CHARACTERIZATION OF *XV7* AND *XV9* cDNA CLONES WHICH CONFER OSMOTOLERANCE TO *ESCHERICIA COLI.*

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INTRODUCTION

The survival of sessile plants requires the ability to withstand extreme water deficit stress caused by drought, salinity, and temperature changes (Skriver and Mundy, 1990). Water loss from plant cells is a very critical environmental stress that affects the availability of land for cultivation (Oliver and Bewley, 1997). More than 35% of the global land surface is thought to be arid or semi-arid, therefore, levels of precipitation is unsatisfactory for horticultural practices (Oliver and Bewley, 1997). The development of crops that are more tolerant to water deficit stress while maintaining productivity will become increasingly important in the future (Oliver and Bewley, 1997). Interest in how plants survive desiccation is increasing because this knowledge will lead to the production of drought tolerant crops (Sherwin and Farrant, unpublished). Crop plants may not have evolved the genetic information to broaden their drought tolerance (Oliver and Bewley, 1997). More information could come from understanding how stress-tolerant plants gain tolerance and identifying those genes that contributing to this phenotype (Oliver and Bewely, 1997).

Few plants can tolerate sever water stress or desiccation (Sherwin and Farrant, 1997; Oliver and Bewley; Ingram and Bartels, 1996). Some indigenous plants can tolerate desiccation of their vegetative tissue (Oliver and Bewley, 1997). The genes involved can influence not only desiccation tolerance but also the mechanisms involved which can be exploited to improve vegetative tolerance and improve the life span and germination success of future seed reserves (Oliver and Bewley, 1997). Desiccation tolerant cells have the ability to withstand sever water loss of the cells and revive from

the air dried state (Oliver and Bewley, 1997). At least 60 species of angiosperms exhibit desiccation tolerance (Oliver and Bewley, 1997). Gymnosperms are the only major class of vascular plants that do not have a desiccation tolerant representative, although they do produce desiccation tolerant seeds and pollen (Oliver and Bewley, 1997). Organisms that are desiccation tolerant can withstand a number of stresses resulting directly or indirectly from cellular water loss (Oliver and Bewley, 1997).

Seeds

Desiccation characterises the final maturation stage of seeds development (Ingram and Bartels, 1996). These "orthodox" seeds lose as much as 90% of their original water and achieve dormancy with undetectable metabolism (Ingram and Bartels, 1996). Seeds are usually dispersed from the parent plant and are viable for many years in the dry state (Oliver and Bewley, 1997). This dry state viability allows the plant to survive severe environmental conditions and allows for maximum dispersal (Ingram and Bartels, 1996). Seeds acquire desiccation tolerance prior to the drying stage itself (Oliver and Bewley, 1997). Seeds undergo metabolic changes during the onset of tolerance, including synthesis of many sugars and proteins, their role in achieving tolerance is still controversial (Oliver and Bewley, 1997).

Pollen

Desiccation tolerance in pollen has been recently discovered and plays a significant role in limiting the damage induced by desiccation (Hoekstra *et al.*, 1992). Pollen can be classified as desiccation tolerant (bicellular) and desiccation sensitive (tricellular) pollen (Oliver and Bewley, 1997). The protective measures include sucrose accumulation, increase in antioxidants, and an increase in membrane acyl group unsaturation (Hoekstra *et al.*, 1992). However, these do not fully explain desiccation

tolerance in pollen, therefore, other factors need to be investigated such as repair mechanisms on rehydration of dried pollen (Oliver and Bewley, 1997).

Resurrection plants

Angiosperms have a unique group of desiccation tolerant plants called resurrection plants. The leaves of these plants have the ability to survive at 2% relative water content (RWC) (Ingram and Bartels, 1996). Desiccation tolerant or resurrection plants are named so because their protoplast recovers after severe water deficits (Sgherri *et al.*, 1993). All true resurrection plants can lose water at varying rates and survive air-dryness in a physiological state referred to as anabiosis (Sgherri *et al.*, 1993; Oliver and Bewley, 1997). The most extensively researched resurrection plant is the African resurrection plant, *Craterostigma plantagineum* Hochst., which survives desiccation of its vegetative tissue, if slowly dried (Oliver and Bewley, 1997). These plants do not survive rapid desiccation Oliver and Bewley, 1997). Recently, the *Xerophyta* genera has been investigated. *Xerophyta viscosa* is a desiccation tolerant angiosperm which can lose its chlorophyll during desiccation (poikilochlorophyllous) (Sherwin and Farrant, 1998). The aim of many studies is to understand the mechanism whereby resurrection plants can tolerate desiccation stress in order to ultimately genetically engineer crops for tolerance.

Stresses associated with desiccation tolerance

Vacoular water loss causes a mechanical stress in which the cytoplasm contract inwards creating tension between the cell wall and plasmalemma (Sherwin and Farrant, unpublished). This could lead to the collapse of the cell wall and rupture of the plasmalemma (Sherwin and Farrant, unpublished). Characteristically, solutes concentration increases and the aqueous medium becomes less viscose (Sherwin and

Farrant, unpublished). Denaturation of nucleic acids, proteins and polar lipids is lethal (Vertucci and Farrant, 1995).

Mechanical stress

Mechanical stress tolerance is thought to depend on the reduction of vacoule volume (Vertucci and Farrant, 1995). In orthodox seeds the vacoules, storage proteins and other insoluble reserves accumulate in the cytoplasm. These are thought to minimise mechanical stress (Sherwin and Farrant, unpublished). Hydrated leaves of resurrection plants have substantial vacoules. Vacoules of *X. viscosa* remain relatively large at 5% RWC stress (Sherwin and Farrant, unpublished). Sherwin and Farrant, (unpublished) suggests that the filled vacoules provide volume within the cytoplasm and return the pressure against the cell wall.

Physiological and biochemical stresses:

Water stress induces changes in photosynthetic activity due to a series of co-ordinated events such as stomatal closure, reduced activity of photosynthetic enzymes, and reduction CO_2 fixation.

Root and shoot growth

Avoidance is dependent primarily upon specialised adaptations in root and shoot design and phenology (Shriver and Mundy, 1990). Cell expansion is one of the most sensitive processes in plants and is sensitive to drought which causes an imbalance between water uptake and water loss in the plant (Frensch, 1997). The complexity between root performance and low plant water potentials is much less understood (Frensch, 1997).

Stomata closure

Closure of stomata during drought stress is thought to be triggered by abscisic acid

(Taylor et al., 1995). Several researchers suggest that there is a redistribution of abscisic acid (ABA) between compartments (Taylor et al., 1995). Redistribution of ABA is water stress-induced compartmental pH shifts which itself is due to inhibition of proton motive forces such as ATPase at the plasmalemma (Taylor et al., 1995). The guard cell ABA receptor faces the apoplasm and that that a 2- to 3-fold increase of ABA in guard cell walls is sufficient to induce stomatal closure (Tabaeizadeh, 1998). It is possible that the accumulation of ABA in guard cells is extensive and fast enough to induce stomatal closure during drought stress (Tabaeizadeh, 1998). Sunflower stomatal control depends only on the concentration of ABA in the xylem sap (Cellier *et al.*, 1998). In drought tolerant line R1 and drought sensitive line S1, stomatal closure in response to exogenous ABA were equivalent in both lines, indicating that R1 and S1 plants display similar sensitivity to ABA with regard to this physiological response (Cellier *et al.*, 1998).

Effect of Water stress on photochemical reactions:

A significant decrease in O_2 evolution has been observed in intact leaves exposed to water stress suggesting that water stress directly affect the photochemical reactions of photosynthesis (Tabaeizadeh, 1998). Several studies have been done on the effect of drought stress on Photosystem II (PSII). PSII is to some extent, tolerant to water stress but inhibited by severe stress (He *et al.*, 1995). According to He *et al.*, (1995) the deterioration of PSII reaction centre proteins can be considered as the main limiting factors for PSII function during water stress (Tabaeizadeh, 1998). During water stress, stomata closure can bring about the transfer of excitation energy from chlorophyll to oxygen resulting in oxygen free radicals being formed. Poikilochlorophyllous resurrection plants lose their chlorophyll and their thylakoid membranes are

dismantled during dehydration (Sherwin and Farrant, 1996). This could possibly be an adaptive mechanism to prevent photo-oxidation under conditions when photosynthesis is not possible (Sherwin and Farrant, 1996). Resurrection plants vary in their response to antioxidants during desiccation and rehydration (Sgherri *et al.*, 1994). Sherwin and Farrant, (1998) compared the tolerance mechanisms between two different genera, *Craterostigma* and *Xerophyta* under high light conditions. Both physical and chemical changes in *C. wilmsii* were induced for protection against free radical damage. Ascorbate peroxidase (AP) activity increased but declined once desiccation reached 5% relative water content (RWC). Rehydration revealed significant increases in superoxide dismutase (SOD) and gluthione reductase (GR) activities (Sherwin and Farrant, 1998). The authors suggest that the plant is protected from free radical damage until complete rehydration and metabolic activity is recovered (Sherwin and Farrant, 1998). In *X. viscosa,* protection from free radicals occurred due to a four-fold increase in anthocyanin content and increased activities of AP, GR and SOD (Sherwin and Farrant, 1998). Sherwin and Farrant, (1998) suggested that the increase in anthocyanin concentration may prevent free radical damage while the thylakoid membrane and chloroplast are restored during rehydration.

Reduction of CO_2 fixation

The activity of enzymes involved in CO_2 reduction are reduced during water stress (Tabaeizadeh, 1998; Bartholomew *et al.*, 1991) One of the main photosynthetic enzymes, Ribulose-1,5-biphosphate carboxyalse (Rubisco) nuclear genes coding for the small subunit of Rubisco, *rbcS*, is primarily affected by water stress at the transcriptional level (Bartholomew *et al.*, 1991). Genes coding for *chl* a/b-binding

proteins, *cab* genes, mRNA levels drop to 70% in drought stressed tomato plants compared to well watered plants (Bartholomew *et al.*, 1991). The activity of fructose 1,6-biphosphatase has also been shown to be inhibited by stress (Tabaeizadeh, 1998). Stromal acidification was suggested as mediator of inhibition, which in turn is facilitated by osmotically induced chloroplast shrinkage (Tabaeizadeh, 1998).

Metabolic Stress

It is thought that proteins and sugars are responsible for maintaining the cellular integrity during desiccation stress (Sherwin and Farrant, unpublished). Angiosperm resurrection plants prevent damage caused by desiccation by protecting the subcellular milieu during dehydration (Sherwin and Farrant, unpublished) . Therefore the need for cellular repair upon rehydration is minimised (Ingram and Bartels, 1996; Vertucci and Farrant, 1995; Oliver and Bewley, 1997). Lots of work has been done on proteins and compatible solutes which are considered as protective components.

Research done thus far:

LEA proteins

These 'late embryogenesis abundant' (LEA) proteins are expressed in vegetative tissue during water deficit, and seeds when developing desiccation tolerance during the maturation stage (Bray, 1997). LEA proteins have been predicted to play a functional role in; maintenance of protein or membrane structure; sequestration of compatible solutes; binding water; and functioning as a molecular chaperone (Bray, 1997). HVA1, protein from barley; and LE25 from tomato when overexpressed, improves drought and salinity tolerance in transgenic rice plants (Bray, 1996).

Dehydrin (RAB) (D-11), another LEA protein has been identified as responsive

transcriptionally or translationally to a variety of stresses. It could possibly act as a chaperone (Ingram and Bartels, 1996). However, direct experimental evidence that LEA proteins are protective towards cellular structures or influence the effects of desiccation has yet to be confirmed (Ingram and Bartels, 1996). Damaging crystallisation of cellular components due loss of water may be counteracted by LEA proteins (Ingram and Bartels, 1996). They could therefore be considered as compatible solutes.

Osmotic adjustment:

Organic osmolytes are a wide range of compounds, most commonly polyhydroxylic compounds (saccharides and polyhydric alcohols) and zwitterionic alkylamines (amino acids and quartenary ammonium compounds) (Hare *et al.*, 1998; Yeo, 1998). The enzymes involved in the synthesis of these osmolytes permit the net accumulation of solutes (osmotic adjustment) (Yeo, 1998). There is increasing evidence that a novel role for osmolytes may include radical scavenging (protection against oxidative damage (Hare *et al.*, 1998) and the use of reducing power and / methyl donor groups (Kishor *et al.*, 1995).

Proline

In *Arabidopsis thaliana* the synthesis and metabolism of proline is controlled by water deficit (Bray, 1997; Iyer and Caplan, 1998; Hare *et al.*, 1998). P5C synthase, one of the enzymes which catalyse the synthesis of proline, is induced by water deficit, salinity and abscisic acid (ABA) (Yoshiba *et al.*, 1995). A gene encoding a specific proline transporter, ProT2 is induced by water deficit (Bray, 1997) which may indicate that proline distribution may be an important factor of osmolyte function. Proline may

interact with enzymes to maintain protein structure and activity within the cell (Iyer and Caplan, 1998). In vitro studies have shown that high concentrations of proline reduces enzymes that denature during heat and salinity stress (Iyer and Caplan, 1998). Hare *et al.*, (1998) suggests that proline accumulation may be the result of metabolic adjustment after stress. Proline may proved carbon, nitrogen and energy for recovery once the stress has been relieved (Hare *et al.*, 1998).

QAC's and TSC's

Glycine betaine

The quaternary ammonium and tertiary sulfonium compounds (QAC's) and (TSC's) accumulate in certain genera of higher plants during water deficit (Tabaeizadeh, 1998). Glycine betaine stabilises the oxygen evolving activity of Photosystem II protein complex (Tabaeizadeh, 1998). Glycine betaine stabilises macromolecules under dehydration stress as well as conferring thermal tolerance, therefore, considered to be an osmoprotectant (Yeo, 1998). Glycine betaine is catalysed by choline monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) (Tabaeizadeh, 1998). Both are soluble chloroplast enzymes and are induced by water deficit stress caused by salinity, drought or cold (Tabaeizadeh, 1998; Wood *et al.*, 1998). The induction of BADH1 and BADH15 mRNAs under drought stress reported by Wood *et al.*, (1998) appear to be similar in salt stressed spinach and sugar beet. BADH mRNA levels cannot fully account for the 26-fold increase in Glycine betaine. It has been suggested that other responses contribute to accumulation of this solute (Wood *et al.*, 1998).

Polyols and Sugars:

Many studies with seeds have shown the accumulation of soluble sugars during the onset of desiccation tolerance (Ingram and Bartels, 1996). In the resurrection plant, *C. plantagineum*, 90% of the total sugar in hydrated leaves is converted to sucrose (Ingram and Bartels, 1996). Koster and Leopold, (1988) showed that sucrose and large oligosaccharides were consistently detected during the tolerant stage of soybean (*Glycine max* L. Merr. cv Williams), pea (*Pisium sativum* L. cv Alaska), and corn (*Zea mays* L. cv Merit) seeds. They also reported that desiccation tolerance was lost as the oligosaccharides were lost(Koster and Leopold, 1988). There results suggests that sucrose may be an important component of desiccation tolerance in seeds, with larger oligosaccharides prevent sucrose from crystallising (Koster and Leopold, 1988). Sugars possibly protect the cell during severe desiccation by glass formation: These sugars form a supersaturated liquid that has the mechanical properties of a solid (Ingram and Bartels, 1996).

Mannitol

In nature mannitol is the most widespread sugar alcohol (Stoop *et al.*, 1997). Mannitol producing plants are highly tolerant of salt stress due to its action as a compatible solute (Stoop *et al.*, (1997). Stoop *et al.*, (1997) show that mannitol accumulation in celery is enhanced by salt stress when grown in nutrient solution containing the equivalent of 30% sea water. Stoop and Pharr, (1993) showed that mannitol containing cells show more than twice the resistance to growth inhibition by NaCl ass sucrose containing cells. This data suggests that mannitol may be acting as an osmoprotectant rather than as a simple osmolyte.. Low levels of mannitol in transformed tobacco plants are significantly more tolerant than untransformed tobacco (Stoop *et al.*, 1997). Recent developments clearly show that mannitol can play an important role in plant

growth and in response to abiotic and biotic stresses (Stoop *et al.*, 1997). Substantial progress has been made in elucidating the unique biochemistry of mannitol metabolism in plants. However, there remain critical gaps like the short range (intercellular) or long range (phloem translocation) movement of mannitol in plants (Stoop *et al.*, 1997).

Sorbitol

Sheveleva *et al.*, (1998) reported on the performance of tobacco after the expression of *Stpd1*, a cDNA encoding sorbitol-6-phosphatase dehydrogenase under optimal growth conditions and during salt stress treatment. High amounts of sorbitol reduced growth and showed symptoms similar to those that have been reported for plants expressing invertase (von Schaewen *et al.*, 1990; Sheveleva *et al.*, 1998). These findings suggest that high amounts of osmolytes may not necessarily protect organisms during osmotic stress that are not adapted for metabolic accumulation.

Genetic Studies

Genetic model systems have been one of the major approaches in the examination of dehydration tolerance. These systems utilise the detailed genetic information, a wide range of mutants, and the potential of positional gene cloning (Ingram and Bartels, 1996). The role of the phytohormone ABA in desiccation tolerance has been studied extensively. ABA-deficient mutants *flacca* (tomato, *Lycopersicon esculentum*) and *droopy* (potato, *Solanum tuberosum*). (for reviews see Ingram and Bartels, 1996; Giraudat *et al.*, 1994). Mutations relating to ABA action has been used to analyse the ABA-mediated drought responses. ABA insensitive mutants have been characterised for *A. thaliana, (abi* loci) and maize (*vp1)* (Giraudat *et al.*, 1994; Ingram and Bartels,

1996). The extensive genetic information available for *A. thaliana* facilitated the isolation of *ABI1 and ABI3* genes by positional cloning (Giraudat et al., 1994). *ABI3* is specifically induced in seeds and probably encodes a transcription factor that activates *lea*-type genes (Bray, 1997). *ABI1* encodes a calcium-regulated phosphatase (Ingram and Bartels. 1996). DNA elements and sequence-specific DNA binding proteins have been investigated extensively (Bray, 1997). Presently, two classes of DNA elements have been elucidated: the ABA-response element (ABRE); and the dehydration-inducible response element (DRE) (Bray, 1997; Shinozaki and Yamaguchi-Shinozaki, 1996). However, more elements are involved in the control of genes that are induced by water deficit (Bray, 1997).

Hormones:

Hormones , have also been of great interest to researchers. Here is a brief account of some of the research on jasmonic acid (JA) and ABA and their role in water stress.

Jasmonic acid

Jasmonates are widely regarded as endogenous plant growth substances which play a role in plant growth development and responses to environmental stress (Loake, 1996). In response to water deficit, JA rapidly accumulates in dehydrated tissue. It is suggested that it reduces tugor pressure (Loake, 1996). Jasmonates have been shown to transduce stimuli resulting from exposure to diverse environmental stresses including water deficit (Loake, 1996). Therefore jasmonates influence many physiological and developmental processes influenced also by ABA (Loake, 1996). Both share common actions at the level of gene expression by inducing similar polypeptides (Loake, 1996).

Abscisic acid

ABA influences various developmental and physiological processes, including embryo maturation and germination. It also influences the response of vegetative tissue to osmotic stress as previously described (stomatal closure) (Skriver and Mundy, 1990). Its role as an inducer of genes responsive to osmotic stress has been extensively investigated as previously described. It has also been implicated in the development of desiccation tolerance in resurrection plants (Vertucci and Farrant, 1995). Endogenous ABA concentration increases when tissues are subjected to water deficit stress resulting from temperature changes, salinity and desiccation (Bray, 1996; Skriver and Mundy, 1990). ABA induces specific gene expression under these stressful conditions (Shinozaki and Yamaguchi-Shinozaki, 1996). Some of these genes are normally expressed during seed desiccation Skriver and Mundy, 1990). In resurrection plants, ABA concentration increases significantly on drying (Sherwin and Farrant, unpublished). Resurrection plants are able to activate the genetic information which is normally only induced during embryogenesis of orthodox seeds) (Sherwin and Farrant, unpublished). Perhaps ABA plays a role in the ability of resurrection plants to survive severe desiccation (Sherwin and Farrant, unpublished).

Signal transduction

Understanding the mechanism whereby plants perceive and transduce a stress signal is the key to understanding water deficit responses (Walker, 1994). Ca^{2+} and IP_3 are possible second messengers in water stress responses in plant cells (Shinozaki and Yamaguchi-Shinozaki, 1996). Phosphorylation processes have been proposed as functional in several signal transduction cascades in plants, yeast and animals cells (Shinozaki and Yamaguchi-Shinozaki, 1996). Various protein kinases have been characterised and are thought to function in the phosphorylation process in various

signal transduction pathways including water stress and ABA response (Shinozaki and Yamaguchi-Shinozaki, 1996). Several recent reports implicate protein kinase in the signal transduction pathways of various environmental signals (Hong *et al*, 1997; Holappa *et al.*, 1995; Walker, 1994). These receptors are typically composed of an extracellular ligand-binding domain, a transmembrane domain, and a cytosolic kinase domain (Walker, 1994) Receptor-like protein kinases containing Leu-rich residues (LRR) are considered to be critical components in the signal transduction pathway of developmental and environmental signals (Hong *et al.*, 1997).

However not all the components of ABA signal transduction have been identified (Bray, 1997) A MAP kinase has been implicated in ABA-regulated gene expression (Bray, 1997). New components suggested to play a role in ABA signal transduction pathway include , protein farnesyl transferase (Bray, 1997). The mechanisms and components involved in desiccation tolerance are very complex and a lot more research is necessary before desiccation tolerance is fully understood.

In our lab 9 genes were cloned from the resurrection plant *X. viscosa* using the strategy of "Complementation by Functional Sufficiency" (Mundree *et al*, unpublished). The aim of my project was to characterise the expression of two of these genes , *XV7* and *XV9*. *X. viscosa* was exposed to various environmental stresses: ABA treatment, temperature changes, and drought stress.

MATERIALS AND METHODS

Plant Material

Xerophyta viscosa Baker plants were collected from the Buffelskloof Nature Reserve (Mpumalanga Province, South Africa) and were grown under glasshouse conditions as described by Sherwin and Farrant (1996).

Isolation of cDNAs encoding for *XV7* and *XV9*

The strategy of "Complementation by Functional Sufficiency" was used by Mundree et al. (Unpublished) to isolate the cDNA encoding for *XV7* and *XV9*.

Isolation of genomic DNA from *Xerophyta viscosa*

Genomic DNA was isolated from *X. viscosa* leaves according to the protocol of Dellaporta *et al.*, (1983). 0.5g of leaf tissue was weighed and quickly frozen in liquid nitrogen. A 3 inch mortar and pestle was used to grind the leaf tissue to a fine powder. The powdered tissue was transferred to a 30 ml oak ridge tube. 15 ml of Extraction Buffer (100mM Tris pH 8, 500mM EDTA[ethylene diamine tetraacetic acid] pH8, 500mM NaCl, 10mM mercaptoethanol) was added and the tube was vortexed for 1minute. 1.0 ml of 20% Sodium Dodecyl Sulphate (SDS) was added and the tube was vortexed for 1 minute and thereafter incubated at 65°C for 10 minutes in a waterbath. 5.0 ml 5M potassium acetate was added and the tube was vortexed for 1 minutes and then incubated at -20°C for 60 minutes. The tube was centrifuged at 12000rpm for 20 minutes at 4°C using benchtop microcentrifuge.. The supernatant was poured through a Miracloth filter (Calbiochem) into a clean 30 ml tube to remove precipitated proteins

and polysaccharides. The pellet was resuspended in 10mL of sterile dH_20. An equal volume isopropanol was added. The tube was gently inverted 2 to 3 times and incubated at -20°C for 30 minutes. The tube was centrifuged for 20 minutes at 12000 rpm in a super speed centrifuge (Beckman J2-21 M/E Centrifuge) at 4°C. The supernatant was poured off and the remaining pellet was dried by inverting the tube on paper towels for 10 minutes. 15 ml of 70% cold Ethanol was added to the dried pellet and centrifuged at 12000rpm for 5 minutes. The supernatant was removed without disturbing the pellet. The tube was stored on ice for 10 minutes to allow the DNA pellet to dry. The DNA pellet was dissolved in 2mL of TE[TrisCl-EDTA (pH 8)]. 10ul of RNase (10mg/mL) solution was added. The tube was incubated at 37°C for 30 minutes. A phenol:chloroform extraction was performed using 500ul phenol (saturated with TE pH8) vortexed for 1 minute then centrifuged for 5 minutes. The aqueous layer was removed and transferred to a new eppendorf tube. The procedure was repeated with 500ul phenol:chloroform (1:1) and 500ul chloroform. The genomic DNA in the aqueous layer was precipitated by adding 50ul 3M Sodium acetate (NaOAc) and 1mL of cold Absolute ethanol. The tubes were kept at -20°C overnight. The eppendorf tube was centrifuged at 4°C for 15 minutes and the supernatant discarded. The remaining pellet was washed with 500ul of cold 70% ethanol and centrifuged at 4°C for 5 minutes. The DNA pellet was resuspended in 50ul of sterile dH_20

DNA was quantified spectrophotometrically (Beckman DU-64 Spectrophotometer): The DNA was diluted in dH_2O (1:50) and absorbances at 260nm and 280nm were obtained. The concentration of DNA (ug/uL) was determined as follows:

2ul genomic DNA (gDNA) +98ul of dH_2O= 100ul (1:50)

gDNA $260_{nm}/280_{nm}$=0.538/0.473=1.13

DNA_{conc}=absorbance at 260nm ×50ug/mL× dilution factor]/ 1000

(1:50) = $[A_{260nm}$×50ug/mL× 50]/ 1000

= [0.538 × 50 × 50]/ 1000

= 1.35ug/mL

DNA was viewed with UV transilluminator and photographed

Isolation and quantitation of total RNA from *X. viscosa.*

Total RNA was isolated from hydrated, dehydrated (37°C relative water content-[RWC]), Abscisic Acid (ABA) treated (10uM for 24 hours), cold-shocked (4°C for 24 hours), heat shocked (37°C for 24 hours) and Sodium Chloride (NaCl) treated (100mM for 24 hours) *X. viscosa* leaves according to the protocol described by Chomczynski *et al.*, 1987.

X. viscosa leaf tissue was ground in liquid nitrogen using motar and pestel. 3g was transferred to an oakridge tube. 15ml of solution B(2M Guanidine thiocyanate, 25mM Sodium Citrate, 0.5%Sarkosyl,

β-mercaptoethanol [120ul]) was added to each oakridge tube and vortexed for 1 minute. 1.5ml of Solution C (2M NaOAc), 15ml Solution D (Phenol), 3ml Solution E (chloroform isoamyl alcohol)) was added. The tube were vortexed again for 1 minute

and cooled on ice for 15 minutes. The tubes were centrifuged at 10 000rpm at 4°C for 20 minutes. The aqueous phases were transferred to new tubes and equal volume of isopropanol was added. The tubes were incubated at -20°C for 1 hour. The tubes were centrifuged at 10 000rpm at 4°C for 20 minutes. The supernatants were discarded and the pellets washed with 70% cold ethanol and air dried. The pellets were resuspended in 3mL diethylpyrocarbonate (DEPC)-treated dH_2O. 1mL of 8M Lithium chloride (LiCl). The tubes were incubated overnight at 4°C

The tubes were centrifuged at 10 000rpm at 4°C for 20 minutes. The pellet was washed with 70% cold ethanol.

RNA was quantified spectrophotometrically: The RNA was diluted in dH_2O (1:50) and absorbances at 260nm and 280nm were obtained. The concentration of RNA(ug/uL) was determined as follows:

2ul total RNA +98ul of dH_2O= 100ul (1:50)

RNA $260_{nm}/280_{nm}$=0.538/0.473=1.13

RNA_{conc}=absorbance at 260nm ×40ug/mL× dilution factor]/ 1000

(1:50) = $[A_{260nm}$×40ug/mL× 50]/ 1000

= [0.538 × 40 × 50]/ 1000

= 1.76ug/mL

RNA was viewed with UV transilluminator and photographed

Southern Blot Analysis

14 ug of gDNA from *X.. viscosa* was digested with EcoRV, and PvuII. The restriction digestions were incubated at 37°C overnight. Lambda DNA restricted with λ Pst was used as marker The digested DNA was electrophoresed on a 0.8% agarose gel (0.24g agarose per 30ml 0.5×Tris-borate[TBE] Buffer) at 20 volts overnight.

Following overnight electrophoreses, the gel was photographed. The gel was cut along the well and nipped on one side to indicate relative position of DNA. The gel was placed in a plastic container and covered with depurination solution (500mL of HCl) and gently agitated for 5 minutes. The denaturing solution was poured off and the gel was washed with sterilised dH_2O. Denaturing Solution (500mL [1.5M and 0.5M NaOH]) was added and gently agitated for 30 minutes. The denaturing solution was poured off and the gel was rinsed with sterilised dH_2O. Neutralising Solution (300-500mL [1.5MNaCl and 1.0M Tris base and HCl to pH 8.0]) was added and gently agitated for 30 minutes. While the gel was neutralised, charged nylon membrane(MSI, 0.45u) was cut to the size of the gel.

Three pieces of 3MM Whatman filter paper was cut a little larger than gel. Nylon membrane and paper towels were cut according to gel size. A wick of Whatman 3MM paper was also cut. After 30 minutes, the neutralising solution was poured off and 500mL of 10×SSC (1×SSC is 150mM NaCl, 17mM Sodium Citrate) was added. The blotting apparatus was assembled. The electrophoresis tray was used as glass plate. The wick was pulled through SSC and wrapped around the glass plate, both ends of the wick was placed in buffer. Gel was placed face down, followed by Nylon

membrane (wetted with 10×SSC). 3×3MM Whatmann paper was placed on top (wetted with 10×SSC)followed by 10cms of towel paper. A glass plate was used to cover transfer apparatus and finally a heavy object(sigma catalogue) was placed in the centre of the lid and left overnight. The nylon membrane (MSI, 0.45u) containing gDNA was exposed to UV light (short wavelength) for 5 minutes to crosslink the nucleic acid onto membrane

Northern Slot-Blot Analysis

Fifteen micrograms of total RNA from hydrated, dehydrated, ABA-treated, cold-shocked, heat shocked and NaCl-treated *X. viscosa* leaves were loaded onto a Slot-Blot apparatus (Biorad). Three replicates of the above treatments were prepared. The nylon membrane (MSI, 0.45u) containing total RNA was exposed to UV light (short wavelength) for 5 minutes to crosslink the nucleic acid onto membrane.

Preparation of radioactively Labelled XV7, XV9 and β-actin inserts:

Electroelution of XV7, XV9 and β-actin inserts

After the gel had been photographed, the target bands were carefully cut out using a scalpel. One end of a dialysis tubing was clamped and filled with 500ul of TE. The target gel fragment was placed in a dialysis tubing and clamped at the other end. The sealed tubing was placed in an

electrophoresis chamber containing 0.5×TE. Electroelution took place at a constant voltage of 2 V/cm between the two electrodes. After electroelution was complete, the polarity of the electrodes was reversed by turning on voltage at 100V for 30 seconds. The tubing was carefully opened and the TE buffer was collected and placed into an

eppendorf tube.. A phenol:chloroform extraction was performed as described earlier.

Radioactive labelling of *XV7*, *XV9*, and β-actin inserts

The *XV7*, *XV9* and β-actin inserts were labelled with [^{32}P] dCTP by random primer labelling (Boehringer-Mannheim) as follows:

The *XV7* and *XV9* DNA was denatured by heating for 10 minutes at 95°C and subsequently cooled on ice. The following reagents were added to make up a the final volume of 20ul: 5ul [^{32}P] dCTP, 2ul reaction mixture,1ul dATP, 1ul dTTP, 1ul dGTP(0.5mmol/L). 9ul DNA (denatured at 95°C),1ul Klenow enzyme. The reaction mixture was incubated for 30 minutes at 37°C. The reaction was stopped by heating to 65°C for 10 minutes.

Removal of unincorporated nucleotides

The above mentioned mixture was passed through a Sephadex G-50 column to separate radioisotope-labelled DNA from unincorporated nucleotides. Two 1ml disposable syringe were plugged with small amounts of sterile glass wool. A column Sephadex G-50 equilibrated in STE (10mMTrisCl ph7.5, 10mmNaCl, 1mmEDTA) buffer was prepared by adding Sephadex G-50 into a 1mL syringe. The 1mL syringe was placed inside a 10mL disposable tube. The tube was centrifuges for 1 minute at 1600g (4000 U/min). The previous two steps were repeated until a column bed volume of 0.9ml was reached. 0.1ml of STE was added to the column and recentrifuged at the same speed as before. The probed DNA was prepared by adding tracking dye and 40ul STE buffer (total volume of 100ul). An eppendorf tube(without cap) was placed at the bottom of the centrifuge tube so that the syringe emptied into the eppendorf tube.

DNA was loaded to the column and centrifuged for 4 minutes. The tracking dye moved with the sample as follows: The Blue Dextran moved with the DNA probe, while the Orange G migrated with the unincorporated nucleotides on the column.

Southern Blot and Northern Slot Blot Hybridization

The container was sealed and incubated at 65°C for 4 hours with agitation. The nylon membrane containing DNA or RNA was transferred to a plastic container containing Pre -Hybridization Buffer(1% BSA [Bovine Serum Albumin], 1mM EDTA, 0.5M $NaHPO_4$, 7%SDS [Sodium Dodecyl Sulphate]).The labelled DNA inserts were denatured by placing it at 95°C for 5 minutes and then rapidly cooled by placing directly on ice. The denatured probes were added to containers, sealed and incubated for 18 hours at 65°C with agitation. Buffer was added to each probed DNA. Small plastic bags filled with buffer and probe DNA were placed in plastic containers and maintained in 60-65°C shaker. The membrane was sealed in a plastic bag and not allowed to dry. The sealed membrane was transferred in an autoradiograph cassette containing enhancer screens. X-ray film (Kodak X-OMAT) over the bag containing the membrane. This was done in dark room with X-ray light on. The cassette was sealed and placed at -70°C for 60 minutes to overnight. The cassette was thawed for 30 minutes prior to development. The cassette was opened in the dark room with X-ray safety light on and fed film into automatic X-ray developer. The film was placed manually in Kodak X-ray fixer for 5 minutes. Washed for 10 minutes in water and then air dried.

DNA Sequencing Analysis

Template DNA of *XV7* and *XV9* (double-stranded plasmid DNA) was purified using a plasmid DNA purification kit (QIAGEN Inc., Hilden, Germany). Sequencing was performed by an ALFexpress™ automated DNA sequencer AMV3.0 (Pharmacia Biotech. AB, Uppsala, Sweden) with T3 Forward primer and T7 Reverse primer. Sequence Similarity Searches of the GenBank and the EMBL database were performed using programs based on the BLAST algorithm. (Altschul *et al.*, 1990).

RESULTS

Sequence analysis of *XV7* and *XV9*

The two cDNA clones contained ATG (translation start codons) at their 5' end and stop codons at their 3' ends (Fig. 1 and 2).

The longest open reading frame in the *XV7* cDNA encodes a protein of 240 amino acids residues with a predicted molecular weight is 28.01kDa. The predicted isoelectric point (pI) is 8.93. A search for homolgy at the nucleotide and amino acid levels for *XV7* shows a 42/131 (30%) identity and 64/37 (46%) positive homology to a putative protein kinase regulator, a syntaxin-like protein with a putative protein kinase regulator function. Protein kinase C phosphorylation site are underlined in Figure 1. *XV7* has a high leucine (Leu) composition (13.4%) and serine (Ser)(9.8%) composition. *XV7* has a 45% probability of being targeted to the cytoplasm.

The longest open reading frame in the *XV9* cDNA encodes a protein of 196 amino acids residues with a predicted molecular weight of 22.22kDa. The predicted pI is 10.41. *XV9* shows no significant homology to any known protein from the Genbank. It has a highglycine (Gly)composition (10.3%) and Arginine (Arg) (9.7%.). *XV9* has a 56.5% probability of being nuclear.

Hydropathic plots

The predicted, possible transmembrane helices for *XV7* were found from sequence position 137 to 157 inside to outside (see graph). Outside to inside helices were found from 135 to 156. Tha amino acids that form part of the region from 137-157 represents the transmembrane domain, as seen in the hydropathic plot (Fig. 4).

Possible transmembrane helices for *XV9* showed that two were found from inside to outside, 129 to 147 sequence position and, 177 to 196 sequence position. From outside to inside two were found, 129 to 161 sequence position and, 166 to 195 sequence position. The inside to outside orientation was predicted as the preferred orientation with the N terminus inside. Therefore the strongly preferred model: N terminus inside-2 strong transmembrane helices,

(1) 129 to 147 and (2)166 to 195

The N terminus of both *XV7* and *XV9* are hydrophyllic regions (Fig. 4 and 5).

DNA Gel-Blot Analysis of *XV7*

The radioactively labelled *XV7* insert hybridised to at least two bands in *X. viscosa* *D*NA digested with EcoRV (lane 1) and three bands digested with PvuII. Suggesting that the *XV7* gene occurs most likely as a 3 copy gene in the haploid genome. The molecular size of the bands varried from approximately 2-4.8kb.The probe also shows that the cDNA *XV7* sequence originates from the *X. viscosa* genome (Fig. 9).

The distinct hybridisation pattern of the 5.9 kDa band probably represents a larger DNA fragment to which the probed *XV7* has hybridised. The intense bands correspond to the *XV7* gene. The presence of faint bands suggest the existence of additional *XV7*-related genes in *X. viscosa.*

DNA Gel-Blot Analysis of *XV9*

The radioactively labelled *XV9* insert hybridised to at least three bands in *X. viscosa* DNA digested with EcoRV while five bandswere observed when digested with PvuII. At least 2 related genes encode the gene product of *XV9* (Fig. 10). This suggests that

XV9 is present in at least 2-3 copies within *X. viscosa* genome.

Northern Slot Blot Analysis

RNA Blot used to analyse the expression of both *XV7* and *XV9* gene. *XV7* is constitutively expressed under hydrated conditions as shown by lane 1 for Fig. 6. Dehydration (35%RWC) and ABA treatment shows equally significant increases in *XV7* mRNA expression. Heat shock also shows significant increases in *XV7* mRNA levels while cold and salt treatment mRNA levels increase but not as significantly as ABA treatment and dehydration. These treatments were salt (10mM NaCL) and cold treatment (4°C for 24 hours). A marked increase of mRNA transcript level of *XV7* within 24 hours after treatment was observed but not to the extent of ABA treatment, dehydration. and heat shock.

XV9 is constitutively expressed as well but not induced by stress treatments except at dehydration (35%RWC) (Fig 7).

RESULTS

ATG GAG ATT ACG CTA CCA CCG CGA GGG CTA ACT CCC AAT CGG CGG CGA ATA AAG GAA
M E I T L P P R G L T P N R R R I K E

GAA GAC GAC GGT AAT GGC GGG GAA GTG GGA GCA AGC GAG AAA TCT CAA CGA AAT TCT CAA
E D D G N G G E V G A S E K S Q R N S Q

GCC TTT CTA CAA ACG AGC TTC CGA GGC CGA AGA GCG TTT GGC AAA GTT CGG AGG AGT CGT
A F L Q T S F R G R R A F G K V R R S R

TTG CCA ATA AAA AAG ATG ACG CCG GGA GCC TGA AGA CGT CCT CTA TCA TAC AAG ATT TCC
A P I K K M T P G A L R R P L S Y K I S

AGT CGA AGC TTC AAA TTG CAC AGA CTG ATC TTC TTT CAG AGA GAG AGA AGG GCT ACA AAG
S R S F K L H R L I F F Q R E R R A T K

GAG ATT CAG AAG CTG ACA GTT GAA GTA GGA CGG ACC ACA GGG AAC TTG AAG AAG CTT CCC
E I Q K L T V E V G R N T G N L K K L P

TTT CAT CCG TAC GTT GGA AAG GAT ATC CCC AGT ACC CTG TAT TAC ATT GGG TTT GTG TTT
F H P Y V G K D I P S T L Y Y I G F V F

TGT TGC ACG CAT AAA ACG GGT GGC GGA CAG GTG GAT ATG ATA AAT TTT GGG ATG TGT TTG
C C T H K T G G G Q V D M I N F G M C L

TAC TCT TAC GTG AAT ATT GAT ATA TTT TCC GTT GGA TTT GCT CAA GTT ATC TAT CAA CTT
Y S Y V N I D I F S V G F A Q V I Y Q L

GTT AAT TTT AAC CTT CCA AGC GCA TCC ATG CAT GTC TTG CAA TTG TAT **TAG** TAA CAA TTA
V N F N L P S A S M H V L Q L Y - -

AACTCAAAAAA

Figure 1. Nucleotide and predicted amino acid sequnces of cDNA clone *XV*9. The amino acid sequence is shown under the corresponding nucleotide triplets.The start and stop codons are bold.

ATG CTT CAT AAC TTA AGA TCC AGG GTA AAG CAG ATG GGT TCC AGT ATG AGC ATG TCG TCT
M L H N L R S R V K Q M G S S M S M S S

TTT GCT AAC AGG GAA GAG TTA CTT GGA CCA AAC AAG AAA GGT GAT GAT ATG AGT AGG GTT
F A N R E E L L G P N K K G D D M S R V

CAG GGT TTG GAT AAC TGG GGC ATT GTT AGC GTA CAG AGA CAA ATT ATG AAA GAG CAA GAT
Q G L D N W G I V S V Q R Q I M K E Q D

GAA GGC CTT GAG AAG CTG GAG GAT ACA GTT TTG AGT ACG AAG CAC ATT GCA CTA GCA GTT
E G L E K L E D T V L S T K H I A L A V

AAT GAA GAG CTG GAT TTG CAT ACA AGA CTA ATT GGT GAC TTG GAT GAA CAT GTG GAT CAA
N E E L D L H T R L I G D L D E H V D Q

GAC TCC CGT CTT CGA CGA GTG CAA AAG AGG CTG GTA GCA ATG AAT GCA CGA GCA AGA GGA
D S R L R R V Q K R L V A M N A R A R G

GGT TGC TCT TGT TTC GCT TTG CTT TTG GGG GGG TTG TCG CAA TTG TAC TTC TAC CTT TTA
G C S C F A L L L G G L S Q L Y F Y L L

TCA TTT ATG CTC TCA TCA AGT ACT TGT AGA ACA TGC AGA ATC CAC CTG CTA GTT GGT TCC
S F M L S S S T C R T C R I H L L V G S

GAT GTT CTC GCC TGT GAC GGA GAA GAA TTC CGG TCG TGC TTG TGC AGA TAC ATT AGC GTG
D V L A C D G E E F R S C L C R Y I S V

CTC AAA GAA AGC AAC CGA AGG ATG ATG TAT ATT CTT CAA CAT TAC TAT CAC AAT TGT TTT
L K E S N R R M M Y I L Q H Y Y H N C F

AAA CAT ATG TAT TAT TAT TAT GCT TGC AGA TAT CGA TAT TGT GTT TTC AAG TTT AGC TCT
K H M Y Y Y Y Y A C R Y R Y C V F K F S S

TGC TTG ATG TGA ATATAACCAGTATGTATTAGCATCTCTGTGTCTTGGTCTCTTGGTCTAAAAAAA
C L M -

AAAAAAAAAAAA

Figure 2. Nucleotide sequence and deduced amino acid sequence of *XV7*. The amino acid sequence is shown under the corresponding nucleotide triplets.The start and stop codon are bold. The protein kinase C phosphorylation sites are underlined.

```
XV7:     13 KRQEMLHNL--RSRV-----KQMGSSMSM--SSFANREELLG-PNK-KGDDMSRVQGLDN 61
            +RQ +L +L  R R+      K  G+   +  SS  + E   G PN     ++     +GL
PKR:     78 RRQNLLDDLVTRERLLLASFKNEGAEPDLIRSSLMSEEAKRGAPNPWLFEEPEETRGLGF 137

XV7:     62 WGIVSVQRQIMKEQDEGLEKLEDTVLSTKHIALAVNEELDLHTRLIGDLDEHVDQTDSRL 121
              I   Q++I++EQD GL+ L   +   K +   +  ELD    +I DL   V+ TD +L
PKR:    138 DEIRQQQQKIIQEQDAGLDALSSIISRQKQMGQEIGNELDEQNEIIDDLANLVENTDEKL 197

XV7:    122 RRVQKRLVAMNARARGGC 139
             R   +R V M  R    C
Sbjct:   198 RNETRR-VNMVDRKSASC 214
```

Figure 3. Amino Acid comparison of XV7 with related proteins. Letters represent "identity" with the XV7 sequence while a positive(+) represent "similarity" with XV7. The percentage identity to XV7 is 36%, and the percentage similarity is 46% as obtained from a computer search using the BLAST network service (Altschul *et al.*, 1990).

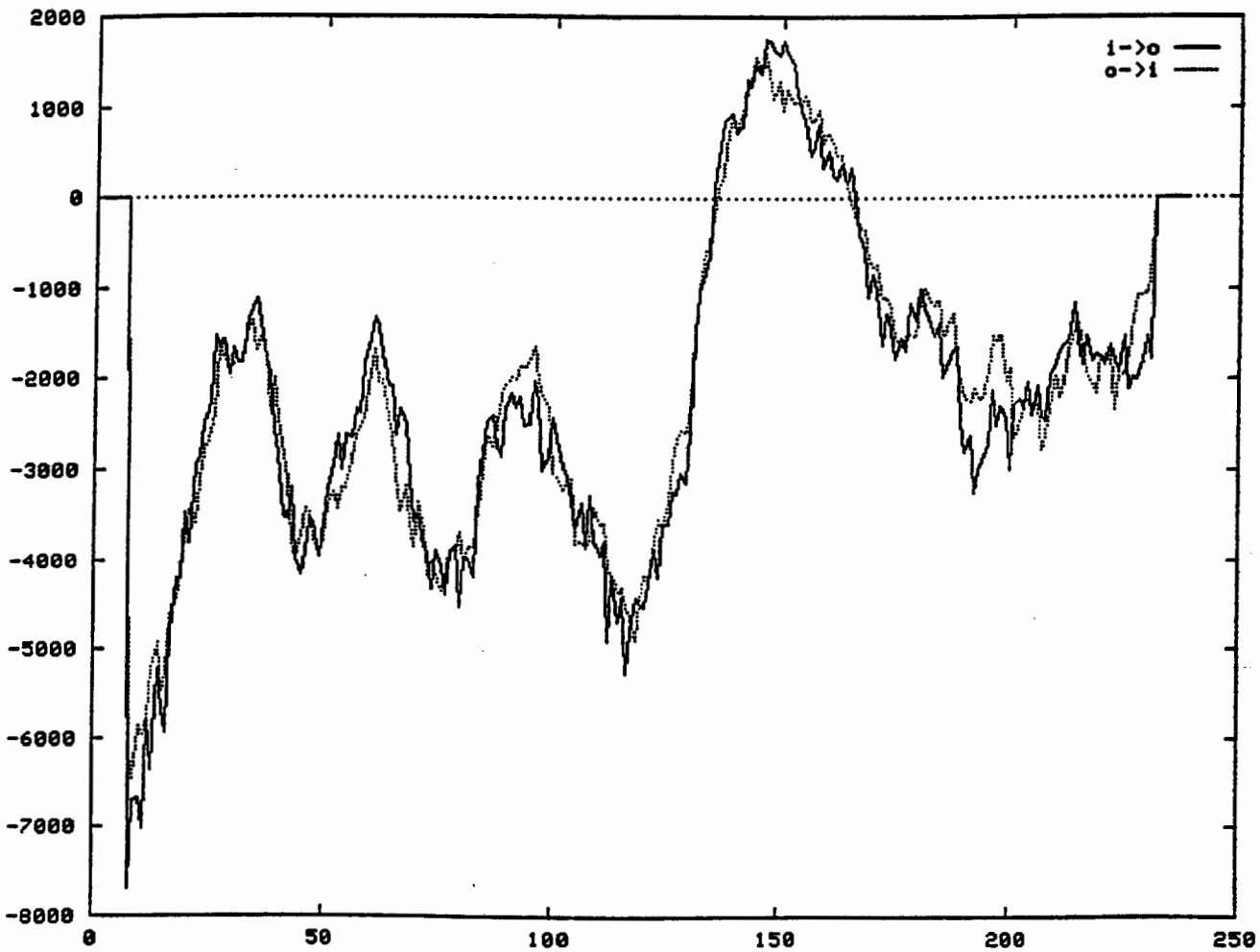

Figure 4. Hydropathic plot of the deduced polypeptide of *XV7*. The hydropathic plot was predicted to the rules of Kyte and Doolittle (Kyte and Doolittle, 1982).

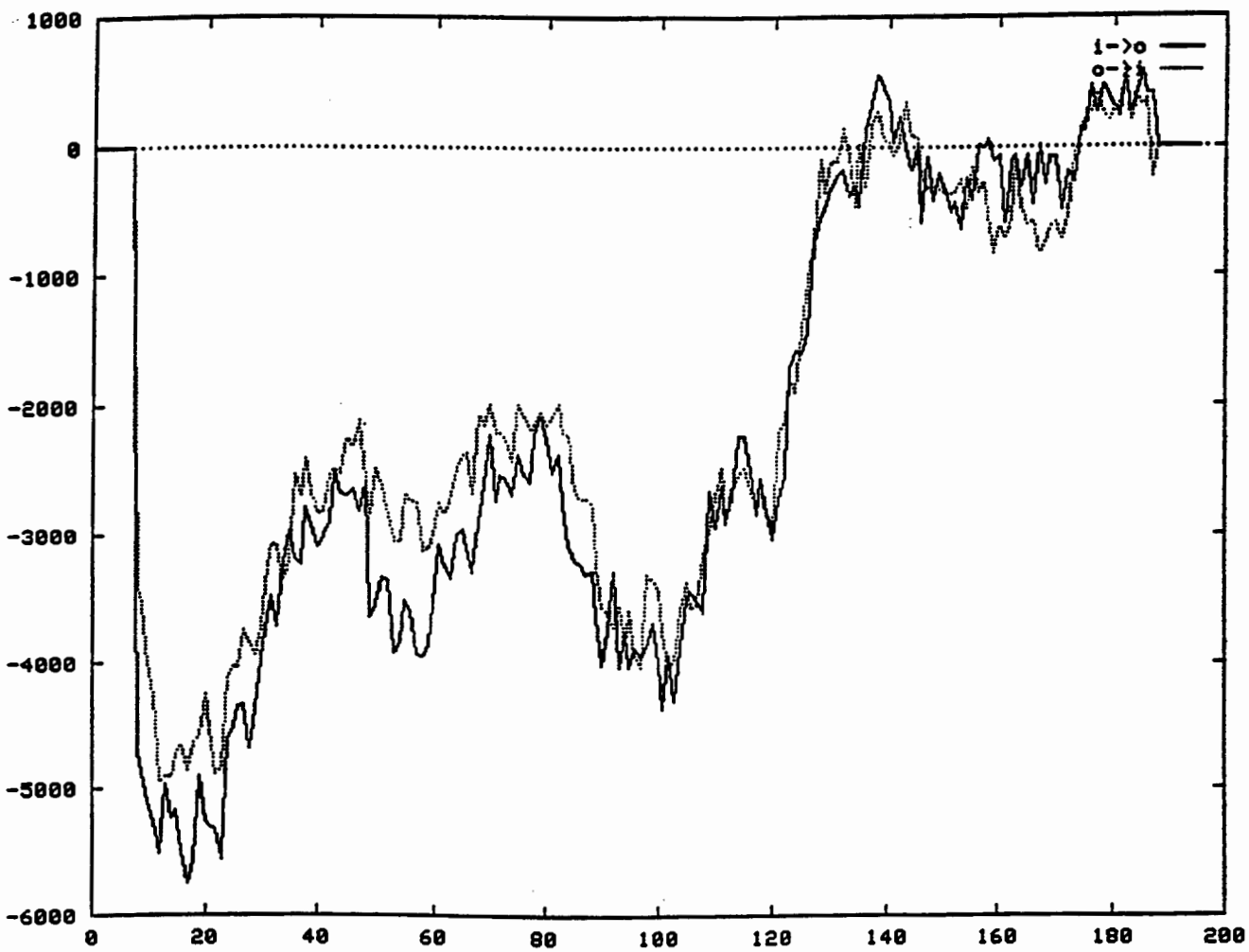

Figure 5. Hydropathic plot of the deduced polypeptide of *XV*9. The hydropathic plot was predicted to the rules outlined by Kyte and Doolittle (Kyte and Doolittle, 1982).

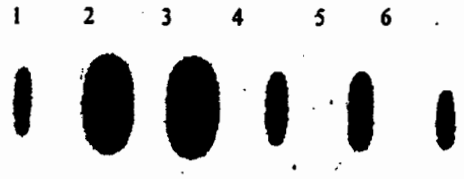

Figure 6. Northern blot analysis of *XV* 7 mRNA levels in *X. viscosa* Baker plants under different environmental stresses. 1, hydrated leaves, 2, leaves were dehydrated to 35% relative water content, 3, ABA treated(10ng), 4, cold treated at 4^0C(24 hours), 5, Heat shock at 37^0C(24 hours), 6, salt treated with 100mM NaCl.

Figure 7. Northern blot analysis of *XV* 9 mRNA levels in *X. viscosa* Baker plants under different environmental stresses.1, hydrated leaves 2, salt treated with 100mM NaCl, 3, cold treated at 4^0C(24 hours), 4, heat shock at 37^0C, 5, ABA treated(10ng)(24 hours), 6, leaves were dehydrated to 35% relative water content.

Figure 8. Slot blot analysis determined levels of selected mRNA sp. Poly (A^+) RNA are not seperated on agarose gel but instead blotted directly onto nylon membrane using commercial slot blot device.

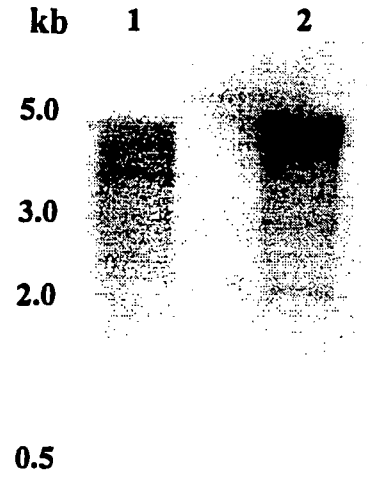

Figure 9. Southern blot analysis of genomic DNA from *X.viscosa* leaves. 14 µg of DNA was restricted with EcoRV (lane 1) and PvuII (lane 2), electrophoresed on a 0.8% agarose gel, transferred to a nylon membrane and probed with *XV7* insert.

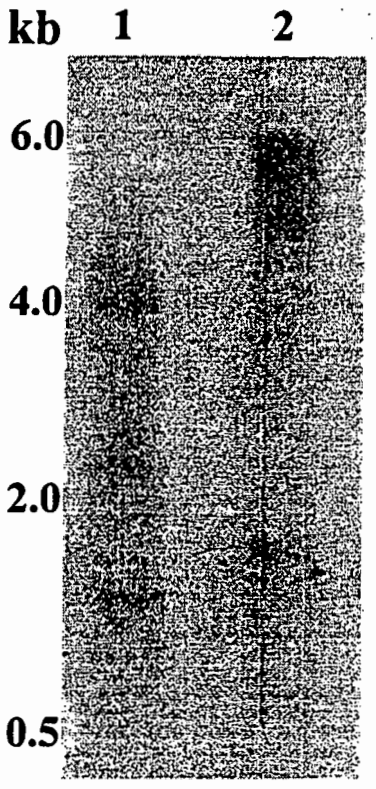

Figure 10. Southern blot analysis of genomic DNA from *X.viscosa* leaves. 14 µg of DNA was restricted with EcoRV (lane 1) and PvuII (lane 2), electrophoresed on a 0.8% agarose gel, transferred to a nylon membrane and probed with *XV9* insert.

DISCUSSION

XV7 is constitutively expressed as shown in lane 1 of Figure 6. *XV9* is also constitutively expressed as well but not induced by stress treatments except at dehydration (35% RWC). Therefore it confirms that *XV9* is dehydration-inducible. The basal levels of expression of *XV9* mRNA may indicate that minimal XV9 protein are present under nonstressed conditions. If this is the case, a low level of this protein may be active during moderate stress or be available for rapid activation during severe stress. One potential target for *XV7* predicted protein kinase is the LEA proteins, which accumulate in response to environmental stresses and are known to be phosphorylated. *XV7* shows 30% sequence identity to a syntaxin-like protein kinase regulator which was recently published (Thoreau *et al.*, unpublished) It has a high Leu composition (12.7%) like *XV7.*

The signal for cellular response involves regulation and activation by posttranslational modifications such as phosphorylation (Bray, 1997). Upregulation of *XV7* transcripts in response to environmental stresses provides additional regulatory control points. It also suggests that the kinase has a specific role in ABA or stress signal transduction.

ABA has been implicated to function in plant responses to many environmental stresses, including dehydration, high salinity, and low temperatures (Skriver and Mundy, 1990; Giraudat *et al.*, 1994) It is well established that an increase of ABA concentration is one important physiological response to the environmental stresses. Furthermore, many of the ABA-induced genes are also induced by environmental stresses (Giraudat *et al.*, 1994; Ingram and Bartels, 1996). The data in Figure 6 shows that the expression of *XV7* markedly induced under dehydration treatment (35% RWC*)*. This confirms that the *XV7* gene is dehydration-regulated. The results show

that *XV7* gene expression is induced by both dehydration and exogenous ABA. Since dehydration -induced expression of many of the ABA- and dehydration-induced genes is dependant on ABA (Giraudat *et al.*, 1994). Further research will determine whether the dehydration-induction of *XV7* is also ABA dependant. Considering *XV7* to be a protein kinase regulator, this observation suggests that *XV7* may function in the transmission of ABA and various environmental stress signals into intracellular reactions. Also, regulation of the gene by a variety of environmental stresses and ABA may indicate that the gene is involved in a general stress response, as suggested for a *Arabidopsis* protein kinase, receptor-like protein kinase (RPK1) (Hong *et al.*, 1997). Whereas only a part of the signal transduction pathway of these environmental stresses has been revealed in plants (Walker, 1994), the discovery of protein kinases induced by various environmental stresses, as described here, may reveal some facts about the mechanism. Understanding the mechanisms whereby plants perceive and transduce the stress signals is the key to understanding these responses, and, ultimately genetically improve stress tolerance (Ishitani *et al.*, 1997). Osmotic stress or water stress is experienced by plants who are subjected to dehydration, high salt, and low temperature. High salt causes osmotic stress by reducing water potential. Cold stress is also implicated in osmotic stress by reducing the water potential from roots to green tissue (Shinozaki-Yamaguchi-Shinozaki, 1996). ABA is also implicated in osmotic stress response that is the result of different environmental stressors. Thus it is possible that induction of the *XV7* gene is due too a common osmotic stress and that the gene is involved in an osmotic stress response rather than in a general stress response. We have demonstrated in this experiment that the gene, *XV7* is regulated by water availability, it is induced when dehydrated and, also expressed constitutively as shown

under hydrated conditions. Plant responses to dehydration, including gene expression appears to involve both ABA-dependent and ABA independent pathways (Shinozaki and Yamaguchi-Shinozaki, 1996; Shinozaki and Yamaguchi-Shinozaki, 1997; Giraudat et al., 1994) Further research is required to asses whether the *XV7* gene is ABA-dependant or ABA-independent using ABA-deficient and ABA-insensitive mutants. *XV7* is responsive to both ABA and dehydration but is expressed independently of ABA.

The sequence similarity between *XV7* and the mammalian syntaxin-like protein kinase receptor shows that *XV7* may function similar to that of animal protein kinases. The syntaxin-like protein kinase receptor was recently entered into the Genbank (Thoreau, unpublished), therefore not much is known about its characteristics. It does show similarity to a myotonin protein kinase, MtPK (Thoreau, unpublished). The plant receptor-like protein kinases (RLKs) are structurally related to the polypeptide growth factor receptors of animals (Walker, 1994). Although several ABA and stress-responsive gene have been characterised, their physiological role in the initial perception and transduction of stress signals is not well defined Recent reports suggest that characterising phosphorylation/ dephosphorylation responses and investigation of stress-responsive protein kinases may contribute towards identifying the pivotal regulatory steps in plant responses to environmental stress.

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