10	1,89E-09				2,16E-10	2,74E-08	2,51E-09	6,2E-11	1,75E-10
	1,21E-12	2,85E-11	1,49E-10							8,92E-14	1,09E-10
	6,29E-12	3,01E-11									1,74E-09
		5,11E-12									6,91E-10
Day 6 Drought	9,17E-09	1,58E-09	4,4E-09	3,07E-09	4,43E-09	Day 12 Drought	3,75E-08	1,82E-08	6,14E-09	6,22E-09	9,56E-09
	2,58E-08	1,06E-09	4,22E-09	1,6E-09	4,81E-09		2,69E-08	1,35E-08	5,85E-09	8,5E-09	1,02E-08
	2,24E-08	1,25E-09	1,67E-09	2,15E-11	1,88E-09		2,61E-08	1,25E-08	1,98E-09	6,09E-09	9,97E-09
	1,82E-08	3,24E-09	1,7E-08	5,89E-09	1,36E-08		1,06E-08	1,33E-08	9,43E-09	9,11E-09	1,18E-08
	2,12E-08	2,37E-09	1,82E-08	8,5E-09	1,52E-08		1,27E-08	8,44E-09	9,76E-09	1,28E-08	1,71E-08
	1,75E-08	6,58E-09	1,33E-08	5,05E-09	1,51E-08		9,56E-09	7,15E-09	6,31E-09	9,05E-09	1,62E-08
		4,75E-09	5,16E-09	6,67E-09	9,56E-09		9,83E-09	6,76E-09	7E-09	7,77E-09	7,88E-09
		2,54E-09	8,92E-09	5,57E-09	1,01E-08		6,95E-09	6,95E-09	1,09E-08	1,25E-08	1,06E-08
		3,7E-09	1,31E-08	2,63E-09	1,19E-08		1,15E-08	1,15E-08	6,18E-09	5,73E-09	9,17E-09

Table 3.18. Results of two tailed independent Welch's t-test between control and drought group samples.

Name	Mean Normalized Cq (Equation 3.14)	N	SD of Normalized Cq (Equation 3.4)	Welsh t value (Equation 3.15)	DF (Equation 3.16)	Critical t values for given dF (p=0.05) (One tailed)
6C EXPA1	1,40E-09	5	2,47E-09	6,86	7,07	1,89
6D EXPA1	1,90E-08	6	5,69E-09			
12C EXPA1	9,03E-10	3	1,05E-09	4,45	8,45	1,86
12D EXPA1	1,68E-08	9	1,06E-08			
6C EXPA2	9,27E-11	6	8,05E-11	4,85	8,05	1,86
6D EXPA2	3,01E-09	9	1,80E-09			
12C EXPA2	1,46E-08	3	1,38E-08	0,39	2,16	2,92
12D EXPA2	1,14E-08	7	4,17E-09			
6C EXPB1	6,83E-10	4	8,43E-10	4,30	8,67	1,86
6D EXPB1	9,55E-09	9	6,06E-09			
12C EXPB1	8,67E-10	3	1,42E-09	5,11	7,04	1,89
12D EXPB1	7,06E-09	9	2,67E-09			
6C EXPB2	1,71E-09	2	1,06E-09	2,25	4,70	2,13
6D EXPB2	4,33E-09	9	2,69E-09			
12C EXPB2	4,75E-10	4	7,70E-10	8,59	10,38	1,81
12D EXPB2	8,64E-09	9	2,61E-09			
6C EXPB3	1,18E-09	2	1,08E-09	4,68	8,61	1,86
6D EXPB3	9,63E-09	9	4,91E-09			
12C EXPB3	4,62E-10	6	6,73E-10	10,00	9,05	1,83
12D EXPB3	1,14E-08	9	3,17E-09			

Results of the Welch's T-tests shows that, calculated t values between normalized Cq values control and drought groups among each target genes is higher than the critical t value (p= 0.05) except EXPA2 at 12th day as can be observed at Table 3.18. This result forces us to reject the Null hypothesis and shows there is difference between expression of expansins control and drought groups of each target gene, except EXPA2 at 12th day where we cannot claim there is significant difference between the expression of EXPA2 between control and drought conditions.

4. DISCUSSION

In this study the aim was identification and analysis of barley expansin genes with the new available data and tools that would uncover the barley expansin genes that hadn't been covered by previous studies. With achieving this aim we also wanted to show the relationships between drought stress on barley plants and expression of barley expansins that were not sufficiently covered in the literature. For this purpose WES data were investigated from several studies via the BarleyExpDB [77] tool for understanding the expression profiles of barley expansins especially on root tissues involving water stress trials primarily drought and additionally salt and water deficiency stresses. Beside that we had designated some of the newly discovered barley expansin genes as differential expression analysis targets as listed on Table 2.1. between watered and drought treated barley roots to further support our hypothesis of linking expansins with the barley drought response mechanism.

Identification of candidate list of sequences as barley expansin genes were investigated using BLAST [89] tool in order to find homologs of relatively better studied rice and wheat expansins with the sequences obtained from databases including UniProt [90], GenBank [91] and Ensembl [69]. These lists were expanded with inclusion of previously identified barley expansins [66,67] and the list were narrowed by further analysis in order to not include genes from some pollen allergens or GH45s that have high similarity with expansins into our study. In total we had identified 78 barley expansins as listed in Table 3.1. Barley expansins are proteins that have varied lengths, most of them fell in the 250-275 range that is the commonly accepted range for plant expansins [54] as it can be observed on Figure 3.1. In order to understand the evolutionary relationship and classify the found genes under expansin sub-groups, phylogenetic analyses were done on barley expansin protein sequences. Results obtained with global alignment via the MUSCLE tool [74] of the sequences and by tree generation by maximum likelihood method via MEGA11 [75], three groups of expansins were identified from barley expansins and found genes were classified by their homologs in other plants which were EXPA with 39 genes, EXPB with 33 genes and EXLA with six genes. No EXLB were identified from the found barley expansins via homolog alignment search done with known EXLB sequences from mentioned sources, unlike *Arabidopsis thaliana* or rice genomes that contains EXLB genes [96]. Dispersion of barley expansins were diverse between chromosomes and most of the barley expansins were located closer to the ends of chromosomes in euchromatic regions and many barley expansins were located closely with each other on chromosome as can be observed on Figure 3.6. , Figure 3.7. and Figure 3.8. .

Phylogenetic analyses showed that compared to EXPA, the EXPB genes more similar with each other as can be seen in Figure 3.2. by comparison of the branch lengths in sub groups. Also we can observe on sub trees of EXPA on Figure 3.3. and EXPB on Figure 3.4. , there are many expansin genes that have very high similarity between each other that seems to be correlating with the adjacency of chromosomal locations of genes as can be seen in Table 4.1. Extent of similarity between some genes including their adjacency could be an indicator of recent duplication of some barley expansins in the barley genome. The expression profiles of genes grouped by similarity on Table 4.1. had very similar expression profiles among the 16 tissue sample on Table 3.3. Exception of this case is the genes without non expressing genes on Table 3.3. and the similarity group 7 on Table 4.1. which only expression was detected from HORVU.MOREX.r3.2HG0107640. Similarities, expression profiles and adjacencies of mentioned barley expansins supports the claim that these genes being duplication, however the possibility of multiple annotation by the processing of WES cannot be disregarded and more analysis including on promoter regions of the genes is required for classifying them as duplicates or genes that evolved to have some different functions but have very recent evolutionary relationship.

Table 4.1. Genes suspected with duplication grouped and total expression in 16 tissues from PRJEB14349 [81].

Similarity Groups	**Gene**	**Group**	**Locations**	**Total Expression FPKM**
1	HORVU.MOREX.r3.3HG0293420	A	3H:509477147-509478450	23.31
	HORVU.MOREX.r3.3HG0293430	A	3H:509496134-509496984	20.21
	HORVU.MOREX.r3.3HG0293450	A	3H:509515974-509516823	17.41
	HORVU.MOREX.r3.3HG0293480	A	3H:509594791-509605128	0.06
	HORVU.MOREX.r3.3HG0293490	A	3H:509627774-509628622	13.61

2	HORVU.MOREX.r3.3HG0250010	A	3H:145511692-145512567	8.49
	HORVU.MOREX.r3.3HG0250020	A	3H:145565696-145566637	9.66
	HORVU.MOREX.r3.3HG0250040	A	3H:145625472-145626355	3.2
	HORVU.MOREX.r3.3HG0250050	A	3H:145691256-145692071	0.00
3	HORVU.MOREX.r3.5HG0532120	A	5H:575833568-575835784	5.91
	HORVU.MOREX.r3.5HG0532140	A	5H:575851320-575852371	5.08
	HORVU.MOREX.r3.5HG0532150	A	5H:575892617-575893487	7.11
4	HORVU.MOREX.r3.3HG0250010	A	3H:145511692-145512567	8.49
	HORVU.MOREX.r3.3HG0250020	A	3H:145565696-145566637	9.66
	HORVU.MOREX.r3.3HG0250040	A	3H:145625472-145626355	3.2
	HORVU.MOREX.r3.3HG0250050	A	3H:145691256-145692071	0.00
5	HORVU.MOREX.r3.1HG0054170	A	1H:361887174-361888390	3.81
	HORVU.MOREX.r3.1HG0054180	A	1H:362068330-362069269	1.15
	HORVU.MOREX.r3.1HG0054190	A	1H:362150973-362151849	3.76
6	HORVU.MOREX.r3.3HG0318560	B	3H:592818991-592820493	30.61
	HORVU.MOREX.r3.3HG0318580	B	3H:592827799-592829147	58.69
	HORVU.MOREX.r3.3HG0318870	B	3H:593707506-593708521	7.15
	HORVU.MOREX.r3.3HG0318880	B	3H:593718367-593719404	36.53
7	HORVU.MOREX.r3.2HG0107600	B	2H:25684571-25685662	0
	HORVU.MOREX.r3.2HG0107610	B	2H:25730775-25731730	0
	HORVU.MOREX.r3.2HG0107640	B	2H:25741953-25742679	4.05

Expression profiling of barley expansin genes as mentioned were done in order to understand the expression motifs of specific and groups of barley expansins in different tissues of the barley plants and see the change of expression of barley expansins in roots under drought stress to support our initial hypothesis. For these purposes the PRJEB14349 [81] WES data that had samples from 16 different tissues of barley under different stages of growth were analysed as can be seen on Table 3.3. Firstly, the data showed that 14 of 78 barley expansins didn’t had detectible expression on any given of the 16 tissue sample when the threshold of expression was set as FPKM<1 [97] as can be observed on Table 4.2. Considering the diversity of the tissues that the WES data obtained from, it can be claimed that the mentioned 14 barley expansins could be silent genes. However in order to confirm their non-expressing status more expression data from more tissue and conditions is required to be analysed.

Table 4.2. Genes without expression (FPKM<1) on any of 16 tissue samples from PRJEB14349 [81].

Gene ID	Group
HORVU.MOREX.r3.1HG0054180	A
HORVU.MOREX.r3.3HG0250050	A
HORVU.MOREX.r3.3HG0293480	A
HORVU.MOREX.r3.4HG0401050	A
HORVU.MOREX.r3.4HG0401290	A
HORVU.MOREX.r3.4HG0401310	A
HORVU.MOREX.r3.7HG0722730	A
HORVU.MOREX.r3.1HG0055780	B
HORVU.MOREX.r3.2HG0107600	B
HORVU.MOREX.r3.2HG0107610	B
HORVU.MOREX.r3.2HG0214300	B
HORVU.MOREX.r3.3HG0293090	B
HORVU.MOREX.r3.6HG0592630	B
HORVU.MOREX.r3.7HG0749130	LA

Conserved domain analysis showed as expected almost all barley expansin genes that we had identified had the DPBB in their 5’ terminus and C-domain at their 3’ terminus that are the model for expansin family across plants [98] as can be observed on Figure 3.9. , Figure 3.10. and Figure 3.11. The exceptions of sequences that lacked any of mentioned domains are listed in Table 3.2. Genes of HORVU.MOREX.r3.6HG0592630 which has a very short sequence that has no available space for DPBB in 5’ terminus and HORVU.MOREX.r3.7HG0749130 which has an incomplete C-domain at 3’ end of the domain that can be observed on Figure 3.10. have easily observable sequence defections for an expansin protein. This two genes had no detectible expression on WES data and are suspected to be expansin genes with defections. Among the identified expansins with missing conserved domains, five of them which are HORVU.MOREX.r3.5HG0532120, HORVU.MOREX.r3.4HG0401070, HORVU.MOREX.r3.5HG0532150, HORVU.MOREX.r3.1HG0054870 and HORVU.MOREX.r3.7HG0749090 had expression on PRJEB14349 [81] except HORVU.MOREX.r3.3HG0293090. This may show that of the nine genes which had missing conserved domains three of them could be classified as defective and remaining six of them are suspected for defection that may result in inactivity of the translated proteins from these genes even if they were translated. However further confirmation for lacking conserved domains should be done in order to confirm their defections since only conserved domain matches with highest match "specific match" were used in this analysis from CDD[93] and perhaps some of those genes had mentioned domains and were not annotated by the system because of high threshold of identity.

The total expression of all barley expansins by the tissues on Table 3.4. shows that tissues that have extensive cellular growth like the lodicule, rachis, developing tillers that are associated with the early plant development and germination processes had very high expansin expression. This indicates the role of expansins which are up-regulated in cells that undergo cellular expansion in barley in cellular growth as expected. The lower expression of expansins in more mature tissues like epidermis or shoots from seeding at 10 cm seedling and also in senescing leaves also support this claim. Another interesting pattern that can be derived from Table 3.4. is that the tissues which are associated with high cellular growth that are exposed to the light like lodicule, rachis and developing tillers had higher total expression of expansin genes than the tissues that are not exposed to the sunlight like root sample and etiolated seedlings. This is most likely caused by expansin genes being associated with auxin induced cellular growth mechanism [24] which perhaps both increase the expression of expansins and acidify the cell wall matrix [99] which increases the activity of expansin proteins [24]. The activation of the mentioned auxin-induced growth mechanism in presence of light [100] could be the difference between the tissues which are exposed and un-exposed to light.

Further analysis on PRJEB14349 [81] showed that firstly there are multiple barley expansins which not expressed among any analysed tissues as can be observed on Table 4.2. In order to have a better representation of expression of expansins genes by groups the mean expression of found genes without genes with non-expression at Table 4.2. were analysed. Results of this as can be observed on Table 4.3. showed that in most tissues the mean expression of EXPB genes were higher than other groups. Exception to this claim is lodicule tissue which EXPA had slightly more expression than EXPB and EXLA being the expansin group with the highest mean expression on epidermis tissue. Even though since EXLA subgroup had only 8 genes so the mean of expression might not be a great indicator for comparison between expansin groups this finding is still interesting especially considered with the different expression pattern of EXLA genes to the EXPA and EXPB by having a relatively higher expression pattern in more mature and non-light exposed tissues like epidermis, roots, senescing leaf and etiolated seedling. This difference of pattern could be associated with different regulation factors effecting EXLA compared to EXPA and EXPB which are possibly more dominantly regulated by auxin or perhaps auxin-abscisic acid interaction [101]. The expression profiles of EXPA and EXPB as groups on Table 4.2. shows both groups expressions are relatively correlating by the rations of expression of the group between the tissues where EXPB group have comparatively higher expression compared to the EXPA group expansins. Exception to this claim is the expression of EXPA and EXPB genes in lodicule tissue which are almost equal. This could be result of exceptionally higher expression of one or two EXPA gene that might that resulted a ratio between EXPA and EXPB that is mostly preserved on remaining 15 other samples especially considered there are only eight EXPA and eight EXPB genes with detectible expression in mentioned tissue.

Table 4.3. Mean expression of barley expansins with non-expressing genes discarded (Table 4.2.) by groups in given tissues from PRJEB14349 [81].

Samples	**EXPA**	**EXPB**	**EXLA**
Bracts removed grain (15DPA)	3.50	12.95	0.81
Grain, bracts removed (5DPA)	4.77	23.08	11.52
Embryos (4d dissected from germinating grains)	5.34	11.81	3.52
Epidermis (4 weeks)	0.55	1.31	6.47
Etiolated (10 day old seedling)	2.10	4.28	4.27
Young Inflorescences (5mm)	5.53	7.05	1.38
Inflorescences (1-1.5cm)	3.68	8.18	0.58
Shoot from the seedlings (10cm shoot stage)	2.30	4.88	3.42
Lemma (6 weeks PA)	3.92	7.26	3.77
Lodicule (6 weeks PA)	21.96	21.13	2.33

Developing tillers (six leaf stage)	6.68	30.59	4.22
Palea (6 weeks PA)	11.44	25.08	2.19
Rachis (5 weeks PA)	12.45	25.80	1.78
Root (4 weeks)	5.43	20.64	6.05
Roots from the seedlings (10cm shoot stage)	6.19	16.66	2.95
Senescing leaf (2 months)	0.17	0.18	1.65

Another important aspect of that could be derived from PRJEB14349 [81] data is that, some expansins as it can be observed on Table 3.4. have different patterns of expression specific to the tissues even if there are some genes in each group that are expressed significantly in each of the samples that are true for all barley expansin groups. This may indicate expansin genes may have evolutionarily been tailored to specific functions in specific tissues and conditions rather than having an applicable “everywhere and every time” behavior. This also may explain why there are significant number of barley expansins that have relatively high similarity in structure. This can be better observed from PRJNA496380 [84] at Table 3.5. and Table 3.6. which shows expression of barley expansins in radicule tissue during germination, where the expression of the expansin in each group have a very interesting profile that can be significantly changed in periods of time as low as 8 hour. These findings shows that barley expansins are very specialized genes in regarding to tissue and condition specific regulation.

PRJEB13621 [83] which give expression of genes in meristematic, elongation and maturation zone of barley roots 3 days after germination with salt stress treatment as can be seen on Table 3.7. provides important expression information about the genes in the different parts of the roots that has different cell growth rates with aspect of salt stress which has similar impacts on plants as drought stress even with the differences between them [102]. The expression of the barley expansin genes from this WES data shows us that, as it can be observed on Table 3.7. and Table 4.4. EXPB has a higher expression rate in all given root compared to EXPA. Both EXPA and EXPB were up regulated in meristematic and elongation zone by the salt stress treatment and were down regulated in maturation zone significantly. This shows that both EXPA and EXPB were up regulated by cells in tissues with high rate of cell growth by salt stress, especially considering higher expression rate in elongation zone compared to meristematic zone which grows faster [103]. The relationship between the water potential loss stress tolerance mechanism in growing cells and EXPA and

EXPB is clear. Expression of EXLA again had a different pattern than EXPA and EXPB which it had very low expression in meristematic zone and had the highest in maturation zone while in elongation zone was down regulated by salt stress supporting differences between the regulation mechanism of EXLA with expansins (EXPA and EXPB) as can be observed on Table 3.7. and Table 4.4. The data from PRJEB13621 [94] that shows expression of gene in barley seminal roots from different parts including 0-12.5%, 25-37.5% and 50-62.5% of seminal root length shows the relationship between the rate of expression EXPA and EXPB and growth rate. In the part of seminal root with the highest plant division 0-12.5% and the part which cell expansion 50-62.5% [104,105] expression of EXPA and EXPB was significantly high. Also in both parts of seminal root water deficiency stress by PEG-8000 treatment resulted in up regulation of EXPA and EXPB. This correlates with the findings of study on different zones of barley roots under salt stress as mentioned. EXLA again had different motif of expression in association with the rate of growth and water potential loss in plants as can be observed on Table 4.5. .

Table 4.4. Total expression of barley expansin genes by groups with detected in different root parts of Clipper variant barley 3 days after germination, where control plants were treated with 0 mM NaCl nutrient medium and salt group were treated with 100 mM NaCl nutrient medium. WES data is obtained from PRJEB13621 [83] via BarleyExpDB [77] and given as FPKM.

	Root Meristematic zone		**Root Elongation zone**		**Root Maturation zone**	
	0 mM NaCl	**100 mM NaCl**	**0 mM NaCl**	**100 mM NaCl**	**0 mM NaCl**	**100 mM NaCl**
EXPA	500,30	645,35	468,16	779,43	246,20	154,02
EXPB	715,09	846,56	1269,63	1480,34	423,98	258,12
EXLA	1,51	2,45	38,69	38,51	41,37	69,25

Table 4.5. Total expression of barley expansin genes by groups with detected expression in different parts of seminal roots where water deficient group barleys were treated with PEG-8000 in their nutrient medium compared to control group which were untreated. Parts of the roots that samples were taken are 0-12.5%, 25-37.5% and 50-62.5% of the total seminal root length. WES data is obtained from PRJNA439267 PRJEB13621 [94] via BarleyExpDB [77] give as in FPKM.

	0-12.5% of the total seminal root length		**25-37.5% of the total seminal root length**		**50-62.5% of the total seminal root length**	
	Control	**Water Deficit**	**Control**	**Water Deficit**	**Control**	**Water Deficit**
EXPA	353,43	623,19	146,39	90,75	277,83	491,14
EXPB	325,22	549,11	170,88	79,29	234,15	294,72
EXLA	15,81	41,38	21,86	32,84	30,75	24,35

Target genes for differential expression analysis between the control and drought treated barley roots were selected by criteria of high expression rate roots in EoRNA [72] database and a wide coverage on barley groups (two EXPA and three EXPB) which expansin genes with relatively evolutionarily separated were chosen as can be observed on Figure 3.2. Names of EXPA1, EXPA2, EXPB1, EXPB2 and EXPB3 were designated for the genes selected as target in order to ease understanding the results for reader. As reference gene we had used MYB1 which has a relatively stable expression confirmed on the WES data used on this study via BarleyExpDB [77]. The selection of MYB1 as sole reference gene for normalization were unfortunately caused by the RNA degradation problem that will be discussed in next paragraph which caused no expression on control samples of other planned reference genes for the experiment including barley GAPDH, actin and tubulin genes. Optimal Tm values of used primers for target and reference gene was determined by gradient qPCR as can be observed in Table 3.11. Because the optimal Tm was selected for each primer the the Pfaffl PCR efficiency rate [87] was accepted as 1.

In order to confirm the drought stresses impact on the drought group plants for differential expression of selected barley expansins several physiological observations and analyses were performed before and during the drought stress treatment. Before the period of stress treatment begun, plants of both control and drought groups were cut from watering until daily monitored soil moisture in each pot was measured as dry. After the confirmation of the dryness of the soil, stress treatment begun with watering of the control groups every 2 days. During the stress treatment as it can be observed on Figure 3.12. as expected drought group barley leaves droop and their color turned from green to yellow progressively while control groups had continued their phenotype and their leaves continued growing. The RWC analysis that can be observed on Figure 3.13. showed control groups had relatively stable RWCs in their leaves while drought plants progressively lost their water content as expected [106]. Between day 0 and 4 as can be observed on Figure 3.13. the control plants had increase of their water content by approximately 20% RWC increase which caused by starting of watering after mentioned cutting of plants from watering before stress treatment in order not have water inside the pot soils interfere with the stress treatment. Drought groups plants kept their water content around the 60-65% until the 8th day which than lost approximately 4% of RWC until day 15, which indicates the drought plants had relatively tolerated the impact of drought stress until around 6th to 8th day and the negative impact of drought stress is higher between day 8th and 15th. This observation is also confirmed by chlorophyll content analysis results that can be observed on Figure 3.14. where both control and drought plants had relatively same stable photosynthesis rate until around 6th and 8th days were drought plants started progressively differentiating negatively by the expected impact of drought stress [106]. By the RWC and chlorophyll content analyses the days which the samples for differential expression will be used was determined as day 6th which was a good indicator for the barley plants under relatively mild impact of drought stress and 12th which would be a good indicator for high impact of drought stress. The stress treatment was completed at 15th day since the drought plants by observation were determined in terminal stage because of the impact of drought stress. Additionally, the chlorophyll content analysis showed the used variety Efes-98 is a drought tolerant considering in drought group plants until the day 10 photosynthesis rate was kept stable. Considering the drought group plants were cut from watering for 24 days including the pre-treatment period, we can claim the used barley variety in this study is relatively drought tolerant.

Root samples were taken by harvesting of three plants from each groups from approximately 0-10 cm length from the surface of soil with caution to not harm little root hairs and placed quickly in liquid nitrogen until its long term storage in -80˚C. Unfortunately despite the attention given not to damage root hairs during cleaning of the roots from soil by brushing after harvesting, the control group plants that were being watered during harvesting had formed sticky mud that were held together by the barley roots that resulted in un desired soil contamination in the samples taken. Effect of the contamination can be clearly seen on Table 3.12. where because of significant amount of samples used for RNA isolation had soil contamination in control plants compared to drought group root samples taken the control groups had significantly lower isolated nucleic acid concentrations. Soil contamination also effected purity of isolated total RNA samples as can be observed on Table 3.12. where drought plants had 260nm/280nm and 260nm/230nm absorbance rates in desired ranges of around 2.0-2.10 and 2.0-2.20 respectively with some deviations, but control samples had significantly lower than ideal 260nm/280nm and 260nm/230nm absorbance rates which indicate protein and organic compound contamination [107]. Additionally, during the storage of both root samples at before RNA isolation and isolated RNA samples before reverse transcription at -80˚C problems occurred with the -80˚C fridge that the samples were kept. During these instances both the storage temperature of the mentioned samples increased several time in some instances more than a day period to temperatures up to – 30 ˚C and the transfer of the samples to another-80˚C fridge exposed the samples to room temperature which can degrade highly unstable RNA. This may have resulted in lower detection expression samples in cDNA that were reverse transcribed from isolated total RNA used for qPCR that were especially problematic on control samples that already had very low concentrations of RNA isolated which were contaminated. The difference between the drought and control samples were tried to be mitigated by using same amounts of reverse transcribed total RNA sample by using higher volumes of control groups samples and using 40ng per qPCR well which is relatively high in order to detect even very minute levels of expression.

The impact of mentioned problems can be observed on the Cq data from qPCR results on Table 3.14. where control groups had detectible expression less than total nine replications (3 biological x 3 technical) done for each sample as can be observed and SD values of control samples were very high which lowers the confidence about the results derived from differential expression analysis. The problem with the control group expression results caused calculations for both relative and differential expressions of target genes to be immensely big and unfortunately meaningless for comparing the change of expression between control and drought conditions as aimed. However the drought group samples were less affected by the mentioned problems caused by soil contamination and had relatively decent levels of expression by Cq data that can be observed on Table 3.14. with lesser variation. From the qPCR results of drought samples it was possible to compare the change of expression of target expansin genes between 6th and 12th day and with each other as can be seen on Figure 4.1. .

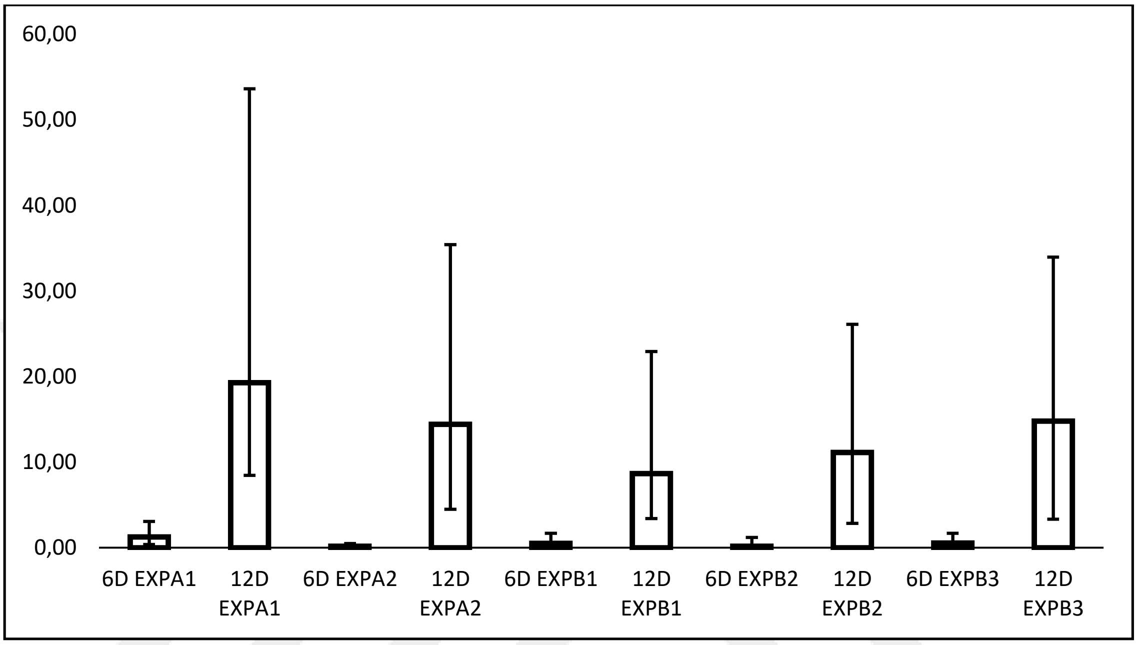


Figure 4.1. Relative expression of five target barley expansins in barley roots at 6th and 12th days of drought stress treatment without control groups. Error bar based on SD and data used were taken from Table 3.15. .

Expression of five selected expansin from PRJNA439267 [94] and PRJEB13621 [83] at Table 4.6. shows that similarly to the findings of relative expression except EXPA2 under water deficiency, water deficiency and salt stress up regulated the expression of the selected barley expansin genes. Even though salt and water deficiency stresses don’t have exactly same impacts on plants [102], it is possible to say these WES data supports the findings from relative expression analysis. Also, the Welch’s t Test results as can be observed on Table 3.18. supports the increase of target expansins genes under drought stress compared to control groups.

Table 4.6. Expression of selected barley expansins in 0-12.5% of the total seminal root of barley with water deficiency treatment (PEG 8000) from PRJEB13621 [94] and in root meristematic zone 3 days after germination with NaCl treatment from PRJEB13621 [83]. Expression were given as FPKM values.

Name	**0-12.5% of the Total seminal root Length**			**Root Meristematic Zone**		
	Control-Root	**Water Deficit-Root**	**Change between control to water deficient**	**Control (No NaCl)**	**100mM NaCl**	**Change between control to 100mM NaCl**
EXPA1	7.68	10.84	41.1%	7.07	9.26	31.0%
EXPA2	13.83	6.59	-52.3%	88.56	103.15	16.5%
EXPB1	13.4	29.07	116.9%	78.24	82.52	5.5%
EXPB2	61.56	93.24	51.5%	83.72	114.57	36.8%
EXPB3	22.86	41.75	82.6%	41.63	51.81	24.5%

5. CONCLUSION

In conclusion, with this study we had identified 78 barley expansin genes that many were not identified as expansins in barley in previous studies [66,67] Table 3.1. that were classified under three groups of EXPA with 39 genes, EXPB with 33 genes and EXLA with six genes. 14 of the 78 barley genes are suspected to be silent genes as can be found on Table 4.2. by their expression data on tissues and conditions employed in this study and we had identified possible duplications of 16 of 78 genes as can be observed on Table 4.1. Expression profiles of the found expansin genes from different tissues and conditions showed that EXPA and EXPB has clear relationship with cellular growth, light exposure (associated with auxin mediated growth) and water stress response mechanisms in barley. EXPB had higher expression compared to EXPA group in barley tissues, especially in roots indicating a more important role for barley plants. EXLA expression showed different motifs compared to EXPA and EXPB indicating different regulation mechanisms and possible functions in barleys. The relative expression results obtained from the roots of drought treated barley plants and total RNA isolated from these root samples showed all five genes which are EXPA1 (HORVU.MOREX.r3.7HG0636890), EXPA2 (HORVU.MOREX.r3.2HG0104860), EXPB1 (HORVU.MOREX.r3.6HG0601160), EXPB2 (HORVU.MOREX.r3.1HG0056580) and EXPB3 (1 HORVU.MOREX.r3.4HG0409870) were up-regulated between the days of 6th and 12th of drought stress treatment and these findings are supported with WES data.

In order to expand the findings of this study, future studies on the regulation mechanisms of barley expansins including hormonal regulation, investigating silencing or increasing expression of barley expansins (especially the genes with highest expression) in root tissues effect on drought tolerance and structural analysis of the identified expansin proteins with regard of found associations with tissue specific, stress response and growth rate related relationships would be important.

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APPENDIX A: WHOLE EXPRESSION SEQUENCING DATA

Table A.1. Expression of barley expansin genes in 16 different tissues obtained from PRJEB14349 [81] WES data via BarleyExpDB [77] from. Definitions of abbreviations are CAR15 (bracts removed grain 15 days post anthesis (DPA)), CAR5 (bracts removed grain 5 DPA), EMB (embryos at 4 day dissected from germinating grains), EPI (epidermis at 4 weeks), ETI (etiolated 10 day old seedling), INF1 (young inflorescences at 5mm), INF2 (inflorescences between 1-1.5cm), LEA (shoot from the seedlings at 10cm stage), LEM (lemma 6 weeks PA), LOD (lodicule 6 weeks post anthesis (PA)), NOD (developing tillers at six leaf stage), PAL (palea at 6 weeks PA), RAC (rachis at 5 weeks PA), ROO (root at 4 weeks), ROO2 (roots from the seedlings at10cm shoot stage) and SEN (senescing leaf at 2 months)

ID (MorexV3)	CAR15	CAR5	EMB	EPI	ETI	INF1	INF2	LEA	LEM	LOD	NOD	PAL	RAC	ROO	ROO2	SEN
HORVU.MOREX.r3.1HG0054170	0,00	0,00	1,61	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,31	0,89	0,00
HORVU.MOREX.r3.1HG0054180	0,00	0,00	0,77	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,07	0,31	0,00
HORVU.MOREX.r3.1HG0054190	0,00	0,00	2,24	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,76	0,76	0,00
HORVU.MOREX.r3.1HG0054870	0,00	2,46	1,47	3,96	3,31	0,33	0,08	0,72	13,01	4,16	8,99	6,43	5,57	0,95	8,57	0,00
HORVU.MOREX.r3.1HG0055780	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,88	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.1HG0056570	3,88	0,99	97,19	21,18	77,99	0,00	0,00	40,56	3,46	3,24	333,20	4,20	8,79	131,90	156,14	0,27
HORVU.MOREX.r3.1HG0056580	0,00	0,00	16,10	0,00	0,28	0,00	0,00	0,00	0,04	0,00	0,00	1,41	0,00	24,70	50,94	0,00
HORVU.MOREX.r3.1HG0056600	0,00	1,10	0,49	0,00	1,87	0,00	0,00	14,30	0,98	0,00	27,89	1,00	2,10	21,51	13,38	0,00
HORVU.MOREX.r3.1HG0056620	59,85	131,20	42,25	9,07	23,49	3,68	2,14	32,84	150,05	41,44	476,89	590,19	343,10	77,86	41,34	0,00
HORVU.MOREX.r3.1HG0056630	26,45	213,92	47,33	0,83	18,64	79,13	90,88	27,43	63,52	568,06	56,13	169,98	301,50	51,75	53,05	0,00
HORVU.MOREX.r3.1HG0071050	17,16	27,26	15,91	8,86	35,60	35,50	6,07	23,17	37,84	183,63	86,01	122,88	22,37	5,95	1,22	5,16
HORVU.MOREX.r3.2HG0104860	0,00	2,97	37,77	0,00	0,00	0,00	0,00	0,13	0,04	11,23	0,00	0,00	0,00	11,91	12,88	0,00
HORVU.MOREX.r3.2HG0107600	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0107610	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00

HORVU.MOREX.r3.2HG0107640	0,00	4,05	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0183770	0,71	3,41	8,59	0,52	0,42	12,93	16,95	1,95	1,15	28,15	1,25	2,08	25,51	5,44	3,77	0,00
HORVU.MOREX.r3.2HG0185850	1,77	10,97	9,03	4,06	6,42	11,04	8,98	6,40	8,37	9,25	30,99	9,39	54,23	12,77	13,89	0,29
HORVU.MOREX.r3.2HG0189470	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	5,37	0,00	0,00	12,83	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0189530	0,00	0,00	8,43	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,72	1,60	0,00
HORVU.MOREX.r3.2HG0189540	0,00	0,00	1,73	0,00	0,00	0,00	0,00	0,13	0,00	0,00	0,00	0,00	0,00	0,81	0,06	0,00
HORVU.MOREX.r3.2HG0195810	0,00	0,00	2,27	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,64	1,21	0,00
HORVU.MOREX.r3.2HG0214300	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0219910	2,45	213,85	0,00	0,72	0,20	0,00	0,00	0,00	0,00	0,00	2,15	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0223260	0,00	0,00	2,05	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	1,48	0,61	0,00
HORVU.MOREX.r3.3HG0250010	6,13	0,00	1,66	0,00	0,09	0,00	0,00	0,00	0,00	0,07	0,06	0,00	0,00	0,20	0,28	0,00
HORVU.MOREX.r3.3HG0250020	7,71	0,00	1,41	0,00	0,05	0,00	0,00	0,00	0,00	0,08	0,00	0,00	0,00	0,10	0,31	0,00
HORVU.MOREX.r3.3HG0250040	0,00	0,00	2,44	0,00	0,00	0,00	0,00	0,00	0,00	0,05	0,00	0,00	0,00	0,13	0,58	0,00
HORVU.MOREX.r3.3HG0250050	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0252190	0,00	0,76	3,94	0,00	0,41	0,00	0,00	0,00	1,52	1,61	0,08	5,85	0,68	7,26	14,17	0,00
HORVU.MOREX.r3.3HG0252250	0,00	0,00	2,70	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,30	0,00
HORVU.MOREX.r3.3HG0256620	0,94	0,00	7,97	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	11,75	17,36	0,00
HORVU.MOREX.r3.3HG0293090	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0293420	0,00	0,00	3,35	0,00	0,00	0,00	0,00	0,00	0,65	0,00	0,00	5,06	0,00	3,13	11,12	0,00
HORVU.MOREX.r3.3HG0293430	0,00	0,00	2,08	0,00	0,00	0,00	0,00	0,00	0,20	0,00	0,00	2,97	0,00	4,33	10,63	0,00
HORVU.MOREX.r3.3HG0293450	0,00	0,00	0,91	0,00	0,00	0,00	0,00	0,00	0,05	0,00	0,00	0,33	0,00	2,91	13,21	0,00
HORVU.MOREX.r3.3HG0293480	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,06	0,00
HORVU.MOREX.r3.3HG0293490	0,00	0,00	2,46	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,78	8,37	0,00
HORVU.MOREX.r3.3HG0297700	15,72	30,28	32,32	6,51	33,67	1,73	0,93	46,46	46,03	378,71	78,60	170,49	146,66	55,77	35,13	1,11
HORVU.MOREX.r3.3HG0318560	0,00	0,00	0,00	0,00	0,10	0,00	0,00	1,55	0,00	0,00	0,00	0,00	0,00	28,55	0,41	0,00
HORVU.MOREX.r3.3HG0318580	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,73	0,00	0,00	0,00	0,00	0,00	52,95	5,01	0,00
HORVU.MOREX.r3.3HG0318870	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	6,45	0,70	0,00
HORVU.MOREX.r3.3HG0318880	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	32,31	4,22	0,00
HORVU.MOREX.r3.4HG0347880	0,00	0,00	1,42	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,08	0,00	0,00	0,40	0,39	0,00
HORVU.MOREX.r3.4HG0396570	16,86	0,00	1,92	0,00	0,00	0,00	0,00	0,00	0,00	0,38	0,00	0,00	0,00	0,12	0,30	0,00
HORVU.MOREX.r3.4HG0401040	0,00	1,61	5,08	0,14	0,00	0,00	0,00	0,27	0,00	0,00	0,00	0,00	0,00	8,19	14,00	0,25
HORVU.MOREX.r3.4HG0401050	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0401060	0,00	0,00	10,77	0,00	0,06	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	12,67	18,58	0,00
HORVU.MOREX.r3.4HG0401070	0,00	0,00	8,65	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	17,80	23,09	0,00

HORVU.MOREX.r3.4HG0401290	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,24	0,00
HORVU.MOREX.r3.4HG0401310	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,48	0,00
HORVU.MOREX.r3.4HG0409710	1,32	2,33	2,87	6,18	2,61	0,09	0,08	4,63	1,69	0,52	2,74	0,71	0,55	5,28	1,40	4,40
HORVU.MOREX.r3.4HG0409760	0,00	39,03	0,00	0,00	0,00	0,00	0,00	0,00	0,06	0,00	0,00	0,54	0,16	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0409870	2,71	13,03	11,97	21,03	7,44	6,49	2,73	10,77	4,11	6,53	5,89	3,03	2,02	23,97	4,79	3,83
HORVU.MOREX.r3.4HG0415820	15,42	11,71	4,36	3,54	1,49	6,17	5,16	6,13	0,14	0,59	0,48	2,44	6,06	10,27	2,79	0,00
HORVU.MOREX.r3.4HG0415870	3,23	56,05	83,21	0,00	0,32	99,10	120,46	2,48	0,34	21,25	4,53	0,55	55,28	90,72	75,44	0,00
HORVU.MOREX.r3.5HG0439640	0,87	4,63	3,98	1,14	2,87	11,19	13,32	1,51	3,65	4,00	4,69	4,03	3,96	2,43	1,48	5,11
HORVU.MOREX.r3.5HG0508200	6,25	5,27	1,70	0,00	1,06	8,40	9,47	0,19	0,71	5,04	0,02	0,95	2,83	0,77	1,07	0,00
HORVU.MOREX.r3.5HG0519910	0,00	2,02	0,07	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,70	0,00	0,10	0,76	0,00
HORVU.MOREX.r3.5HG0526530	63,90	31,39	8,53	0,49	1,70	18,95	25,92	2,10	6,35	24,26	3,34	16,20	56,09	5,79	5,41	0,00
HORVU.MOREX.r3.5HG0532120	0,00	0,00	0,54	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,66	2,71	0,00
HORVU.MOREX.r3.5HG0532140	0,00	0,00	0,21	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,37	1,92	2,58	0,00
HORVU.MOREX.r3.5HG0532150	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,12	0,00	0,00	4,16	2,83	0,00
HORVU.MOREX.r3.5HG0533630	0,00	0,00	4,86	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	13,07	9,04	0,00
HORVU.MOREX.r3.6HG0543540	0,00	0,21	0,06	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	13,27	20,94	0,00
HORVU.MOREX.r3.6HG0543550	0,00	0,00	0,07	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	15,53	21,06	0,00
HORVU.MOREX.r3.6HG0592630	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.6HG0592640	0,59	1,59	2,04	0,00	0,08	1,74	0,40	0,95	0,00	0,04	0,00	0,39	0,56	20,25	3,82	0,00
HORVU.MOREX.r3.6HG0598380	0,00	3,65	2,36	0,00	0,00	0,10	0,14	2,49	0,14	0,63	0,00	1,33	9,36	1,05	0,74	0,00
HORVU.MOREX.r3.6HG0601090	288,22	59,82	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,41	0,00	9,13	9,63	0,00
HORVU.MOREX.r3.6HG0601160	5,87	24,79	34,61	5,03	8,07	2,31	1,66	15,12	2,60	3,62	46,01	8,33	53,37	37,29	49,63	0,48
HORVU.MOREX.r3.6HG0601180	0,58	2,16	21,24	0,00	0,00	0,87	1,10	7,85	0,29	0,07	25,59	2,30	13,29	22,31	14,84	0,00
HORVU.MOREX.r3.6HG0615400	2,02	68,97	21,31	1,20	3,62	144,18	96,88	11,08	49,12	246,28	61,14	105,36	196,13	15,91	12,82	0,00
HORVU.MOREX.r3.7HG0636890	0,00	0,00	12,67	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	2,62	0,75	0,00
HORVU.MOREX.r3.7HG0704780	0,64	8,91	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.7HG0722730	0,00	0,00	0,12	0,00	0,25	0,00	0,00	0,00	0,00	0,00	0,26	0,00	0,00	0,00	0,26	0,00
HORVU.MOREX.r3.7HG0723940	3,54	1,08	1,43	0,07	0,05	4,17	4,77	0,00	2,61	0,77	0,03	6,77	8,95	3,58	5,17	0,00
HORVU.MOREX.r3.7HG0749090	0,00	0,75	1,31	1,18	7,97	0,00	0,00	0,98	0,00	0,46	3,48	0,24	0,62	0,06	0,00	0,00
HORVU.MOREX.r3.7HG0749130	0,00	0,08	0,00	0,00	0,00	0,59	0,76	0,00	0,00	0,00	0,00	0,10	0,44	0,00	0,00	0,15

Table A.2. Expression of barley expansin genes with detected expression in different parts of seminal roots where water deficient group barleys were treated with PEG-8000 in their nutrient medium compared to control group which were untreated. Parts of the roots that samples were taken are A (0-12.5% of the total seminal root length), B (25-37.5% of the total seminal root length) and C (50-62.5% of the total seminal root length). WES data is obtained from PRJEB13621 [94] via BarleyExpDB [77] from in FPKM.

ID (MorexV3)	Given Name	Control-Root A (0-12.5% of the total seminal root length)	Control-Root B (25-37.5% of the total seminal root length)	Control-Root C (50-62.5% of the total seminal root length)	Water Deficit-Root A (0-12.5% of the total seminal root length)	Water Deficit-Root B (25-37.5% of the total seminal root length)	Water Deficit-Root C (50-62.5% of the total seminal root length)
HORVU.MOREX.r3.1HG0054170	A	3,12	2,05	8,39	1,83	0,53	3,01
HORVU.MOREX.r3.1HG0054180	A	0,6	0,3	1,82	0,65	0,12	0,97
HORVU.MOREX.r3.1HG0054190	A	2,67	1,29	5,93	2,66	0,44	3,05
HORVU.MOREX.r3.1HG0054870	LA	3,3	5,25	8,92	8,11	5,42	4,96
HORVU.MOREX.r3.1HG0055780	B	0	0	0	0	0	0
HORVU.MOREX.r3.1HG0056570	B	82,81	27,44	40,91	118,86	8,44	43,42
HORVU.MOREX.r3.1HG0056580	B	61,56	39,96	58,44	93,24	10,75	78,59
HORVU.MOREX.r3.1HG0056600	B	1,88	0,8	0	5	0,4	0,65
HORVU.MOREX.r3.1HG0056620	B	13,6	23,72	5,89	13,55	18,73	6,33
HORVU.MOREX.r3.1HG0056630	B	22,86	10,1	10,11	41,75	7,55	9,51
HORVU.MOREX.r3.1HG0071050	A	0,96	0,5	0,36	1,57	0,19	1,45
HORVU.MOREX.r3.2HG0104860	A	13,83	0	0	6,59	0	0
HORVU.MOREX.r3.2HG0107600	B	0	0	0	0	0	0
HORVU.MOREX.r3.2HG0107610	B	0	0	0	0	0	0
HORVU.MOREX.r3.2HG0107640	B	0	0	0	0	0	0
HORVU.MOREX.r3.2HG0183770	B	4,48	1,58	1,92	3,28	3,2	2,21
HORVU.MOREX.r3.2HG0185850	A	10,88	9,74	8,78	16,26	11,76	13,7
HORVU.MOREX.r3.2HG0189470	B	0	0	0	0	0	0
HORVU.MOREX.r3.2HG0189530	B	7,15	2,86	12,52	5,17	0,99	7,07
HORVU.MOREX.r3.2HG0189540	B	4,67	0,25	0	27,86	0,83	3,84
HORVU.MOREX.r3.2HG0195810	B	10,3	4,25	7,19	16,01	2,04	17,95
HORVU.MOREX.r3.2HG0214300	B	0	0	0	0	0	0
HORVU.MOREX.r3.3HG0219910	B	0	0	0	0	0	0
HORVU.MOREX.r3.3HG0223260	A	8,83	2,15	4,06	15,61	2,44	14,38
HORVU.MOREX.r3.3HG0250010	A	3,81	1,16	1,22	4,88	0,83	4,63
HORVU.MOREX.r3.3HG0250020	A	2,81	0,9	1,05	3,72	0,8	3,6

HORVU.MOREX.r3.3HG0250040	A	2,5	0,93	1,23	2,97	0,54	3,28
HORVU.MOREX.r3.3HG0250050	A	0	0	0	0	0	0
HORVU.MOREX.r3.3HG0252190	A	8,46	9,07	13,49	13,83	2,73	22,66
HORVU.MOREX.r3.3HG0252250	A	0,8	0	0	0,1	0	0
HORVU.MOREX.r3.3HG0256620	A	22,85	17,18	19,9	41,85	8,09	40,62
HORVU.MOREX.r3.3HG0293090	B	0	0	0	0	0	0
HORVU.MOREX.r3.3HG0293420	A	3,58	2,28	3,9	14,4	1,33	16,84
HORVU.MOREX.r3.3HG0293430	A	3,61	4,72	6,92	11,6	2,45	18,9
HORVU.MOREX.r3.3HG0293450	A	3,5	5,59	10,77	6,15	2,24	14,75
HORVU.MOREX.r3.3HG0293480	A	0	0	0	0,78	0	0,27
HORVU.MOREX.r3.3HG0293490	A	4,25	3,2	5,23	18,07	2,25	18,37
HORVU.MOREX.r3.3HG0297700	A	24,88	10,12	13,45	79,52	14,96	40,51
HORVU.MOREX.r3.3HG0318560	B	0,09	0,39	0	9,05	0,14	2,05
HORVU.MOREX.r3.3HG0318580	B	0,65	1,3	0,62	22,35	0,44	7,59
HORVU.MOREX.r3.3HG0318870	B	0	0,85	0	0,1	0	0
HORVU.MOREX.r3.3HG0318880	B	0,2	1,1	0,14	3,65	0	1,74
HORVU.MOREX.r3.4HG0347880	A	4,24	0,11	0,67	6,59	0,67	4,81
HORVU.MOREX.r3.4HG0396570	A	2,47	0,2	0,45	7,16	0,79	3,5
HORVU.MOREX.r3.4HG0401040	A	29,02	15,54	21,31	45,09	7,34	46,98
HORVU.MOREX.r3.4HG0401050	A	0,48	0	0	2,44	0	0
HORVU.MOREX.r3.4HG0401060	A	44,8	8,92	21,78	64,73	3	37,91
HORVU.MOREX.r3.4HG0401070	A	41,06	13,9	34,09	79,7	5,28	56,02
HORVU.MOREX.r3.4HG0401290	A	3,55	0,68	2,16	4,48	0,12	1,57
HORVU.MOREX.r3.4HG0401310	A	10,03	2,15	7,35	16,17	0,57	7,04
HORVU.MOREX.r3.4HG0409710	LA	2,79	7,99	11,88	8,33	13,63	6,87
HORVU.MOREX.r3.4HG0409760	LA	0	0	0	0	0	0
HORVU.MOREX.r3.4HG0409870	LA	9,72	8,62	9,95	24,94	13,79	12,52
HORVU.MOREX.r3.4HG0415820	B	3,05	7,1	2,58	8,44	7,24	5,93
HORVU.MOREX.r3.4HG0415870	B	64,61	18,32	36,58	88,97	5,85	38,1
HORVU.MOREX.r3.5HG0439640	B	2,29	2,15	2,09	3,53	1,76	2,76
HORVU.MOREX.r3.5HG0508200	B	1,42	0,83	0,92	1,74	0,15	1,56
HORVU.MOREX.r3.5HG0519910	A	3,63	1,77	3,26	3,07	0,5	4,36
HORVU.MOREX.r3.5HG0526530	A	20,38	1,68	6,96	8,79	1,3	1,14
HORVU.MOREX.r3.5HG0532120	A	15,28	7,48	16,8	23,33	1,61	19,07
HORVU.MOREX.r3.5HG0532140	A	7,92	3,18	7,88	15,64	1,01	11,21
HORVU.MOREX.r3.5HG0532150	A	17,46	5,29	14,01	43,03	3,13	25,09
HORVU.MOREX.r3.5HG0533630	A	13,08	4,07	10,91	35,68	4,65	28,46
HORVU.MOREX.r3.6HG0543540	B	10,69	10,69	24,47	14,8	3,05	26,45
HORVU.MOREX.r3.6HG0543550	B	10,28	10,09	22,02	13,44	2,66	23,98
HORVU.MOREX.r3.6HG0592630	B	0	0	0	0	0	0
HORVU.MOREX.r3.6HG0592640	B	1,56	0,09	0,08	14,35	0,16	2,79
HORVU.MOREX.r3.6HG0598380	B	0,34	0,1	0	0,24	0	0,24
HORVU.MOREX.r3.6HG0601090	B	2,49	1,84	0,15	4,45	1,67	0,19
HORVU.MOREX.r3.6HG0601160	B	13,4	3,77	5,92	29,07	2,24	8,53

HORVU.MOREX.r3.6HG0601180	B	4,84	1,3	1,6	10,21	1	3,24
HORVU.MOREX.r3.6HG0615400	A	3,9	2,46	2,53	5,16	2,17	2,98
HORVU.MOREX.r3.7HG0636890	A	7,68	3,57	14,96	10,84	2,11	12,62
HORVU.MOREX.r3.7HG0704780	A	0	0	0	0	0	0
HORVU.MOREX.r3.7HG0722730	A	1,06	1,78	1	2,28	0,97	2,63
HORVU.MOREX.r3.7HG0723940	A	5,45	2,43	5,21	5,97	3,83	4,76
HORVU.MOREX.r3.7HG0749090	LA	0,09	0	0,59	0,4	0	0
HORVU.MOREX.r3.7HG0749130	LA	0	0	0	0,1	0	0

Table A.3. Expression of barley expansin genes with detected expression in different root parts of barley plants 3 days after germination, where control plants were treated with 0 mM NaCl nutrient medium and salt group were treated with 100 mM NaCl nutrient medium. WES data is obtained from PRJEB13621 [83] via BarleyExpDB [77] from in FPKM values.

ID (MorexV3)	**Group**	**Meristematic Zone (Control)**	**Meristematic Zone (Salt)**	**Elongation Zone (Control)**	**Elongation Zone (Salt)**	**Maturation Zone (Control)**	**Maturation Zone (Salt)**
HORVU.MOREX.r3.1HG0054170	A	2,46	2,79	6,86	17,70	1,54	0,49
HORVU.MOREX.r3.1HG0054180	A	0,63	0,92	2,70	5,91	0,49	0,18
HORVU.MOREX.r3.1HG0054190	A	2,39	3,24	7,30	17,79	1,21	0,41
HORVU.MOREX.r3.1HG0054870	LA	0,07	0,00	7,13	4,58	7,79	14,26
HORVU.MOREX.r3.1HG0055780	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.1HG0056570	B	221,72	263,68	622,87	636,46	117,69	50,55
HORVU.MOREX.r3.1HG0056580	B	83,72	114,57	56,32	113,89	24,62	4,57
HORVU.MOREX.r3.1HG0056600	B	0,78	1,36	1,48	1,17	0,56	0,25
HORVU.MOREX.r3.1HG0056620	B	9,79	12,60	111,05	116,11	28,50	25,16
HORVU.MOREX.r3.1HG0056630	B	41,63	51,81	17,18	25,49	37,70	37,31
HORVU.MOREX.r3.1HG0071050	A	1,01	1,50	4,26	2,68	1,92	3,14
HORVU.MOREX.r3.2HG0104860	A	88,56	103,15	33,68	43,44	24,61	1,06
HORVU.MOREX.r3.2HG0107600	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0107610	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0107640	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0183770	B	3,92	3,95	3,74	4,14	3,70	3,59
HORVU.MOREX.r3.2HG0185850	A	16,03	15,88	12,01	11,59	14,52	23,17
HORVU.MOREX.r3.2HG0189470	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0189530	B	7,33	8,01	91,21	88,72	32,06	31,12

HORVU.MOREX.r3.2HG0189540	B	7,25	7,69	3,41	1,82	4,26	2,95
HORVU.MOREX.r3.2HG0195810	B	24,64	27,74	8,79	5,09	10,96	2,85
HORVU.MOREX.r3.2HG0214300	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0219910	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0223260	A	41,12	46,54	15,17	9,24	21,65	7,80
HORVU.MOREX.r3.3HG0250010	A	5,03	4,44	1,63	0,66	2,75	0,48
HORVU.MOREX.r3.3HG0250020	A	4,97	4,13	1,62	0,66	2,95	0,43
HORVU.MOREX.r3.3HG0250040	A	3,85	3,81	1,34	2,18	3,59	3,08
HORVU.MOREX.r3.3HG0250050	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0252190	A	5,39	9,04	15,22	23,37	1,97	1,13
HORVU.MOREX.r3.3HG0252250	A	2,06	3,29	8,23	11,73	0,86	0,53
HORVU.MOREX.r3.3HG0256620	A	38,74	53,73	0,47	5,58	9,78	0,43
HORVU.MOREX.r3.3HG0293090	B	0,00	0,00	0,00	0,07	0,00	0,00
HORVU.MOREX.r3.3HG0293420	A	4,24	10,96	16,48	58,03	2,04	2,70
HORVU.MOREX.r3.3HG0293430	A	5,07	11,67	16,12	60,99	2,55	2,55
HORVU.MOREX.r3.3HG0293450	A	4,03	8,72	10,31	53,07	1,84	2,94
HORVU.MOREX.r3.3HG0293480	A	0,00	0,00	0,00	0,86	0,00	0,00
HORVU.MOREX.r3.3HG0293490	A	5,41	9,18	13,08	34,35	2,16	1,71
HORVU.MOREX.r3.3HG0297700	A	85,33	104,39	52,65	75,53	31,51	15,18
HORVU.MOREX.r3.3HG0318560	B	0,00	0,00	0,20	0,34	0,73	3,82
HORVU.MOREX.r3.3HG0318580	B	0,00	0,00	0,60	1,07	1,33	6,21
HORVU.MOREX.r3.3HG0318870	B	0,00	0,00	0,00	0,00	0,00	0,35
HORVU.MOREX.r3.3HG0318880	B	0,00	0,00	0,00	0,00	0,00	0,18
HORVU.MOREX.r3.4HG0347880	A	6,81	7,23	3,30	2,34	5,07	3,94
HORVU.MOREX.r3.4HG0396570	A	5,10	5,94	2,70	2,55	4,80	3,53
HORVU.MOREX.r3.4HG0401040	A	52,36	67,75	0,00	4,85	13,20	0,45
HORVU.MOREX.r3.4HG0401050	A	0,00	0,45	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0401060	A	43,46	55,18	19,42	26,95	12,55	0,56
HORVU.MOREX.r3.4HG0401070	A	30,70	48,76	44,86	77,64	11,31	3,07
HORVU.MOREX.r3.4HG0401290	A	0,00	0,12	4,28	8,03	0,06	0,46
HORVU.MOREX.r3.4HG0401310	A	0,23	0,84	15,43	26,55	1,27	1,57
HORVU.MOREX.r3.4HG0409710	LA	0,08	0,00	11,55	15,84	15,81	22,02
HORVU.MOREX.r3.4HG0409760	LA	0,00	0,00	0,05	1,32	0,00	0,00
HORVU.MOREX.r3.4HG0409870	LA	1,21	2,25	16,73	13,21	16,50	32,63
HORVU.MOREX.r3.4HG0415820	B	0,49	1,72	1,61	1,52	1,69	5,81
HORVU.MOREX.r3.4HG0415870	B	182,76	203,01	56,63	105,59	52,47	5,60
HORVU.MOREX.r3.5HG0439640	B	2,06	2,48	0,00	0,00	0,55	0,07
HORVU.MOREX.r3.5HG0508200	B	1,71	2,17	0,00	0,00	0,27	0,00
HORVU.MOREX.r3.5HG0519910	A	3,37	3,37	0,00	0,06	0,90	0,00
HORVU.MOREX.r3.5HG0526530	A	0,67	0,81	6,81	4,91	10,44	13,75
HORVU.MOREX.r3.5HG0532120	A	1,71	3,73	14,25	27,33	0,81	1,42
HORVU.MOREX.r3.5HG0532140	A	1,20	3,48	9,38	20,87	0,66	0,87
HORVU.MOREX.r3.5HG0532150	A	0,12	1,66	2,56	14,79	0,00	0,58
HORVU.MOREX.r3.5HG0533630	A	19,03	24,94	0,63	3,05	3,51	0,00

HORVU.MOREX.r3.6HG0543540	B	2,94	4,85	8,78	15,84	2,19	0,63
HORVU.MOREX.r3.6HG0543550	B	3,42	5,25	9,46	17,11	2,43	0,75
HORVU.MOREX.r3.6HG0592630	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.6HG0592640	B	1,42	4,74	4,43	12,20	0,85	3,37
HORVU.MOREX.r3.6HG0598380	B	0,31	0,51	0,00	0,00	0,05	0,00
HORVU.MOREX.r3.6HG0601090	B	0,00	0,00	0,00	0,00	5,73	1,68
HORVU.MOREX.r3.6HG0601160	B	78,24	82,52	159,32	183,84	63,06	46,94
HORVU.MOREX.r3.6HG0601180	B	40,96	47,90	112,55	149,94	32,58	24,89
HORVU.MOREX.r3.6HG0615400	A	10,00	11,18	2,99	3,75	10,14	9,20
HORVU.MOREX.r3.7HG0636890	A	7,07	9,26	112,22	111,20	35,04	35,48
HORVU.MOREX.r3.7HG0704780	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.7HG0722730	A	0,70	1,16	0,68	0,57	0,47	0,26
HORVU.MOREX.r3.7HG0723940	A	1,45	2,11	9,52	8,56	8,03	10,94
HORVU.MOREX.r3.7HG0749090	LA	0,15	0,20	3,23	3,56	1,27	0,34

Table A.4. Expression of barley expansin genes with detected expression in radicle tissue during different stages of seed germination. WES data is obtained from PRJNA496380 [84] via BarleyExpDB [77] from in FPKM values.

ID (MorexV3)	**Group**	**Radicle germinating seed 8h**	**Radicle germinating seed 16h**	**Radicle germinating seed 24h**	**Radicle germinating seed 32h**	**Radicle germinating seed 40h**	**Radicle germinating seed 48h**
HORVU.MOREX.r3.1HG0054170	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.1HG0054180	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.1HG0054190	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.1HG0054870	LA	11,71	6,59	2,23	0,60	0,00	0,00
HORVU.MOREX.r3.1HG0055780	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.1HG0056570	B	0,00	0,51	5,98	112,53	23,67	46,19
HORVU.MOREX.r3.1HG0056580	B	0,00	0,00	0,00	1,07	0,00	0,00
HORVU.MOREX.r3.1HG0056600	B	0,00	0,00	0,33	69,47	3,85	19,56
HORVU.MOREX.r3.1HG0056620	B	0,00	0,00	0,25	5,28	0,00	0,00
HORVU.MOREX.r3.1HG0056630	B	0,00	5,10	9,77	119,30	12,42	75,29
HORVU.MOREX.r3.1HG0071050	A	6,08	19,52	16,20	23,77	14,03	26,21
HORVU.MOREX.r3.2HG0104860	A	0,00	0,00	1,06	10,13	0,54	1,44

HORVU.MOREX.r3.2HG0107600	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0107610	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0107640	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0183770	B	0,00	0,00	0,44	0,56	0,46	0,99
HORVU.MOREX.r3.2HG0185850	A	8,54	9,68	13,24	22,63	13,89	19,79
HORVU.MOREX.r3.2HG0189470	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0189530	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0189540	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0195810	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.2HG0214300	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0219910	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0223260	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0250010	A	0,00	0,00	0,00	0,00	0,00	0,36
HORVU.MOREX.r3.3HG0250020	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0250040	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0250050	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0252190	A	0,00	0,00	0,00	8,14	0,35	1,74
HORVU.MOREX.r3.3HG0252250	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0256620	A	19,04	2,34	6,54	2,34	20,23	5,43
HORVU.MOREX.r3.3HG0293090	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0293420	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0293430	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0293450	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0293480	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0293490	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0297700	A	197,96	188,71	90,32	96,96	41,76	85,90
HORVU.MOREX.r3.3HG0318560	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0318580	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0318870	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.3HG0318880	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0347880	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0396570	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0401040	A	1,61	0,00	0,00	0,00	0,94	0,00
HORVU.MOREX.r3.4HG0401050	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0401060	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0401070	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0401290	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0401310	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0409710	LA	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0409760	LA	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.4HG0409870	LA	23,41	17,59	20,71	45,73	8,03	15,22
HORVU.MOREX.r3.4HG0415820	B	0,00	0,00	0,00	4,90	3,28	4,74
HORVU.MOREX.r3.4HG0415870	B	6,63	57,12	376,85	502,11	169,98	247,76
HORVU.MOREX.r3.5HG0439640	B	1,50	2,26	3,27	1,10	1,76	1,05

HORVU.MOREX.r3.5HG0508200	B	2,66	1,74	2,85	4,70	4,72	5,30
HORVU.MOREX.r3.5HG0519910	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.5HG0526530	A	0,48	1,91	4,01	46,03	8,40	29,86
HORVU.MOREX.r3.5HG0532120	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.5HG0532140	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.5HG0532150	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.5HG0533630	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.6HG0543540	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.6HG0543550	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.6HG0592630	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.6HG0592640	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.6HG0598380	B	0,00	0,00	0,00	0,00	0,40	0,56
HORVU.MOREX.r3.6HG0601090	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.6HG0601160	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.6HG0601180	B	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.6HG0615400	A	20,74	15,01	47,21	30,57	6,46	7,14
HORVU.MOREX.r3.7HG0636890	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.7HG0704780	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.7HG0722730	A	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.7HG0723940	A	1,14	2,14	4,31	4,02	0,00	1,80
HORVU.MOREX.r3.7HG0749090	LA	0,00	0,00	0,00	0,00	0,00	0,00
HORVU.MOREX.r3.7HG0749130	LA	0,00	0,00	0,00	0,00	0,00	0,00