Identification and Analysis of DREB Gene(s) in *Solanum tuberosum* L.

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Declaration

I certify that this research work entitled as "**Identification and analysis of DREB gene(s) in *Solanum tuberosum*"** is my own work. The work has not been presented elsewhere for assessment. The material that has been used from other sources it has been properly acknowledged / referred.

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Abbreviations

DREB	Dehydration responsive element binding
TF	Transcription factor
CBF	C repeat binding factor
ERF	Ethylene response factor
FAO	Food and Agriculture organization
°C	Degree Celsius
bp	Base pairs
MT	Million tones
Ha	Hectare
NARC	National Agricultural Research Centre
PARC	Pakistan Agriculture Research Council
ABA	Abscisic acid
NCBI	National Centre for Biotechnology Information
Cds	Complete coding sequence
PCR	Polymerase Chain Reaction
CTAB	Cetyl trimethyl Ammonium Bromide
PVPP	Polyvinylpolypyrrolidine
IAA	Isoamyl alcohol
TE	Tris-EDTA
TAE	Tris-Acetate-EDTA
ITASSER	Iterative Threading ASSEmbly Refinement
UV	Ultra violet light

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ABSTRACT

Solanum tuberosum generally known as potato is a globally important food crop with high nutritional value. It belongs to Solanaceae family of plants also known as nightshades. It is a drought sensitive crop in comparison to many other plants and in extreme cases drought can contribute to adverse outcomes by negatively influencing survival, biomass production and crop yield. To withstand drought stress plants has evolved several defense strategies that involve morphological as well as genetic changes. For thorough understanding of plant reactions to these stresses, understanding of genetic basis of plant defensive mechanisms against theses stresses is vital. Many transcription factors which control the regulation of numerous genes related with drought has been investigated. The DREB genes are vital genes that are expressed under drought conditions in different plants. This family of transcriptions factors includes distinctive as well as vital proteins that participate in abiotic limitation reactions and resistance in plants. In our recent study we aim to identify and analyze the (DREB) Dehydration Responsive Element Binding genes from Potato. For this purpose many DREB genes were identified in Solanaceae as well as other plant species with help of literature review. From those identified genes DREB3 from model plant *Nicotiana tobaccum* and DREB2 from *Glycine max* are amplified in 7 potato varieties (Desiree, Asterix, Sante, Hermes, 2005-1, Roko, Lady Rosetta) for the purpose of identification of DREB genes in potato. Protein modeling of amplified gene sequences was done by using ITASSER software. Later Phylogenetic tree was generated using MegaX software for the purpose of finding evolutionary relationship between DREB gene sequences which were retrieved by *in silico* analysis. This study will offer foundation for future investigation of these genes in Potato as well as other plants. After identification of DREB genes in potato further studies can be performed to increase drought tolerance of potato as well as other related plants by genetically engineering DREB genes.

1 Introduction

Solanum tuberosum is a member of the Solanaceae family. This family contains sweet pepper (*Capsicum annuum*), tobacco (*Nicotiana tabacum*), tomato (*Solanum lycopersicum*) and eggplant (*Solanum melongena*) as well as petunia along with 2000 other species (Gebhardt, 2016). The genus Solanum is a polymorphous and mainly humid and semi humid genus encompassing greater than 1000 species (Spooner *et al*., 2014). Potato is ranked as the third most produced and consumed crop after rice and wheat and approximately a billion people throughout the world consume it in several forms (Anwar *et al*., 2015). It is an herbaceous, plant 0.4-1.4 m in length and its shape may range from upright to completely horizontal. Twigs show a discrepancy from almost hairless towards compactly hairy and have different colors like purple, green or mottled green. Leaves shapes are pinnate having one terminal leaflet and 3 to 4 sets of big, ovoid leaflets containing smaller ones in middle of them (Struik, 2007). *Solanum tuberosum* plants create rhizomes (generally known as stolons) that contain rudimentary leaves that are normally curved at the edges. These instigate after the basal stem nodes, usually underneath ground, having three rhizomes in a single node (Struik, 2007). Potato is grown everywhere in the world, though in the tropics it is grown in the cool highlands, typically at altitudes over 1000 m, and in the subtropics it is grown in the winters, autumn, as well as spring seasons (Hijmans, 2001).

Potato progresses best in cold weather conditions, as elevated temperatures supports foliar development above tuberization (Haverkort, 1990). It can be grown in a variety of soil natures, however it is sensitive to drought stress and therefore can solitary be grown in regions having adequate rainfalls (Haverkort, 1990; Bohl and Johnson, 2010). Cultivars are carefully chosen with better adaptation against drought and frost because different species have different tolerance range to these stresses. It is a multipurpose, carbohydrate-rich foodstuff greatly famous throughout the globe. Moreover potato is low in fat as well. It contains numerous micronutrients particularly vitamin C that stimulates absorption of iron as well as reasonable source of Fe (iron). Important vitamins like B1, B6 and B3 as well as minerals like phosphorus (P), potassium (k) and magnesium (Mg) are also present in potato in great amount along with

pantothenic acid, folate and riboflavin (FAO 2008). Dietary antioxidants, that may have a role to avoid ailments associated with ageing, and dietary fiber that improves health. Fresh potatoes contain about 20% dry stuff and 80% water. Starch is vital constituent of dry content of potato ranging from 60 – 80%. Protein content on account of dry weight of potato is parallel to cereals on and very great as compare to other tubers and nodes (FAO 2008)

1.1 Potato Statistics of Pakistan

Even though potato was cultivated in the Indian region of subcontinent in 16th and 17th centuries at the time of Pakistan's independence in 1947 growth was limited to some 1000 hectares with total yearly amount produced was not more than the 30,000 tonnes. Newest agricultural statistics (2018) illustrate that potato is cultivated on 187,200 hectares that produce roughly four million tonnes (Pakistan Bureau of statistics). Potato is mainly cultivated in regions of Punjab where presently greater than 95% of the potato growth is centered. Further areas per potato production are Khyber Pakhtunkhwa (KPK), Baluchistan along with Sindh as well. In Pakistan, consumption of potato is displaying an uphill drift nowadays yearly per capita ingestion is more than 15 kg, increased from about 10 kg few years back. In Pakistan, potatoes are used largely as a staple food in many parts and serve as a domestic vegetable available throughout the year. Usually, 3 crops of potato that are spring, summer and autumn are produced in diverse agro-ecological environments of Pakistan ranging from grasslands to high mountains (Khan and Akhtar, 2006). Potato growth process needs minimal labor input and the spell from sowing to harvest is comparatively smaller than other main reaps (not more than 90 days) that marks it as perfect crop for agrarians. Still, besides the availability of appropriate environment, ease of cultivation and low labor requirement, potato production rate in Pakistan is not encouraging in comparison to other developing countries (Khan and Akhtar, 2006).

There are numerous biotic and abiotic stresses that limit potato productivity in the country. Low-temperature stress, drought, salinity, soil problems, improper use of fertilizers and lack of availability of quality irrigation water are some of the fundamental abiotic evils in Pakistan which negatively affect potato productivity. Drought is found to

be major limiting factor in tropical areas (Levy *et al*., 2013). Drought, certainly, is exaggerated by deviation in rainfall and high temperature, which result in additional evaporation and reduced availability of water to potato and other crops (Obidiegwu *et al*., 2015). Major potato budding seasons in Pakistan includes spring, summers and autumn with production range of 07.10%, 15-20% and 70-75% respectively (PARC).

1.2 Production Constraints

1.2.1 Abiotic Stresses

Abiotic limitations are considered to account for 50% losses of normal yields of different crops globally (Wang et al., 2003). Substantial abiotic restraints in the potato production are deprived soil, inadequate usage of pesticides, temperature extremes (cold stress and heat stress), higher salt levels, drought and little allocation of the space for potato cultivation. Temperature has severe influences on yields. Both extremes of temperature (very high and very low) are not perfect for desired tuber yield. Very low temperature (≤0°C) can cause direct injury to seedling, alteration in movement of water in plant, mineral and water uptake and solubility of solutes in soil water consequently dropping tuber yield (Hijmans, 2003; Liao *et al*., 2016) extreme frosts (≥-3°C) can even lead to complete demolition of the whole field of the potato crop (Pino *et al*., 2007). High-temperature exposure often results in physical wilting, high respiration rate, limited photosynthetic activity, slow rate of tuber initiation, abnormalities in enzymatic and metabolic activities of the crop which are generally associated with low yields of potato in the tropics (Hijmans, 2003; Levy and Veilleux, 2007).

Major abiotic constrictions such as drought and fluctuation in temperatures stimulate troubles in physiological and biochemical procedures in addition to physical injury which results in limited growth and production output of crops (Fahad *et al*., 2017). Drought and salinity are among the worst ecological stresses negatively affecting the output of crops throughout the world (Hu *et al*., 2005; Tester and Langridge, 2010). Potato crop is sensitive to drought as well as salinity and substantial yield losses may occur under the influence of these problems (Obidiegwu *et al*., 2015). Problems of drought and salinity are prevalent in some parts of Pakistan, mainly in dry and semi-arid regions and overall nearly 6.3 million hectares area in country is influenced via salt as

well as drought strain (Qureshi *et al*., 2008). A cumulative extent of abnormalities like growth and developmental as well as physiological irregularities of potato and other crops that are challenged due to salinity and drought are broadly described (Heuer and Nadler, 1998; Teixeira *et al*., 2007; Stiller *et al*., 2008; Lipiec *et al*., 2013). Changes of temperature above and below the optimal range, drought and salinity stress affect photosynthesis, respiration, nutrient uptake capacity of potato which has profound effects on tuber formation, number of tubers and their size (Levy and Veilleux, 2007). Low productivity of potato in reply to heat shock, poor water supply, and salt stress alone with drought, are well proven from previous investigations (Yuan *et al*., 2003; Thiele *et al*., 2010; Aksoy *et al*., 2015).

Drought corresponds toward lower yield and production of potato by affecting its leaves and the degree of photosynthesis thus reducing the growth period of the crop (Hirut *et al.*, 2017). Drought is the most significant abiotic limitation of potato that can cause 79% yield losses in extreme conditions (Luitel *et al*., 2015). Such kind of abiotic constraints cause general as well as definite effects development and growth of the plant. Plants reply to such circumstances by adapting a collection of physical, physiological, biochemical and genetic alterations, that permit plants to persist and reproduce (Khan and Equipment, 2011). While grown in various diverse climatic conditions, potato crop is sensitive to drought condition. Water strain imparted due to drought and temperature harshness is a predominant abiotic stress which lessens plant growth and efficiency. Plants react to acclimatize with these circumstances by a collection of biochemical and physiological alterations (Khan and Equipment, 2011). Less water availability during the time of crop progression considerably reduce yield, lessens production period and preventive terrestrial dissemination (Jeknic *et al*., 2013). Drought stress principally harms plant during the course of tuber formation hence affecting different attributes like tuber number, their extent and quality (Lafta and Lorenzen, 1995). Drought conditions negatively alters tuber production and primarily reduce vegetative growth, length of shoots, magnitude and number of leaves (Weisz *et al*., 1994; Deblonde and Ledent, 2001) Drought condition, along with stomata closure also diminishes gaseous exchange by reducing transpiration and rates of photosynthetic (Ekanayake and Midmore, 1992; Kiziloglu *et al*., 2006).

Water intake of potato plant is limited due to its shallow root system (Ekanayake and Midmore, 1992; Dalla Costa *et al*., 1997). The reaction of plants toward drought tension is an intricate procedure comprising numerous genes, and signaling trails besides different gene products. Response toward drought condition in plants incorporates alterations in physical attributes, internal structures and amendments in composition and biochemistry which happens at countless stages, reaching from single cell to photosynthetic structures, and complete assembly of the plant (Nakashima *et al*., 2009) . The substantial roles of numerous transcription regulators in the plant replies to ecological constraints have been well recognized by investigations by the use of genetic as well as molecular biology tactics (Singh et al., 2002; Shinozaki *et al*., 2003)

1.2.2 DREB Genes

DREB genes are the transcription factors which exhibit a vital character in prompting expressions of various abiotic stress affected genes (Yamaguchi-Shinozaki and Shinozaki, 1994)**.** The DREB (dehydration responsive binding factor) genes that are members of ERF subfamily has a crucial part in the plants reactions against abiotic constraints and hence gained substantial consideration in previous times (Agarwal et al., 2017). Proteins that belong to DREB subfamily are additionally allocated in six small classifications termed as A-1, A-2, A-3, A-4, A-5 and A-6, of these A-1 and A-2 are the two leading groups. DREB1/CBF genes, that falls in the A-1 subclass and genes like DREB2, that falls in A-2 subgroup is primarily involved expression of osmotic and cold stress responsive genes correspondingly, hence a crosstalk exist among them (Akhtar *et al*., 2012).

1.3 Structural Characteristics and Regulation of DREB/CBF Genes

All transcription factors contain DNA binding domain having small peptide region, known as DNA binding motif (Agarwal et al., 2017). The amino acid sequence of DNA binding motif is mostly preserved in the family. Proteins that belongs to the CBF/DREB has extremely preserved DNA-binding domain named as ERF/AP2 domain, involving approximately 60 amino acids (Okamuro et al., 1997). Such ERF domain is reflected as specific to plants (Riechmann and Meyerowitz, 1998). The amino acids like valine (present on location 14) and glutamic acid (19) of AP2/ERF domain are fairly preserved

and has a dominant role to play in recognition as well as specific binding of DRE *cis* elements (Zhuang et al., 2011).

The *CBF* governing system is very intricate and it is best examined in *Arabidopsis*. Ruling of transcription factors often works through binding of the regulatory proteins with *cis* acting elements found upstream to the functional genes (Zhuang et al., 2011). A 9 bp conserved sequence (TACCGACAT) is revealed by promotor enquiry of the drought-inducible, increased salinity-inducible as well as cold-inducible genes such as *RD29A/COR78?LT178* in the *Arabidopsis thaliana* (Shinozaki and Yamaguchi-Shinozaki, 2000). This conserved sequence institute DRE, a drought response element. This sequence is present in promotor section of several genes that are induced either by cold or by drought stimulus (Thomashow, 1999; Shinozaki and Yamaguchi-Shinozaki, 2000). Parallel cis-acting elements termed as CRT (C-repeat) and LTRE (low response temperature elements) both having the A/GCCGAC motif makes the center of DRE sequence, controls the promotors induced by cold (Thomashow, 1999). DRE element work in ABA- independent gene expression and ABRE (ABA-responsive element) *cis*-acting elements work in ABA-dependent pathway under the influence of abiotic strain circumstances (Narusaka *et al*., 2003). A DREB1/CBF gene holds the preserved AP2 or DNA binding domain that binds precisely with CRT/DRE sequences thus triggers transcript of many genes governed by the DRE/CRT sequence (Qin *et al*., 2004).

Expression pattern of *DREB2* genes known as *DREB2A* and *DREB2B*, are tempted due to dehydration along with higher salt strains (Lata and Prasad, 2011a). Cis-acting elements of DRE are recognized in promotors of different stress inducible gene of various plants for instance Brassica , tobacco, wheat and certain other grasses signifying occurrence of related regulatory systems of countless further plants as well (Nakashima and Yamaguchi-Shinozaki 2010). DREB like genes has been identified from a range of multiple species (Navarro *et al*., 2009), like *Arabidopsis thaliana* (Sakuma *et al*., 2002a), *Zea mays* (Nguyen *et al*., 2009), *Gossypium hirsutum* (Gao *et al*., 2009), *Oryza sativa* (Chen *et al*., 2008), *Solanum lycopersicum* (Agarwal *et al*., 2006b), *Glycine max* (Chen *et al*., 2007b), *Lolium perenne* (Xiong and Fei, 2006b) *Hordeum vulgare* (Xue and Loveridge, 2004a) *Malus domestica*. (Yang *et al*., 2011) as well as *Bryophyta* (Liu *et al*., 2007b). Numerous genes are controlled under the influence of these molecular directions

and these can be categorized in two main clusters (Fowler and Thomashow, 2002a; Seki *et al*., 2002). First group contains genes encoding proteins to defend cells against abiotic stresses. Other group is mainly composed of genes which controls stress signal transmission along with gene expression systems, for example transcription factors besides protein kinases (Lan Thi Hoang *et al*., 2017). Amongst these DREB genes has been stated as essential stress-responsive transcription factors. DREB genes bind precisely with sequence DRE present in promotors of genes that are induced by stress hence triggering gene expression (Qin *et al*., 2004).

AP2/EREBP family falls among the leading gene families in the plants that entail the different transcription factor encompassing DNA binding domain (Okamuro *et al.*, 1997). Different associates of family perform crucial parts in many trails comprising flowering regulator, environmental stress signaling as well as hormone responses. Above 100 AP2 genes are reported in the plant species like Arabidopsis, grape, rice and that can be distributed in four different subfamilies like DREB, AP2, ERF, and RAV (Lee *et al*., 2012). Transcription factors cooperate with the cis-acting elements that are existent in promotor section of several stress-responsive genes hence, trigger cascades or a complete linkage of genes to improve tolerance to several stresses at once (Ibraheem *et al*., 2010)**.** The family AP2/ERF comprises distinctive subfamilies of transcription factors of the plants having an elementary part in the biotic and abiotic stress reactions (Xie *et al*., 2019). Dehydration responsive element binding factors that are also known as *DREB* transcription factors are the affiliates of AP2/ERF family that involve various essential guiding and stress reacting genes (Nakano *et al.*, 2006)**.**

The DREB genes that are recognized in diverse plant species persuade the expression of different functional target genes that are involved in abiotic stresses (Sakuma *et al.*, 2006b) . The APETALA 2 family is the big set of transcription factors which are specific to plants (Sakuma *et al*., 2002a). Of these, several stress-inducible DREB subfamily participants are identified and investigated. It has been recognized that these are primary elements that are tangled in plant reaction against abiotic stress through regulating gene expression by the cis-acting elements (Yamaguchi-Shinozaki and Shinozaki, 2006a). The participation of members of ERF family that bind with ethylene-responsive element (ERE), as response to abiotic strain has been well studied as well

(Mizoi et al., 2012). Overexpression of DREB genes prompts the downstream stress responsive gene's expression thus enhancing freezing, salt and drought tolerance in Arabidopsis (Jaglo-Ottosen *et al*., 1998; Liu *et al*., 1998a). DREB2A as well as DREB2B are triggered due to high salinity, heat shock and dehydration (Jaglo-Ottosen et al., 1998; Nakashima et al., 2000a; Sakuma et al., 2006c). In result of overexpression of accumulative form of DREB2A triggers the expression of genes that are induced by heat shock as well as dehydration hence enhance drought, heat shock and high salinity tolerance in Arabidopsis (Sakuma et al., 2006c).The presence of DREB genes induced by various stresses in evolutionary distant species illustrates significance of these genes in terrestrial plants along with DRE sequences.

1.3.1 Cell Signaling Pathway of DREB Genes

Plants normally adapt various mechanisms to respond to different environmental stresses. Many kind of biochemical and physiological adjustments are made in order to cope these stresses, these include reduction in area of leaves, deviations in amount of water, leakage of electrolyte, formation of the reactive oxygen species along with gathering of free radicals as well as many other changes (Bartels and Sunkar, 2005). Aside from these changes, ABA (abscisic acid), which is the plant growth manager and stress related hormone, prompts stomatal cessation in leaves to lessen water loss by transpiration. Molecular reactions to abiotic strains, comprises stress observation, signal transmission toward cellular constituents, gene expression eventually leading to metabolic deviations divulging stress acceptance (Agarwal *et al*., 2006a). TFs interrelate by means of cis-acting elements that are present in promotor of numerous stress related genes for up regulating expression of the several downstream genes, hence reporting stress acceptance (Agarwal and Jha, 2010). Molecular studies in Arabidopsis and additional plants divulge numerous pathways that self-reliantly respond toward abiotic strain in both ABA-dependent and ABA-independent pathway hence creating an extremely multifaceted gene network (Fowler and Thomashow, 2002b).

1.3.2 ABA as Key Player in the Stress-related Gene Expression

ABA (Abscisic acid) is a vital plant hormone which has a directing part in various physiological procedures within plants, like enhanced intensities of ABA are elicited by

numerous ecological strains like water stress, cold, salinity, heat stress, and desiccation and wounding. Additionally, this is also demonstrated the fact that ABA is the chief physiological indicator which prompts drought and extraordinary salinity reactions (Gómez *et al*., 1988). Since numerous genes tempted in response to cold and dehydration stress by treatment with ABA (Zhu, 2002), there exist various genes which do not respond to these kind of actions (Zhu, 2002; Yamaguchi-Shinozaki and Shinozaki, 2005) signifying presence of ABA-dependent as well as ABA-independent signal transduction cascade (Fig 1). DRE is the key cis acting element that has a role in ABA-responsive or irresponsive gene expression through the period of abiotic strains (Nakashima and Yamaguchi-Shinozaki, 2009).

1.4 AP2/ERF Family

Many transcription factors having AP2 like DNA binding domains are part of AP2/ERF family and family affiliates are encrypted by 145 loci that is present in *Arabidopsis thaliana* (Sakuma *et al*., 2002a) and 167 loci present in plants of *Oryza sativa* (Sharoni *et al*., 2011). Arabidopsis homeotic gene APETALA2 provided first evidence of this specific domain (Jofuku *et al*., 1994), later a similar domain was observed in tobacco (*Nicotiana tabaccum*) (Ohme-Takagi and Shinshi, 1995). These highly preserved domains contain around 60 residues (Weigel, 1995). Proteins of this family remains precise to plants transcription factors, and the green alga *Chlamydomonas reinhardtii* is the lowermost plant that has AP2/ERF family proteins (Shigyo *et al*., 2006). Binding affinity of DREB1A and DREB2A for the core sequence A/GCCGAC of DRE is illustrated evaluation of these proteins (Sakuma *et al*., 2002a). Moreover, A/GCCGACNT sequence is preferred by DREB1A while ACCGAC is preferred by DREB2A (Sakuma *et al*., 2006a). These kind of binding inclinations were set by exploring the promotors of genes up regulated in genetically modified plants overexpressing every DREB (Maruyama *et al*., 2004; Sakuma *et al*., 2006a). Contrary to this, five ERF proteins exhibit maximum affinity for sequence (AGCCGCC) of GC-box that is the primary sequence of ethylene response elements (Fujimoto *et al*., 2000).

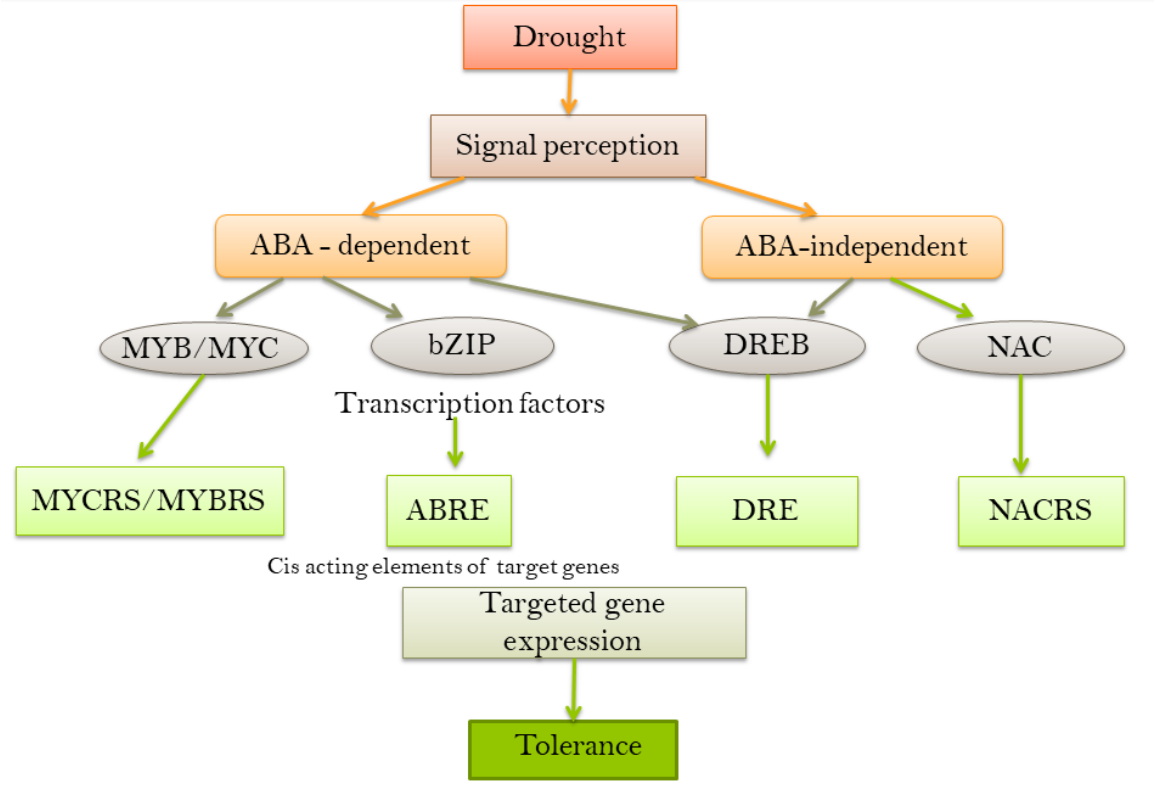


Figure 1: Signal transduction pathway of DREBs. As indicated in figure DREB genes are expressed as result of plant response in ABA- dependent as well as ABA independent manner.

1.5 Objectives

- To identify DREB genes in different plants by *in silico* analysis using NCBI and GenBank
- To identify DREB genes in *Solanum tuberosum*
- To amplify and analyze DREB genes by phylogenetic analysis and protein modeling

2 Literature Review

2.1 History

History of potato originates around 8,000 years in the past near Lake Titicaca that is located around 12,500 ft (3,800 m) beyond sea level in the area of South American Andes mountain ranges. Researches indicates that different groups of hunters who entered the South American continent around 7,000 years ago started domesticating potato plants and started their cultivation around the lake (FAO 2008). Need for preservation of genetic variety of staple foods is greatly emphasized by historical findings about potato. When in 1845-1846 potato crop was devastated by late blight, extensive famine trailed in Ireland due to their dependence on small number of potato varieties. This disaster resulted in death of around 1 million people while more than a million were forced to move overseas. Novel varieties are constantly required for agriculture systems that depend on potato as to fight pests and ailments, to enhance yields and for sustenance of production on marginal lands (FAO 2008). Nonetheless biodiversity of potato is under threats: primeval varieties grown by people of Andean region have been lost due to diseases, climate variation and social disruption. In biodiversity backup of Chiloe archipelago in Chile, around 200 varieties of native potato are cultivated (FAO 2008).

Agriculture practices that were transmitted verbally by generations of mainly female farmers were practiced. International Treaty of potato is part of multilateral system established under FAO's International Treaty on plant Genetic Resources for Food and Agriculture. Treaty which came in 2004 focuses on preservation and sustainable usage of crop plant assortment along with rational and rightful allocation of remunerations obtained from their usage. 2008 was acknowledged as the International Year of Potato by the United Nations (UN).In this international year of potato, UN considered it as a basis of the food for upcoming years to guarantee food security for existing and forthcoming generations (FAO 2008). Potato is a fundamental part of the universal food arrangement already. This is the world's most vital food product, whose yield rates were approaching 325 million tonnes in 2007. Potato's ease of cultivation and

high energy content have made it a valuable crop for millions of farmers and its ingestion rates are gradually increasing reaching up to 50% of overall harvest in some cases (FAO 2008).

2.2 Potato Crop in Pakistan

In Pakistan area under potato production improved to about 10700 ha from 3000 ha at time of independence in 1947. Throughout the course of this time normal potato production rates increased from 9 MT to 20 MT per Ha. Pakistan depends 99% on locally produced seeds for daily consumption and it is self-reliant in potato production. According to a study by PARC (Pakistan Agriculture Research Council) annual domestic production of potato is increased to 1.8 million tonnes, of which 280000 million tonnes is used as seed for further cultivation. The most recent agricultural figures (2018) display that around 4.0 million tonnes of potato is produced on area of 187,200 hectares. According to Pakistan Bureau of statistics Punjab is the highest potato producing province with more than 95% production followed by Khyber pakhtunkhwa (KPK), Balochistan and Sindh. Potato per capita intake is around 15 kg up from a decade ago, hence indicating an upward trend in annual usage (PARC)

2.3 Abiotic Stresses Limiting Potato Growth

Abiotic constraints are frequently threatening plants by negatively influencing their growth, biomass production as well as crop productivity. According to a study (Thakur *et al*., 2010) potato's quality and yield along with limited geographical range are attributed to abiotic stresses like salinity and dehydration. Plant growth and terrestrial ecosystems productivity is greatly threatened by drought, hence it is a sever challenge for plants worldwide (Chaves *et al*., 2003) and various agricultural systems are at verge of facing water scarcity. Plants are influenced by drought stress in both cases when the water flow toward roots is challenging and once the transpiration rate increases. Both these circumstances occur in dry or semi-arid conditions. It is estimated by various climatic models that drought stress will be greatly enhanced in future due to global warming and other climatic changes (Salinger, 2005) though great regional clashes exist (Metz *et al*., 2007) . Frequency and intensity of drought can increase up to 30% in extreme drought

cases by 2100 (Fischlin *et al*., 2007). Poor and marginalized groups with imperfect coping capacities will by greatly affected by these undesirable changes in agronomy. A recent study (Hijmans, 2003) indicates that in 2040-2069 global yield losses of potato due to drought by 18-32%. Recent studies and FAO data clearly indicates effects of drought on cultivation and production of potato. Plant reactions against abiotic stresses are extremely intricate and encompass expression of genes that encode stress related enzymes which are operational in different biosynthetic pathways of osmo protectants and many different stress related metabolites (Fraire Velázquez and Balderas Hernández, 2013). Success rate of achieving abiotic stress tolerance of plants is limited due to the fact that molecular mechanisms governing stress signaling pathways are very intricate and challenging (Khan and Equipment, 2011).

Environmental strains have numerous adversative effects on plant growth. Even if grown in various diverse climates, potato is sensitive against drought stress. Most prevalent abiotic stress is the water scarcity imparted by dehydration and temperature severities resulting in limited growth and productivity. Many biochemical and physiological alterations are adapted by plants to react against these conditions (Khan and Equipment, 2011). Potato's yield is reduced, production period is shortened and topographical dissemination is limited due to less water availability (Jeknic *et al*., 2013). Tuber formation in potato along with number of tubers, size and quality is severely influenced by drought (Lafta and Lorenzen, 1995) along with various other effects like vegetative growth, shoot length number and size of leaves (Weisz *et al*., 1994; Deblonde and Ledent, 2001) Drought stress condense gas exchange by reducing transpiration and photosynthetic proportions apart from closing stomata (Ekanayake and Midmore, 1992; Kiziloglu *et al*., 2006). Shallow root system of potato is the main cause of its vulnerability to drought that limits water intake (Ekanayake and Midmore, 1992; Dalla Costa *et al*., 1997)

2.4 Plants Response to Drought Stress

Plants react against water scarcity and adjust to semiarid drought circumstances by numerous anatomical, physiological biochemical and morphological deviations

containing alterations in the gene expression (Basu et al., 2016). Many plants also acclimatize diverse life approaches to manage drought stress; drought prevention and drought tolerance. Prevention against drought is the capability of plants to retain tissue water content under drought circumstances, whereas if a plant is capable of performing its usual functions even at conditions of less water availability, it is termed as drought tolerance of plant (Hussain et al., 2018).

Morphological fluctuations of plants like reduced stomatal conductance, reduced leaf region, improvement of extensive root systems plays great role in drought prevention of plants (Levitt, 1980). On contrary to this, cell-specific physiological, molecular and biochemical changes which comprises specific expression of gene and accumulation of specific proteins under drought stress are the attributes of drought tolerance of plants. Diverse genes are induced in response to drought stress sequence homology with known proteins help in prediction of function these genes (Bohnert and Sheveleva, 1998). Genes that are induced in response to drought not only protect plant cells against dehydration but also regulate genes in signal transduction pathway against drought (Reddy *et al*., 2004).

2.5 DREB Transcription Factors

The reaction of plants toward drought strain is an intricate procedure comprising several genes, gene products besides signaling pathways (Vishwakarma et al., 2017). Combined response toward drought stress that occurs in plants also encompasses fluctuations in morphology, internal structures as well as changes in physiology and biochemistry that exist on countless different levels including individual cells, photosynthetic organs and entire structure of plants (Rehem *et al*., 2012). Various transcription factors are discovered in expedition of finding genetic factors that work in combination with different abiotic signaling cascades. Some of these important transcription factors responding to low temperature ,drought and high salinity stress include the ethylene responsive element binding factors (ERF), MYC, MYB, basic-domain leucine zipper (bZIP), WRKY binding (WRKY), NAC and DELLA transcription factors (Agarwal *et al*., 2006a; Xiong and Fei, 2006a).

DREB transcription factors has gained most attention among these factors because they are involved in regulation of many stress inducible genes that has a role in initiating response to various stresses (Khan and Equipment, 2011). Genes that plays a role in gene expression and signal transmission include different transcription factors such as DRE (dehydration response elements) (Agarwal *et al*., 2017).Molecular and genetic biology studies are used to investigate role of transcription factors in plant responses to different stresses (Duque *et al*., 2013; Joshi *et al*., 2016). DREB proteins are the transcription factors that ha tors that plays a vital role in persuading the expression of a variety of abiotic stress-inducible genes (Lata and Prasad, 2011b). Ethylene responsive factors (ERF) along with Dehydration responsive element binding factors (DREBs) are key factors governing the expression of genes in diverse signaling pathways due to their altered DNA-binding specificity (Phukan *et al*., 2017). A DNA binding motif of approximately 60 amino acids is present in these transcription factors (Phukan *et al*., 2017). 14^{th} and 19^{th} amino acids are different in DREBs and ERF and they are considered crucial for specific binding to DRE (Phukan *et al*., 2017).

The DREB genes are described by Val (valine) residue at location 14 and Glu (glutamic acid) at location 19. However, ERFs display an Ala (alanine) and Asp (aspartic acid) residue at position 14 and 19, correspondingly. Inspite of these two residues a conserved Ala (alanine) that is present at position 37 in ERF/AP2 domain has also been found as crucial in binding specificity to DRE (Bouaziz *et al*., 2015). Furthermore, in recognition and binding of DREB genes to DRE cis elements DNA binding domain is found to be necessary (Xie *et al*., 2019). DREB genes were classified into six groups (A-1 to A-6) by Sakuma *et al* due to the fact that they are a large multi gene family (Sakuma *et al*., 2002b). A-1 and A-2 are the largest one having DREB1 and 2 type genes that are involved in abscisic acid (ABA)-independent pathways (Barrero *et al*., 2006). Genes present in type DREB1 (like AtDREB1A, AtDREB1B and AtDREB1C) are thought to be involved in cold response trail, whereas type DREB2 (AtDREB2A) are involved in osmotic-responsive pathway (Lan Thi Hoang *et al.*, 2017).

DREB family of transcription factors is categorized into six groups (Table 1) (Sakuma *et al*., 2002b). DREB genes were originally classified in 2 groups; first that respond toward drought (dehydration) and other that react to low temperature (Liu *et al*., 1998b).During the time of last 15 years transcription factors including DREB genes has been extensively studied due to their manipulation in response to abiotic stresses along with their role in instruction of stress related genes that function in defense pathways against stresses in plants

Investigations on genetic manipulations of DREB genes in plants are still beginning. In spite of a great amount of transgenic plants having DREB and preliminary accomplishments in attaining stress tolerance against controlled experimental conditions; several concerns exist that should be determined to completely discover the prospective of DREB-transgenic plants under the influence of regular stress surroundings. Previous investigations represent that some transcription factors participate in controlling manifestation of abiotic stress responsive genes (Figure 1). This indicates ABA independent pathway of genetic expression against drought is controlled by DREB proteins (Shinwari *et al*., 1998; Nakashima *et al*., 2000b). While drought responses that are ABA dependent comprises the ABA responsive element as well as MYB/MYC promoter elements recognized by basic-region Leu-zipper (bZIP) and Myb/Myc transcription factors correspondingly (Abe *et al*., 1997; Razik and Quatrano, 1997; Uno *et al*., 2000; Kim *et al*., 2001). Meanwhile the cDNAs encoding DRE binding proteins; CBF1, DREB1A, and DREB2A were identified by yeast one-hybrid selection from the Arabidopsis (Stockinger et al., 1997; Liu et al., 1998b) a succession of DREB like genes have been identified from an extensive range of plants, like tobacco (Park *et al*., 2001), rice (Dubouzet *et al*., 2003; Oh *et al*., 2005), wheat (Shen *et al*., 2003b), tobacco (Shen *et al*., 2003a), Brassica (Jaglo *et al*., 2001; Gao *et al*., 2002), barley (Xue and Expression, 2002), tomato (Zhang *et al*., 2004), maize (Qin et al., 2004), cotton (Huang *et al*., 2008), tall fescue (Tang *et al*., 2005), soybean(Chen *et al*., 2007a) and many other plants.

Table 1: Members of different subgroups in DREB gene family

Group of DREB Family	**Gene**	**Triggered by**
A-1	DREB1D/CBF4	Salt, dehydration and ABA
A-2	DREB2A /DREB2B	Heat shock, salinity and drought
A-3	ABI4	ABA and high salinity inducible
A-4	TINY HARDY	Drought Drought
A-5	DBF1	Triggered by ABA,weakly induced by high salinity and dehydration
A-6	RAP2.4 RAP2.4B	Drought/high salinity Drought/high salinity

Through genetic manipulations it is realistic to alter stress resistance genes from any source into any plant species. Earlier investigations recommended that the DREB regulons can be implied in advancement of tolerance in innumerable kinds of agriculturally essential crop plants to abiotic stresses through way of gene transfer techniques. For instance, the expression of AtDREB1A and OsDREB1A in genetically modified Arabidopsis directed to superior frost and dehydration tolerance (Liu *et al*., 1998b; Dubouzet *et al*., 2003) . Overexpression of AtCBF4 in Arabidopsis convened higher drought, freezing and stress tolerance (Haake *et al*., 2002). Apart from above mentioned examples there exist countless other transgenic plants which are revealed to have amplified drought tolerance in result overexpression of DREB genes from particular plants.

3 Material Method

3.1 *In silico* Analysis

DREB genes of different plant species were identified by *In silico* analysis with the help of online tools like NCBI and FenBank. After *in silico* identification, accession numbers of DREB genes with complete cds were retrieved from NCBI database. DREB3 (Accession no: EU727157.1) and DREB2 (DQ 208968.1) complete cds was chosen for PCR amplification.

3.2 Primer Designing

For primer designing of DREB3 and DREB2, their sequences were retrieved from NCBI database and then primers were designed manually. After designing primers OligoCalc was used to identify properties like GC content, melting temperature and primer size.

For amplification of DREB3 following primer set was designed and used

STH1(F) GAATAATGGAAATGTGTCGAAGC

STH1(R) GTACTAAGTGATAACCAGAAC

For amplification of DREB2 Following set of primers was used

STB1(F) AGGTGTAATCGTTGTCTTCCTG

STB1(R) CAGAACCCATGATTGGTGGAT

3.3 Sample Collection

Potato tubers were obtained from National Agriculture Research Centre (NARC), Islamabad. These varieties include

Solanum tuberosum .var. Desiree

Solanum tuberosum .var. Asterix

Solanum tuberosum .var. Roko

Solanum tuberosum .var. Hermes

Solanum tuberosum .var. Lady Rosetta

Solanum tuberosum .var. Sante

Solanum tuberosum .var. 2005-1

Seeds were grown in ASAB glasshouse under controlled settings. Temperature was maintained between 18-25°C, watering of plants was done on alternate days.

3.4 Molecular Analysis

3.4.1 DNA Extraction

DNA extraction was performed using CTAB (Cetyl trimethyl Ammonium Bromide) method using leaves from plants. For this purpose 1ml of CTAB+2microlitre of b-Marceptoethanol per sample were preheated at 65°C in the water bath and added to each ground sample with a pinch of PVPP (polyvinylpolypyrrolidine). Samples were than incubated for 30 minutes at 65°C in the heated block, then removed and cooled to ambient temperature for 1-2 minutes. 500 microlitre of chloroform-IAA (24:1) was added to each tube. Tubes were transferred to orbital shaker and shaken on minimum speed for 10-20 minutes. Samples were centrifuged for 10 minutes at 13,000 rpm. Supernatant (upper layer) was removed and carefully transferred to a clean 1.5 ml eppendorf tube. DNA precipitated by adding 600 microlitre of ice cold isopropanol. DNA Pallet was obtained by centrifuging for 10 minutes at 13,000 rpm. Supernatant was removed and 1ml of wash buffer (70% ethanol) was added. Samples were centrifuged for 5 minutes at 13,000 rpm. Wash buffer was drained away by carefully inverting the tube and pellet was dried. Pellet was dissolved in 50 microliter of T.E. DNA quality was checked agarose gel and stored at -20°C.

3.4.2 Gel Electrophoresis

Gel Electrophoresis was used for visualization of DNA. 1.5 % Agarose gel was prepared by measuring 0.75g of agarose and mixing it in 50 ml 1X TAE [0.5mM EDTA (pH 8.0)

and 20mM Tris Acetate], mixture was heated to ensure that agarose is completely dissolved and a clear solution is obtained. After slightly cooling the solution for 1 to 2 minutes at room temperature, 5µl of Ethidium Bromide was added for visualization under ultra violet (UV) light. After the solution was prepared it was transferred in the casting tray having comb in it.

Gel was permitted to cool for 40 minutes. After solidification casting tray was put in gel tank containing 1x TAE. 100 bp ladder was loaded in the 1st well and DNA samples were loaded in subsequent wells by mixing 5µl of sample with 2 µl of loading dye. Gel electrophoresis conditions were set at 80 volts, 500mA current and 45 minutes of time. After 45 minutes gel was viewed in Gel Doc system in order to visualize DNA bands.

3.5 PCR Amplification

For PCR amplification a reaction mixture of 25 microlitre was prepared. Following reagents were added for this purpose.

Reagent	Volume
PCR water	15.5 µl
10x Taq buffer	2.5 µl
$Mgcl_2$	1.5 µl
2.5mM dNTP's	1ul
Forward Primer	1ul
Reverse Primer	1ul
DNA Template	1.5ul
Taq Polymerase	1ul

.

PCR profile was set for 35 cycles and other conditions were as follows

	Step	**Temperature**	**Time**
1.	Initiation	94^0C	1 minute
2.	Denaturation	94^0C	1 minute
3.	Annealing	54^0C	1 minute
4.	Extension	72^0C	2 minutes
5.	Extension	72^0C	10 minutes

Gel electrophoresis with 1.5% gel was used to view PCR results.

3.6 Identification of Conserved Domains

Conserved domains of all 18 sequences which were selected after *in silico* analysis were retrieved by using NCBI Conserved Domain Database (CDD).

3.7 Phylogenetic Analysis

In order to find evolutionary relationship among DREB genes from different plants, phylogenetic tree was generated by using Mega X software.

3.8 Protein Modeling

After PCR amplification of DREB2 and DREB3 their protein models were generated. For this purpose nucleotide sequences were retrieved from NCBI and translated by using Expasy translate tool. ITASSER (Iterative Threading ASSEmbly Refinement) tool was used for protein modeling of DREB2 and DREB 3.

4 Results and Discussion

4.1 *In silico* Analysis

In the present study we performed *in silico* identification of DREB genes in 18 distinct plant species (Table 2). Many stress-inducible DREB genes has been identified from numerous plants, including eudicots like *Solanum lycopersicum* (tomato) and *Brassica napus* (oilseed rape) (Jaglo *et al*., 2001), monocots such as *Zea mays* (maize) (Kizis and Pagès, 2002) and *Oryza sativa* (rice) (Dubouzet *et al*., 2003) as well as *Hordeum vulgare* (barley) (Xue and Loveridge, 2004b) and from *Physcomitrella patens* (moss) (Liu et al., 2007a) as well . The existence DREB genes in distantly related species specify the significance of the DRE sequence and DREB gene in the stress reactions of terrestrial plants.

4.2 Growth of Plants

Plants of seven potato varieties in ASAB glass house (Fig 2). These varieties include

Solanum tuberosum .var.Desiree

Solanum tuberosum.var.Asterix

Solanum tuberosum.var.Hermes

Solanum tuberosum.var.2005-1

Solanum tuberosum.var.Sante

Solanum tuberosum.var.Roko

Solanum tuberosum.var.Lady Rosetta

Table 2: List of genes alongwith their accession numbers and gene length created after *in silico* identification of DREBs from different plants.

	Gene	**Accession number**	**Gene Length**	**Coding sequence**	**Plant**	**Plant Type**
1	OsDREB1D	AY785895.1	762 bp	Complete cds	*Oryza sativa*	Monocot
2	OsDREB1A	JQ885955.1	717 bp	Complete cds	*Oryza sativa*	Monocot
3	LeDREB3	AF506825.1	835bp	Complete cds	*Solanum lycopersicum*	Dicot
4	StDREB1	JN125862.1	657 bp	Complete cds	*Solanum tuberosum*	Dicot
5	StDREB2	JN125858.1	438 bp	Complete cds	*Solanum tuberosum*	Dicot
6	AtDREB1B	AB013816.1	1396 bp	Complete cds	*Arabidopsis thaliana*	Dicot
7	LlaDREB1b	JN698889	642 bp	Complete cds	*Lepidium latifolium*	Dicot
8	OsDREB1F	AY785897.1	660 bp	Complete cds	*Arabidopsis thaliana and Oryza sativa*	Dicot
9	MbDREB1	EF582842.1	2284 bp	Complete cds	*Malus baccata*	Dicot
10	GmDREB3	DQ208969.1	597 bp	Complete cds	*Glycine max*	Dicot
11	SbDREB2A	GU809211.1	1062 bp	Complete cds	*Salicornia brachiata*	Monocot
12	LcDREB3a	EU999998.1	1543 bp	Complete cds	*Leymus chinensis*	Monocot
13	GmDREB2	DQ208968.1	480 bp	Complete cds	*Glycine max*	Dicot
14	BpDREB2	DQ211836.1	1,384 bp	Complete cds	*Broussonetia papyrifera*	Dicot
15	MnDREB2E	KF678401.1	2349bp	Complete cds	*Morus nigra*	Monocot
16	OsDREB2A	JQ341059.1	846 bp	Complete cds	*Oryza sativa*	Monocot
17	HvCBF4	AF298230.1	915 bp	Complete cds	*Hordeum vulgare*	Monocot
18	NtDREB3	EU727157.1	654	complete cds	*Nicotiana tabaccum*	Dicot

Figure 2: Potato plants grown in ASAB glass house

4.3 PCR Amplification

DREB 2 and DREB 3 were amplified in lab by using specially designed STB1 and STH1 primers respectively. DREB2 gene was previously identified from *Glycine max* (soyabean) (Chen et al., 2007a) while DREB3 genes were formerly identified and studied in tomato (Islam and Wang, 2009), soybean (Nasreen *et al*., 2013) and tobacco (Yamaguchi-Shinozaki and Shinozaki, 1994) and other plant species as well. After PCR amplification with DREB 2 and DREB 3 primers, following results were obtained (Fig 3, 4).

4.4 Identification of Conserved Domain

Conserved Domains Conserved domain of selected 18 sequences was retrieved from NCBI conserved domain (CDD) database as investigated by previous studies (Weigel, 1995b; Stockinger et al., 1997).

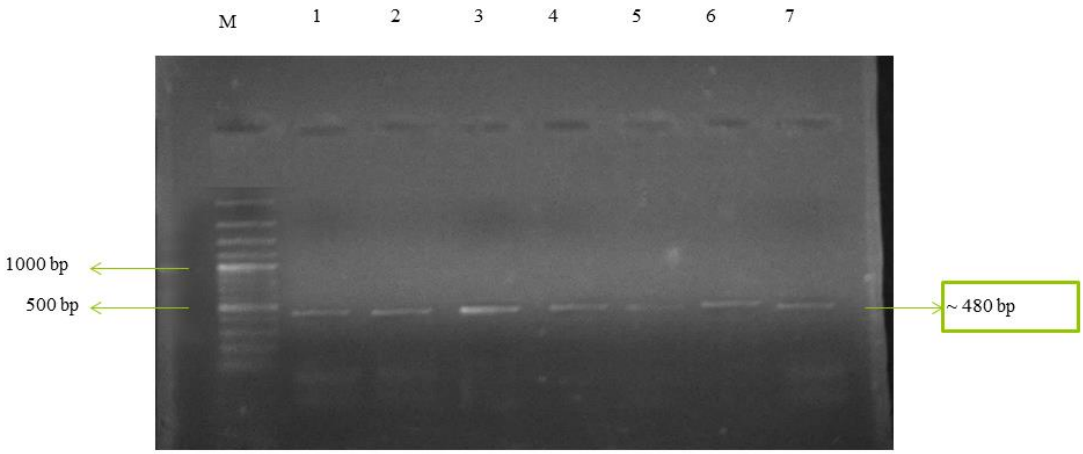


Figure 2: Gel image of PCR amplification of DREB3. M(ladder), 1(Roko), 2 (Hermes), 3 (Desiree), 4 (L.Rosetta), 5 (Sante), 6 (Asterix), 7 (2005-1)

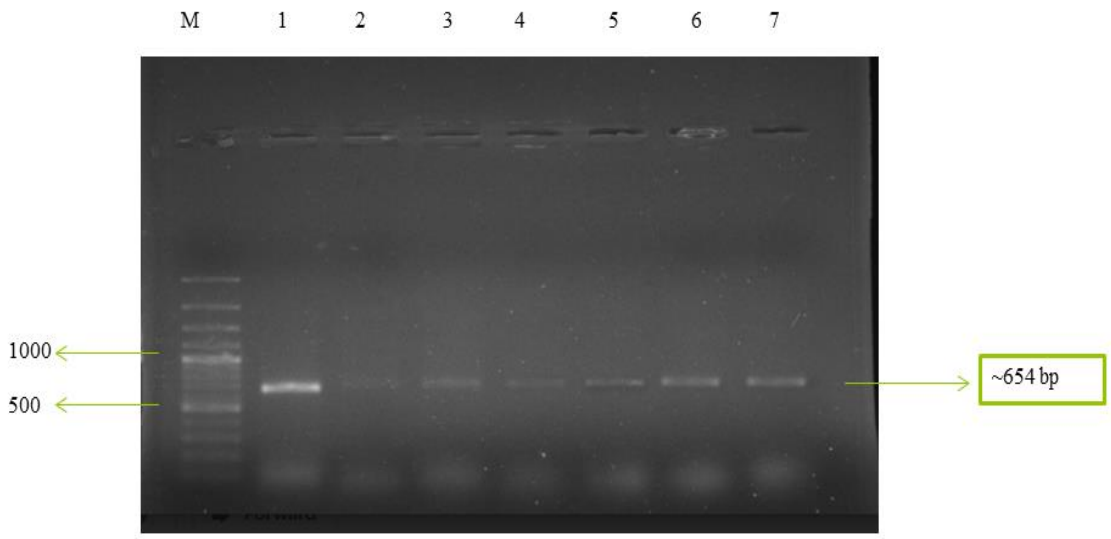
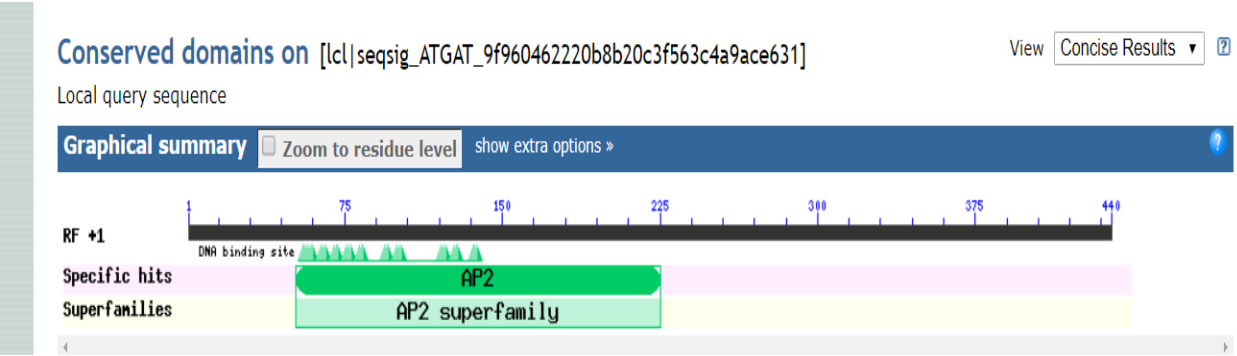
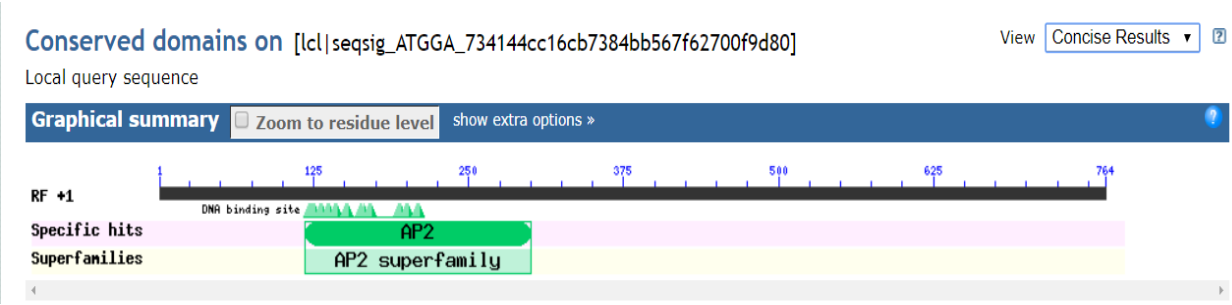


Figure 3: Gel image of PCR amplification of DREB3. M (ladder), 1 (Desiree), 2 (Hermes), 3 (Roko), 4 (Sante), 5 (L rosetta), 6 (2005-1), 7 (Asterix)

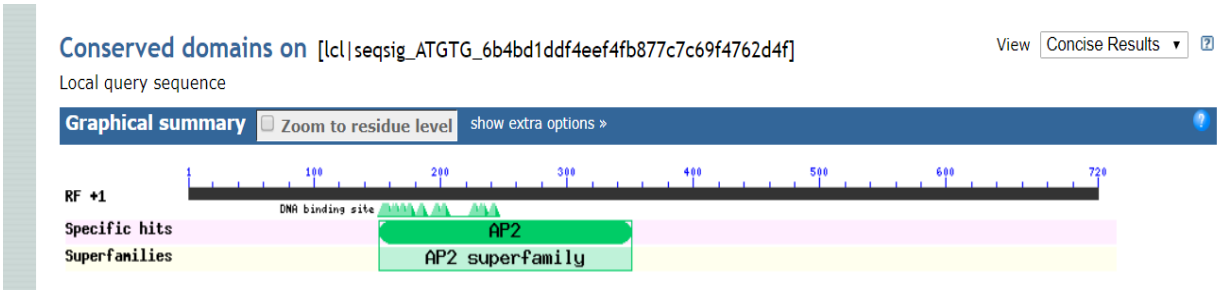
1. *Oryza sativa* DREB1D gene (OsDREB1D)**,** Accession number #AY785895.1



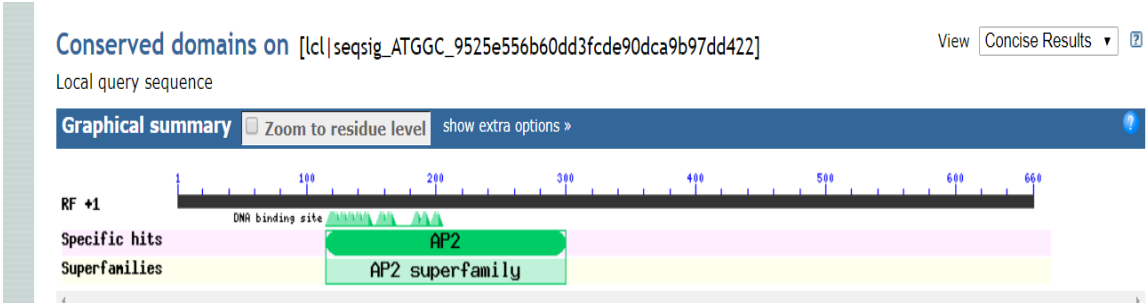
2. *Oryza sativa* DREB1A gene (OSDREB1A), Accession number # JQ885955.1



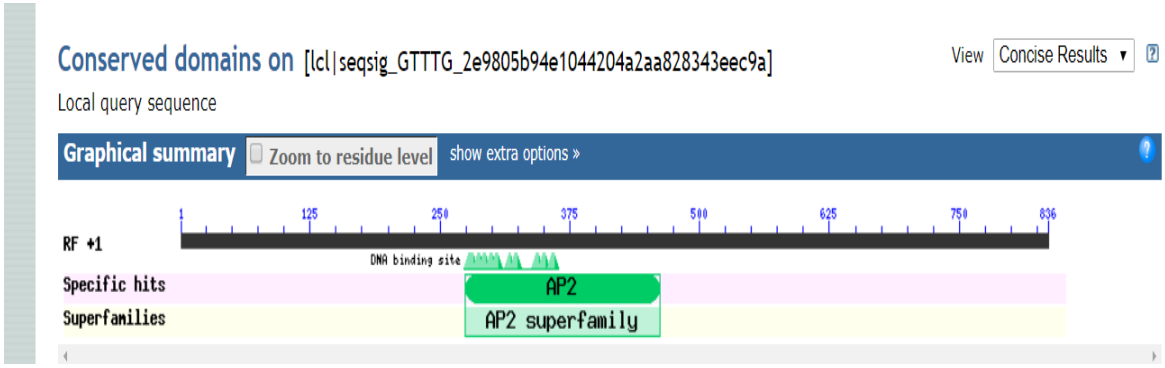
3. *Lycopersicon esculentum* DREB3 gene (LeDREB3), Acccession# AF506825.1



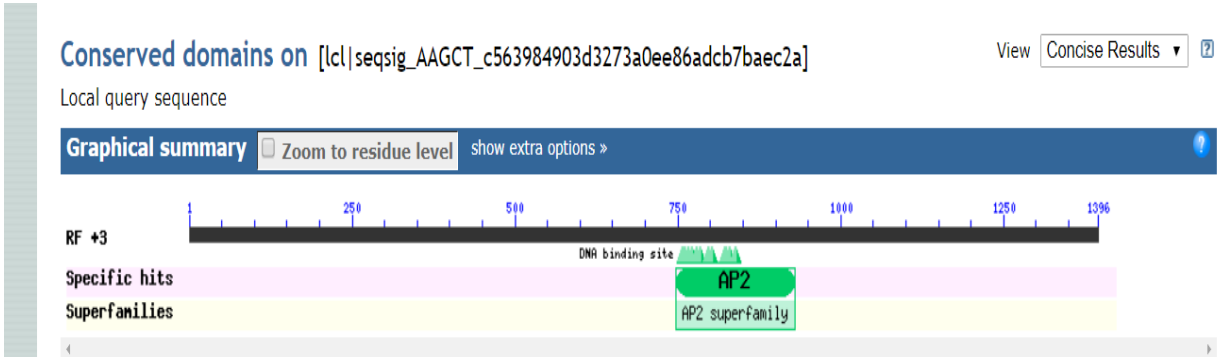
4. *Solanum tuberosum* DREB1 gene (StDREB1), Accession # JN125862.1



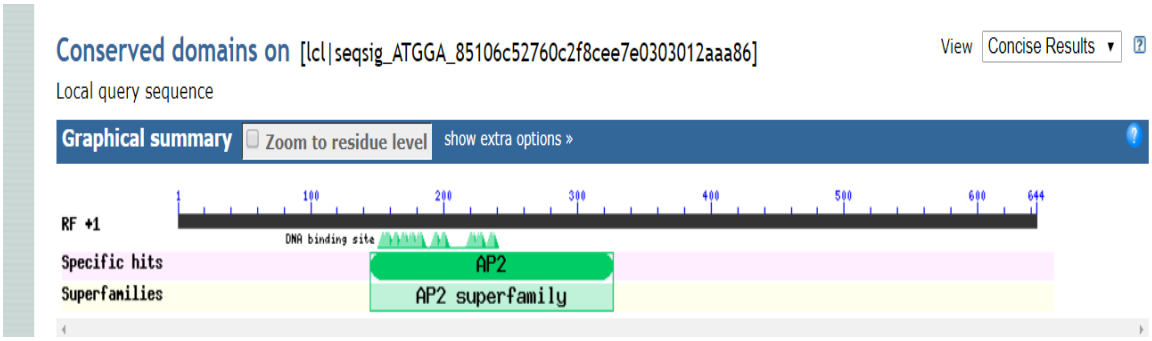
5. *Solanum tuberosum* DREB2 gene (StDREB2), Accessionn # JN125858.1



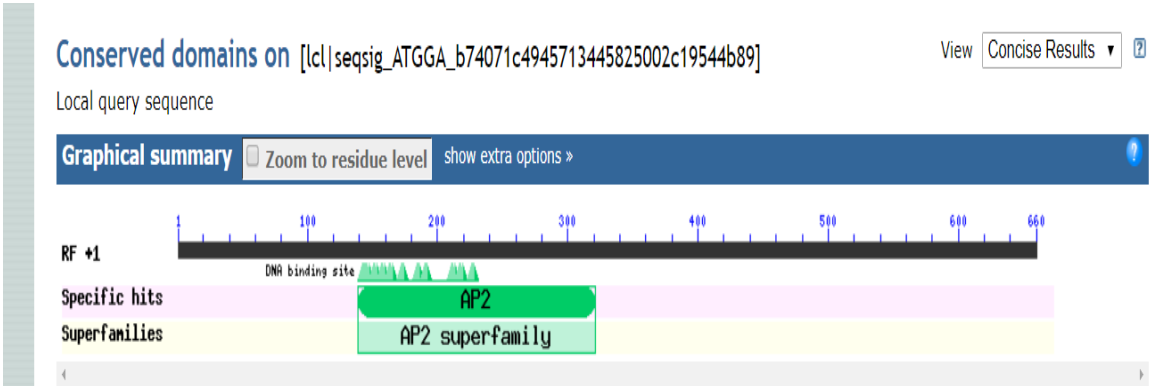
6. *Arabidopsis thaliana* DREB1B gene (AtDREB1B), Accession # AB013816.1



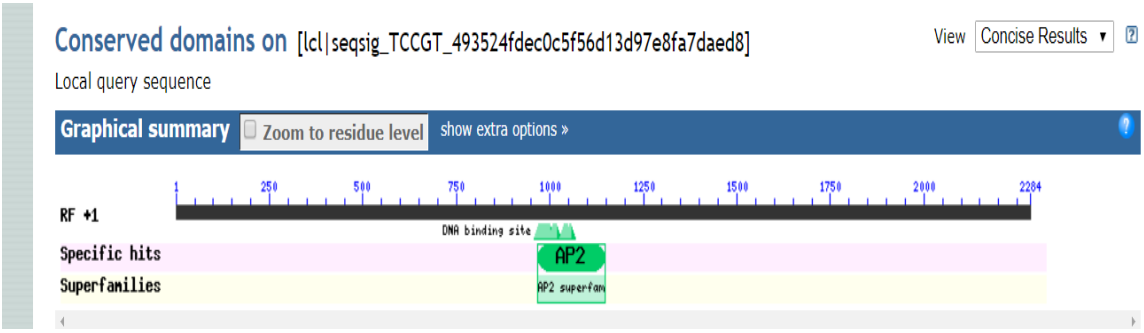
7. *Lepidium latifolium* DREB1B gene (LlDREB1b), Accession # JN698889.1



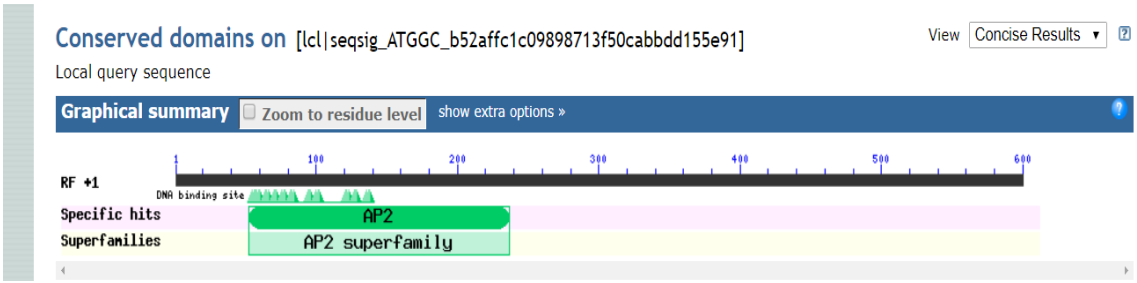
8. *Oryza sativa* DREB1F gene (OsDREB1F) ,Accession # AY785897.1



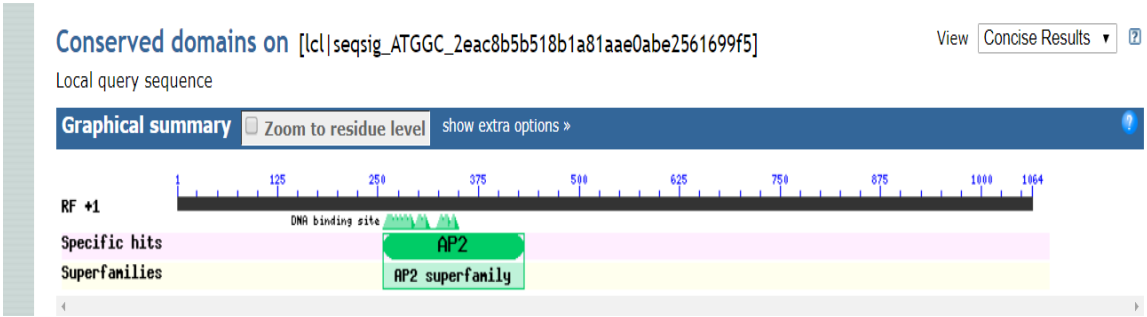
9. *Malus baccata* DREB1 gene (MbDREB1), Accession# EF582842.1



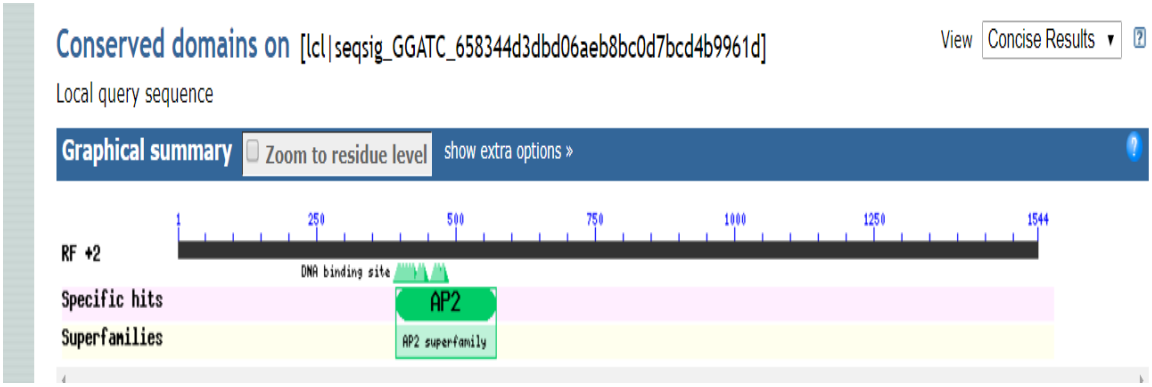
10. *Glycine max* DREB3 gene (GmDREB3), Accession # DQ208969.1



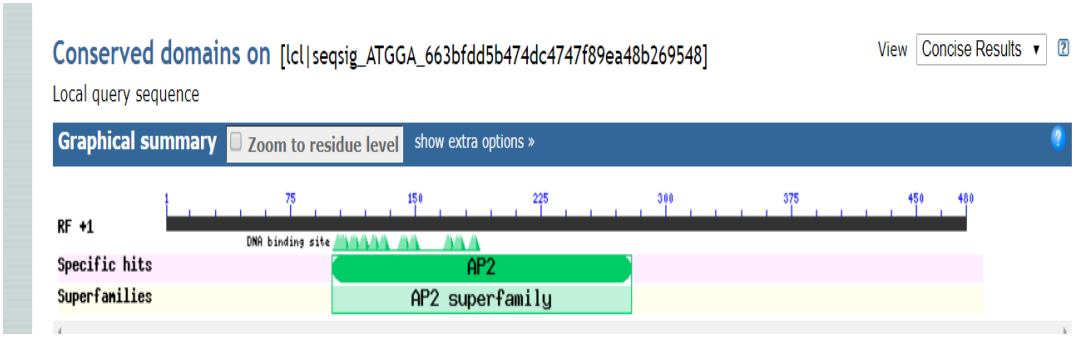
11. *Salicornia brachiata* DREB2A gene, (SbDREB2A), Accession # GU809211.1



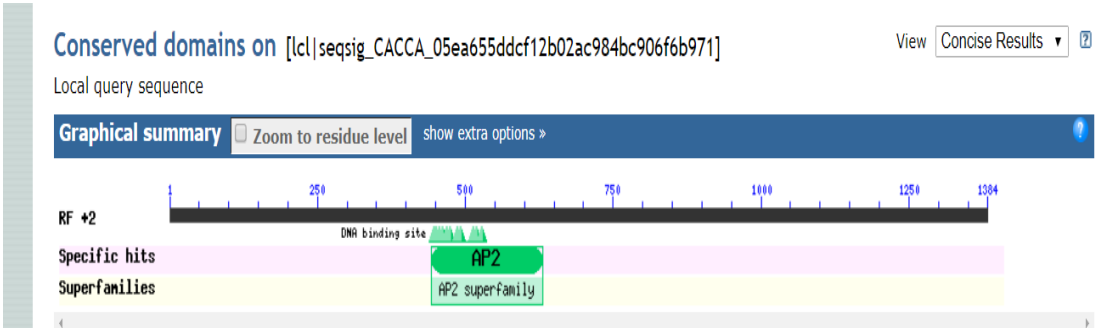
12. *Leymus chinensis* DREB3 gene (LcDREB3), Accession # EU999998.1



13. *Glycine max* DREB2 gene (GmDREB2), Accession # DQ208968.1



14. *Broussonetia papyrifera* DREB gene (BpDREB), Accession # DQ211836.1



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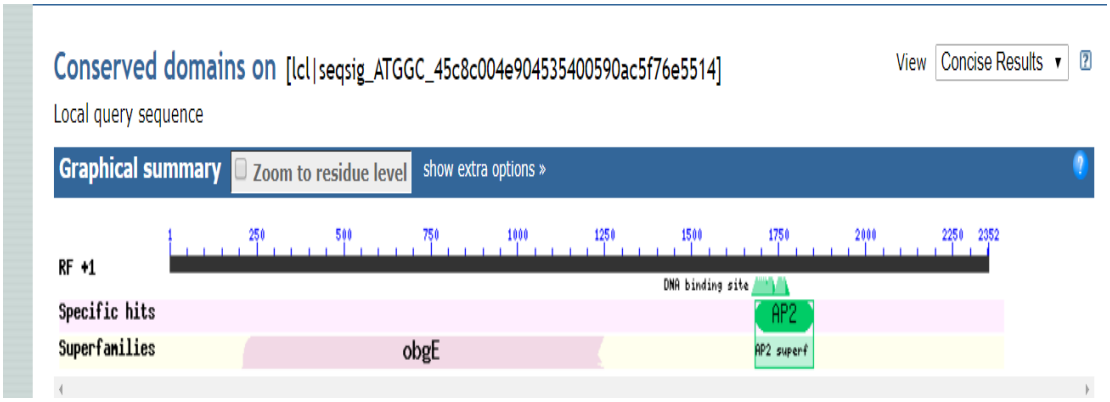
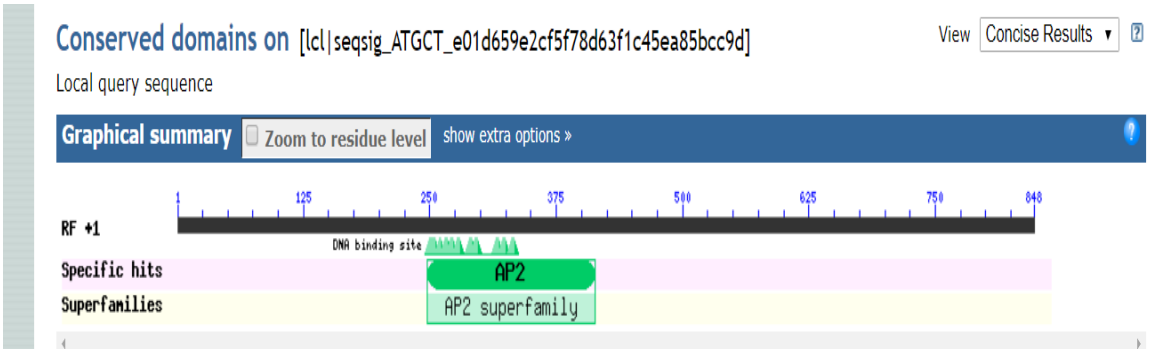
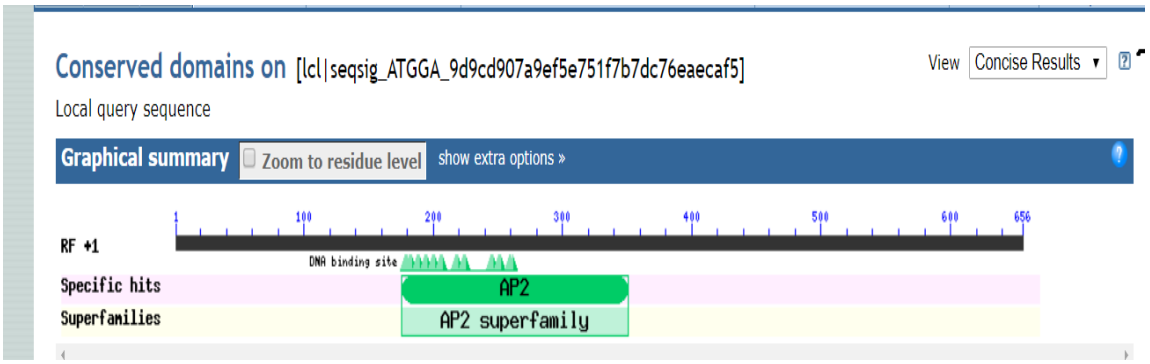
15. *Morus notabilis* DREB2E gene (MnDREB2E), Accession # KF678401.1



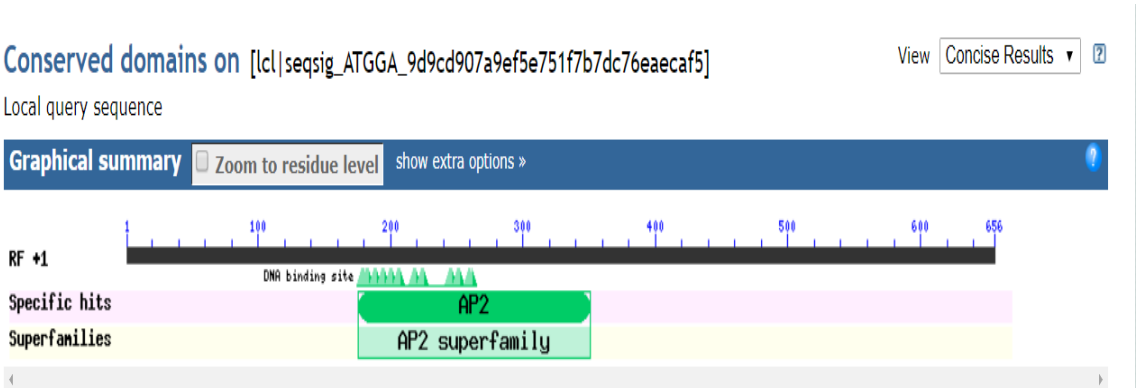
16. *Oryza sativa* DREB2A gene (OsDREB2A), Accession # JQ341059.1



17. *Hordeum vulgare* DREB/CBF1 gene, (HvDREB1), Accession # AF298230.1



18. *Nicotiana tabacum* DREB3 gene, (NtDREB3), Accession# EU727157.1



4.5 Phylogenetic Analysis

The evolutionary history was concluded by the use of Neighbor Joining Method (Saitou *et al*., 1987) . The optimal tree having the sum of branch length =72.50268291 is presented. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). The tree is drawn to scale with branch lengths in the same units as evolutionary distance used to conclude the phylogenetic tree. The evolutionary distances were calculated using the Maximum Composite likelihood method (Tamura *et al*., 2004) and are in the units of the number of base substitutions per site. This analysis involved 18 nucleotide sequences all uncertain positions were removed for each sequence pair (pairwise deletion option). There were a total of 762 positions in the final dataset Evolutionary analysis was accompanied in MegaX (Kumar *et al*., 2018)

4.6 Protein Modeling

Protein models for amplified gene sequences were generated by using ITASSER (Iterative Threading ASSEmbly Refinement).Representative protein models of both sequences are shown (Figure 6, 7).

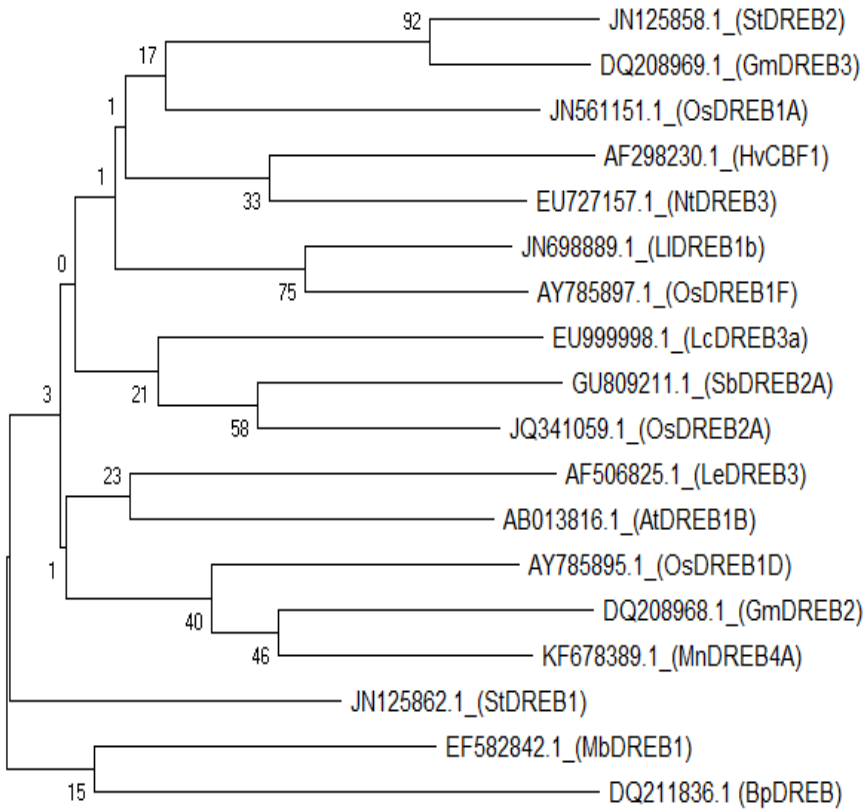


Figure 4 : Phylogenetic Analysis of Selected DREB genes

Figure 6: Representative protein model of DREB2

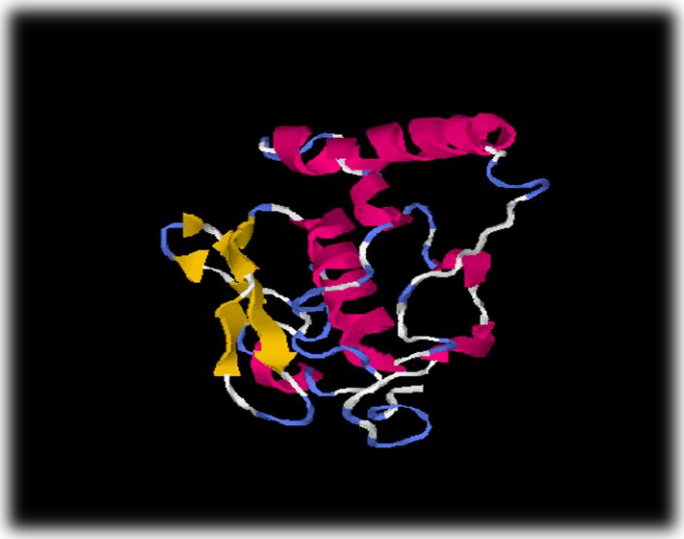


Figure 7: Representative protein model of DREB 3

5. Conclusion and Future Aspects

In order to enhance stress lenience of the plants, it is crucial to understand molecular mechanisms adapted by plants when they respond against abiotic strains like heat, drought, cold and salinity. By studying these mechanisms in productivity of plants can be greatly increase by improving stress tolerance of plants through genetic manipulations. Responses to abiotic stresses are very important as they help in manipulating plants to improve productivity and stress acceptance. To cope with these limitations, numerous genes are synchronized primarily by TFs; their gene products perform their function in enhancing stress lenience of plants. One important class among these TFs is DREB that binds with DRE cis-acting elements. DREB genes are essential TFs of plants that regulate countless gene expressions in reaction to various stresses. These have a crucial part in giving tolerance against many stresses, commonly in an ABA-independent mode through DRE cis-acting elements and AP2/ERF DNA binding domain.

DREB genes can be genetically manipulated to create modified plants having greater tolerance to diverse abiotic constraints using diverse promotors. Molecular examinations of these genes will offer supplementary evidence of multifaceted regulatory networks involved in the abiotic stress responses and the over lapping of different signaling pathways throughout the alteration of plants to withstand innumerable abiotic stresses. In addition, considering DREB genes as aspirant genes and mounting appropriate functional markers that can ultimately be implied for MAS (Marker assisted selection) and other techniques that will pave a path for creating crop varieties by genetic manipulations that will be superior in stress tolerance.

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