MOLECULAR BIOLOGY OF WATER STRESS RESPONSIVE GENES IN WHEAT

by

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ABSTRACT

Drought stress, the major limiting factor in worldwide wheat production, has long been an area of high interest to plant scientists. Drought stress in plants results in various physiological changes including reduced growth, photosynthetic activity and overall protein synthesis. Water deficit also results in accumulation of the plant hormone abscisic acid (ABA) which in turn induces expression of ABA responsive genes. The function of these gene products are not known although they are believed to play a role in osmoprotection and osmoregulation during water stress and desiccation. The objective of this study was to isolate cDNAs corresponding to the *rab* (responsive to ABA) family of water stress inducible genes from winter wheat (*Triticum aestivum* L. cv TAM W-101) and determine changes in steady state RNA *rab* levels during water stress.

A cDNA library was constructed in the lambda Uni-Zap XR vector system using poly $(A)^+$ mRNA isolated from roots of water stressed wheat seedlings. The cDNA library was screened and cDNAs representing two different size members of the *rab* gene family were isolated. Sequence analysis revealed sizes of 781bp and 1068bp with long open reading frames encoding polypeptides of 15,766Da (p*Tawsp*15 (*Triticum aestivum* water stress protein)) and 23,229Da (p*Tawsp*23), respectively. The predicted amino acid sequences of wheat TaWSP 15 and 23 have significant regions of identity with reported amino acid sequence of other reported RAB polypeptides from both monocots and dicots.

Northern blot analysis of total RNA hybridized with p*Tawsp*15 revealed increases in steady state RNA levels with increasing water stress. RNA transcripts corresponding to p*Tawsp* began to accumulate in both leaves and roots after a mild stress (93% relative water content (RWC)) and continued to accumulate even as stress became severe (48% RWC). Upon rewatering of severly stressed seedlings, *Tawsp* transcripts return to control levels within 24 hours.

These results support the idea that ABA responsive gene products may accumulate in order to protect the plant during severe desiccation and that once the stress is relieved and protection is no longer necessary the transcripts are rapidly degraded. This scenario would approximate the events which occur during seed development and maturation. Perhaps through a better understanding of drought responsive genes, we may be able to elucidate the molecular mechanisms involved in drought tolerance and use this understanding to develop more tolerant wheat cultivars using molecular techniques.

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CHAPTER I

INTRODUCTION

Drought stress is a major limiting factor of dryland winter wheat crop production in arid and semi-arid regions. Yield reduction in the U.S. Great Plains is associated with drought stress during reproductive growth, yet water deficiency during early development may also result in decreased tillering leading to fewer seed heads per plant, therefore reducing yield. Despite the large volume of recent work investigating molecular drought stress responses, there is little information available on basic molecular responses to drought stress in winter wheat.

The objective of this study was to characterize water stress responsive genes from water stressed winter wheat (*Triticum aestivum* L.) roots. Previous studies indicated that the *rab* (responsive to abscisic acid (ABA)) gene family is ubiquitous in plants and may play a role in desiccation tolerance in maturing embryos as well as mature plants (Dure et al. 1989, Skriver and Mundy 1990). "TAM W-101" was chosen because of its known drought tolerance characteristics (Ritchie et al. 1990). "TAM W-101" has also been shown to undergo numerous changes in translatable mRNA species as well as large increases in *rab* transcripts in response to drought stress (King et al. 1992b).

Literature Review

Chronic or sporadic periods of drought are prevalent in many of the world's major agricultural regions including many U.S. crop regions. Crop yield is dependent on the timing and amount of rainfall received. Comprehensive reviews include Boyer and McPherson (1975), Turner and Begg (1981), Turner and Kramer (1980), Christiansen and Lewis (1982), Teare and Peet (1983), Schulze (1986), Turner (1986), Boyer (1987), and Kramer (1988). In the U.S., the highest proportion of crop losses can be directly attributed to drought.

Plants are affected by drought stress in a variety of ways including decreased cellular growth, inhibition of cell wall synthesis, decreased nitrate reductase levels, increases in alpha-amylase and ribonuclease activity, increased abscisic acid (ABA) levels, inhibition of photosynthesis and decreases in cell wall extensibility (Matthews et al., 1984, Ritchie et al., 1990). These effects together result in reduced plant growth and the resulting decrease in yield (Rhodes and Matsuda 1976; Boyer 1987; Mason et al. 1988). Drought-induced growth reduction is particularly injurious to yield during early growth and grain filling stages.

Plants adjust to decreased water availability through physiological changes which help to conserve and maintain a supply of water. Plants are able to adjust to lowering water potential in the soil by increasing levels of solutes, thus lowering the water potential within the growing tissues, in a process known as osmotic adjustment (Westgate and Boyer 1985, Johnson et al. 1984). Osmotic adjustment allows the plant to continue extracting water from the soil until the water potential gradient between

the growing tissues and the water source eventually reaches a point which is not sufficient to drive the large uptake of water required to maintain growth (Boyer 1987).

The inadequate water supply and decreased water flow into the plant results in decreased cell turgor. Decrease in cell turgor pressure is believed to be the trigger for ABA biosynthesis and accumulation (Skriver and Mundy 1990). ABA accumulation in turn may play a role in further osmotic adjustment (Quarrie and Lister 1983, Guerrero and Mullet 1986, Pecik and Quarrie 1987, Benson et al. 1988, Creelman et al. 1990). Increased levels of ABA have been linked to numerous physiological changes including stomatal closure and the resulting decreased photosynthetic capacity, increased water conductance through roots, increase in sugar content in roots (Karmoker and Van Steveninck 1979), increase in turgor pressure in root tips (Jones et al. 1987), decreased elongation of stem segments (Wakabayashi et al. 1989), increased water use efficiency (Steuer et al. 1988), decreased polysome content (Benson et al. 1988), decreased shoot growth and the concomitant maintenance of root growth and inhibition of photosynthetic capacity (Seeman and Sharkey 1987).

Developments in molecular biology have made possible investigation of underlying mechanisms involved in the physiological changes previously discussed. Initial studies determined that drought stress results in disaggregation of polysomes (Hsiao 1970, Bewley and Larsen 1982, Guerrero and Mullet 1986), overall reduction of protein synthesis (Mason et al. 1988), accumulation of various organic acids, proline, sugars, and betaine (Hanson and Hitz 1982, Venekamp 1989). These effects are most evident in rapidly growing tissues where levels of polysomes decrease

drastically during drought stress in comparison with more mature tissues in which there is relatively little change in polysomes levels (Bewley and Larsen 1982). Decreased protein synthesis is probably not involved in the initial inhibition of growth since polysome disaggregation appears to takes place after the initial stages of growth inhibition (Mason et al. 1988). Protein synthesis probably does play a role in the later stages of growth reduction since protein synthesis is necessary for auxin stimulated growth (Bates and Cleland 1979, Theologis et al. 1985).

While there is an overall decrease in protein synthesis there have been reports of drought-induced increases in the levels of specific proteins (Dure et al. 1989, Skriver and Mundy 1990). Most of the drought-induced genes thus far characterized have been determined to be inducible by ABA (Skriver and Mundy 1990). Changes in gene expression in response to increased ABA levels were first detected in maturing embryos and have recently been detected in response to environmental stresses such as salt and drought (Dure et al. 1989, Skriver and Mundy 1990).

ABA responsive genes have been classified into a gene family known as LEA (late embryogenesis abundant) consisting of three homology groups (Dure et al. 1989). Group one is represented by cotton *lea* D19, group two is represented by *rab* 21 (responsive to ABA) and cotton *lea* D11 and group three includes various *lea* genes from barley, rape and carrot (Dure et al. 1989). Group two LEA proteins accumulate in response to drought stress in several species including rice, barley, maize, tomato and resurrection plant (reviewed by King et al. 1992a).

The role which ABA responsive genes play in drought stress is not clearly understood at this time. Several ABA responsive genes contain motifs which suggest a role in protein or nucleic acid binding (Skriver and Mundy 1990). The genes may play a role in protecting the plant from drought-induced damage by stabilizing cellular components such as nucleic acids or proteins (Dure et al. 1989, Skriver and Mundy 1990). Stabilized mRNA and proteins could facilitate production of essential cellular components allowing quick recovery and renewed growth following relief of stress. Rapid recovery from drought stress would be particularly important in crop plants such as wheat which may be subjected to stress during various stages of their development.

CHAPTER II

MATERIALS AND METHODS

Plant Materials

Winter wheat (*Triticum aestivum* cv. TAM W-101) seeds were germinated on wetted filter paper in petri dishes for 24 h at 4 °C, incubated at 22 °C for 4 h and sown into pots containing 1.4 kg of coarse sand. Seedlings were grown in a controlled environment growth chamber (Conviron Model E-15) that provided 600 micromole photon m^{-2} s^{-1} during an 18/22 °C night/day regime with a 16 h photoperiod. Plants were fertilized and fully irrigated until the water stress cycle was imposed.

One week after emergence, seedlings were either stressed by withholding water and allowing the sand to dry slowly over several days (stressed plants) or irrigated daily to runoff (control plants). Roots from control and stressed plants were collected when the stressed plants began to visibly wilt (approximately 5 days after last irrigation). Root tissues were immediately frozen in liquid nitrogen for poly $(A)^+$ RNA isolation.

cDNA Library Construction, Screening, and Sequencing

Poly $(A)^+$ RNA was isolated from stressed root tissue using oligo dT chromotography (Sambrook et al. 1989). First strand cDNA was synthesized using isolated mRNA as a template with oligo dT containing an attached Xho I restriction

site as a primer. The reaction was carried out using Moloney-murine leukemia virus reverse transcriptase (M-MuLVRT) in the presence of buffer and nucleotides (dGTP, dTTP, 5-methyl dCTP and ^{32}P dATP) for one hour at 37°C.

Second strand cDNA synthesis was performed by nicking the RNA paired to the first strand cDNA using RNase H. The remaining RNA served as a primer for DNA polymerase I mediated nick translation resulting in second strand cDNA synthesis. Second strand cDNA synthesis reactions were carried out under buffered conditions in the presence of deoxynucleotides (dATP, dCTP, dGTP, dTTP) for 2.5 hours at 16°C.

The ends of the double stranded cDNA were made even by end filling with T4 DNA polymerase. The blunting reaction was carried out in the presence of buffer and deoxynucleotides for 30 minutes at 37°C. EcoR I adaptors were then ligated onto the ends of the blunted cDNA using T4 DNA ligase in the presence of ATP for 16 hours at 4°C. Following ligation, EcoR I ends were kinased (to allow later ligation into the vector) using T4 polynucleotide kinase in the presence of ATP for 30 minutes at 37°C.

The double stranded cDNA was finally prepared for insertion into the vector by digesting the cDNA with Xho I restriction enzyme. The DNA was digested for one hour at 37°C. Following digestion the DNA was run through a Sephacryl spin column to remove the resulting small fragments of DNA from the full length cDNAs containing an EcoR I site on one end and an Xho I site on the other end.

The cDNA was ligated into the vector using T4 DNA ligase for 16 hours at 12°C and then for two hours at room temperature. The ligation mixture contained

cDNA, ligation buffer, rATP, Uni-ZAP XR predigested (EcoR I - Xho I) vector (Stratagene), and T4 DNA ligase. Four micrograms of the resulting ligation products were then packaged into Gigapack II Gold packaging extracts (Stratagene) according to manufacturer's specifications.

The packaged library was titered using PLK-F' cells. The packaged ligation products were pre-incubated with the cells for 15 minutes prior to plating in top agar on agar plates. Following overnight incubation at 37° C, plaques were counted and the number of recombinants plaque forming units (PFU) in the first packaging was estimated to be 3.0×10^4. The remainder of the DNA from the ligations was packaged resulting in 7.5×10^4 additional PFU for a total of 1.05×10^5 PFU. The library was amplified using PLK-F' cells by mixing aliquots containing 5.0×10^4 PFUs with 600 microliters of $O.D._{600}$ cells and growing the plaques in top agar overnight at 37°C. The phage stock was collected and determined to contain 3.1×10^{10} PFU/ml.

The cDNA library was screened using duplicate filter lifts with ^{32}P labeled rice *rab* 16 DNA as a probe (Mundy and Chua 1988). Numerous positives were identified, and these were subclassed into two different size groups. Two of the clones (representing different size groups) were selected, and a series of Exo III/ S1 nuclease generated deletions were constructed using the Erase-a-base system (Promega) according to manufacturers specifications. Individual deletions were sequenced using Sequenase enzyme (U.S. Biochemicals, Cleveland, OH) resulting in overlapping

sequences. The overlapping sequences were spliced to reveal the complete nucleotide sequence of each clone.

Northern Blot Analysis of Drought Stressed Wheat Leaves and Roots

Northern blot analysis was performed on total RNA isolated from control and progressively more stressed wheat leaves and roots. For these studies, TAM W-101 seedlings (3 days old) grown in coarse sand (as previously described) were stressed by withholding irrigation. Samples were collected from control (97% relative water content (RWC)), 3-day (93% RWC), 4-day (70% RWC) and 5-day (48% RWC) stressed leaves and roots. Samples were also collected 24h after rewatering the 5-day stressed seedlings (95% RWC).

Total RNA was extracted from control and water stressed leaves and roots using a phenol extraction and LiCl precipitation method. Tissue was ground to a fine powder and nucleic acids were then extracted with equal volumes of grinding buffer (10 g/L triisopropylnaphthalenesulfonic acid sodium salt, 60 g/L p-aminosalicylic acid, 100mM Tris-HCL, pH 7.6, 50 mM ethyleneglycol-bis-N,N,N',N'-tetraacetic acid, 100 mM NaCL, 10 g/L sodium dodecyl sulfate (SDS), 50 mM 2-mercaptoethanol) and phenol:chloroform:isoamyl alcohol (12:12:1). RNA was precipitated using 4 M LiCl (final concentration 2 M) at 4 °C, resuspended in water, and precipitated at -20 °C in 0.3 M sodium acetate and 2.5 volumes ethanol.

Northern blot analysis was performed by size separating twenty micrograms of total RNA in a 1.2% agarose/formaldehyde gel and transferring the RNA to a nylon

membrane (Zetaprobe Biorad, Richmond, CA) in 20X SSPE. The nylon membrane was prehybridized overnight at 42 °C (5X SSPE, 50ml/100ml formamide, 5X Denhardt's solution, 0.5g/100ml SDS, 10mg/100ml salmon testes DNA) and hybridized overnight at 42 °C in prehybridization solution containing ^{32}P random primed (USB, Cleveland, OH) DNA (smaller size full length cDNA) as a probe. Following hybridization blots were washed at 60 °C for 45 min each in 2X SSPE, 0.1g/100ml SDS; 1X SSPE, 0.1g/100ml SDS; 0.1X SSPE, 0.1g/100ml SDS and visualized by exposure to Kodak X-Omat film at -70 oC.

CHAPTER III

RESULTS AND DISCUSSION

The two selected cDNAs contained sequences of 781 and 1068 base pairs, respectively (Figs. 1 and 2). The 781bp cDNA encodes a polypeptide of 149 amino acids with a molecular weight of 15,766Da and the 1068bp cDNA encodes a polypeptide of 231 amino acids with a molecular weight of 23,229Da. The cDNAs will be referred to as p*Tawsp* (*Triticum aestivum* water stress protein) 15 and 23, respectively.

In p*Tawsp* 15 and 23 the first ATG occurs at positions 25 and 75 respectively (Figs. 1 and 2) which are in optimum context as described before for plant translation initiation codons (Joshi 1987). Both cDNAs contain long open reading frames (450 bases for p*Tawsp*15, 650 bases for p*Tawsp*23) followed by long 3' non coding regions (approx. 300 bases for both cDNAs) (Figs. 1 and 2). These findings are consistent with *dhn* (dehydrin) cDNAs which had the first ATG as the beginning codon (Close et al. 1989).

The predicted amino acid sequences of wheat TaWSP 15 and 23 have significant regions of identity with reported amino acid sequence of other rab, dhn, and lea polypeptides from both monocots and dicots. The highly conserved domains are present between amino acids 52 to 88 and 130 to 150 of the wheat TaWSP 15 amino acid sequence in comparison with RAB, DHN, and LEA proteins from rice (Mundy and Chua 1988), maize (Villardell et al. 1990), barley (Close et al. 1989),

tomato (Goday et al. 1990), resurrection plant (Piatowski et al. 1990), and cotton (Dure et al. 1989)(Fig. 3). The WGCG program Gap was used for these alignments with the default values of gap weight of 3.0 and length weight of 0.1. The conserved region between amino acids 52 to 88, present in all species, has previously been described (Dure et al. 1989). The conserved region between amino acids 130 to 150 is found in all of the proteins with the exception of the cotton LEA D11 protein (Fig. 3). While *Ta*WSP 15 showed maximum amino acid similarity with rice RAB 16 (79%), *Ta*WSP 23 showed very high similarity with *Hv*DHN 18 (95%).

Northern blot analysis revealed that there is an increase in steady state *Tawsp* levels following a mild water stress (93% RWC) in both leaves and roots and that levels continue to increase as desiccation increases (Figure 4). Northern analysis also shows that *Tawsp* transcripts have returned to control levels within 24h after rewatering (Figure 4). These results indicate that there is an increase in steady state levels of *Tawsp* RNA in both leaves and roots during a water stress regime and that both tissues rapidly lose the transcripts once water becomes available to the plants.

Levels of p*Tawsp* transcripts continue to accumulate even as the level of water stress becomes harsh (48% RWC). These results support the idea that ABA responsive gene products may accumulate in preparation for a severe desiccation and that once the stress is relieved and protection is no longer necessary the transcripts are rapidly degraded. This scenario would approximate the events which occur during seed development and maturation. Perhaps through a better understanding of drought

responsive gene regulation, we may be able to elucidate the molecular mechanisms involved in drought tolerance and develop tolerant wheat cultivars using molecular techniques.

```
1    CGAGGTCCAGTTTAGGAGGCAAAGATGGAGTTCCAAGGGCAGCACGACAACCCCGCCAAC   60
                              M  E  F  Q  G  Q  H  D  N  P  A  N

61   CGCGTCGACGAGTACGGCAACCCGTTTCCGTTAGCCGGCGCGTGGGGGGAGCGCACCCGC  120
       R  V  D  E  Y  G  N  P  F  P  L  A  G  A  W  G  E  R  T  R

121  TCCCGGCACCGGCGGGCAGTTCCAGGCCCACAGGGGAGAGCACAAGACCGGTGGATCCTG  180
       S  R  H  R  R  A  V  P  G  P  Q  G  R  A  Q  D  R  W  I  L

181  CATCGCTCCGGCAGCTCCAGCTCCAGCTCGTCTTCCGAGGACGACGGCATGGGCGGGAGG  240
       H  R  S  G  S  S  S  S  S  S  S  S  E  D  D  G  M  G  G  R

241  AGAAAGAAGGGCATGAAGGAGAAGATCAAGGAGAAGCTCCCCGGTGGCCACAAGGACAAC  300
       R  K  K  G  M  K  E  K  I  K  E  K  L  P  G  G  H  K  D  N

301  CAGCAGCATATGGCGACGGGGACAGGGACTGGAGGAGCCTACGGGCCGGGAACTGGGACT  360
       Q  Q  H  M  A  T  G  T  G  T  G  G  A  Y  G  P  G  T  G  T

361  GGTGGAGCCTACGGGCAGCAAGGCCACACAGGAATGGCCGGCGCCGGCACTGGCACGGGC  420
       G  G  A  Y  G  Q  Q  G  H  T  G  M  A  G  A  G  T  G  T  G

421  GAGAAGAAGGGGATCATGGACAAGATCAAGGAGAAGCTGCCGGGACAGCACTGAGCCACC  480
       E  K  K  G  I  M  D  K  I  K  E  K  L  P  G  Q  H  *

481  GGTCCGGCTGGCTGCTTCCTTGTAGCTACGGGTCAAAGCCTTCCAGTTCCACGTGATCTT  540

541  TGTTCAATAATAATAAGATGAAGCTGAACGAAAAGTTGTCTCTGATCGCATCCGCGTCAG  600

601  GGACACTTTTCTGTATACAGCGTGCGTTGTGTCTGTTACGTTTGTCTGTTGTGTCTTCAT  660

661  GTTGAAAAAAACTTAGTATACAACTGAACTTTTTTTTCTATCAAGGAAATCGGGCCCTTG  720

721  TTGATGTTAAAAAAAAAGGAGTCCATGCCATTTTTGTGCCTTAAAAAAAAAAAAAAAAAA  780

781  A  781
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Fig. 1. Nucleotide and deduced amino acid sequence of wheat p*Tawsp* 15

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1    AAGAAGTACAAACAGCAGCATACTAGATTTTGATTCCCCCTGTGACAAGCTCAGCTCAGC   60

61   GCACGCAAGATGGAGTACCAGGGACAGCAGCAGCGCGGCCGCGTCGACGAGTACGGCAAC  120
                M  E  Y  Q  G  Q  Q  Q  R  G  R  V  D  E  Y  G  N

121  CCGGTGGCCGGACATGGCGTCGGCACCGGCATGGAGACGCACGGCGGCGTCGGCACCGGC  180
       P  V  A  G  H  G  V  G  T  G  M  E  T  H  G  G  V  G  T  G

181  GCGGCCGCCGGTGGGCATTTCCAGCCCATGAGGGACGAGCACCAGACCGGCCGTGGGATC  240
       A  A  A  G  G  H  F  Q  P  M  R  D  E  H  Q  T  G  R  G  I

241  CTGCACCGCTCCGGCAGCTCCAGCTCCAGCTCGTCTGAGGATGATGGCATGGGCGGGAGG  300
       L  H  R  S  G  S  S  S  S  S  S  E  D  D  G  M  G  G  R

301  AGGAAGAAGGGCATCAAGGAGAAGATCAAGGAGAAGCTCCCCGGTGGCCACGGTGACCAG  360
       R  K  K  G  I  K  E  K  I  K  E  K  L  P  G  G  H  G  D  Q

361  CAGCACACCGGTGGCACCTACAGACAGCAGGGTACTGGCATGGTCGGCACCGGCGGCACC  420
       Q  H  T  G  G  T  Y  R  Q  Q  G  T  G  M  V  G  T  G  G  T

421  TACGGGCAGAAGGGTCACACTGGGATGACCGGCACCGGCGGCACCTACGGGCAGCAGGGT  480
       Y  G  Q  K  G  H  T  G  M  T  G  T  G  G  T  Y  G  Q  Q  G

481  CACACTGGGATGACCGGCACCGGCGGCACCTACGGGCAGCAGGGCCACACTGGGATGACC  540
       H  T  G  M  T  G  T  G  G  T  Y  G  Q  Q  G  H  T  G  M  T

541  GGCACCGGCGGCACCTACGGACAGCAAGGCCACACCGGGATGGCCGGCACCGGGGCGCAT  600
       G  T  G  G  T  Y  G  Q  Q  G  H  T  G  M  A  G  T  G  A  H

601  GGCACCACGGCCACTGGTGGCACCTACGGGCAGCCGGGCCACACCGGGATGACAGGCACG  660
       G  T  T  A  T  G  G  T  Y  G  Q  P  G  H  T  G  M  T  G  T

661  GGGGCGCACGGCACCGGAGGCACCTACGGGCAGCACGGCACTGACACCGGCGAGAAGAAG  720
       G  A  H  G  T  G  G  T  Y  G  Q  H  G  T  D  T  G  E  K  K

721  GGCATCATGGACAAGATCAAGGAGAAGCTCCCAGGCCAGCACTGAGCGCTGAGGAGAGCC  780
       G  I  M  D  K  I  K  E  K  L  P  G  Q  H  *

781  CGCGGCCGCCACTTCTGAGAGTGGAGGTGCCGGTCGACCACCGTTGCAGAATCAATAATA  840

841  AGATCGCGATACGATACAATAAAATTCCACCATACAACGTGAGCCTAGTTCACCTAGCTC  900

901  ACTTGCGTGTTGGAGGAGCCACTGTATCTAGGCTCAAGTTTACGTGAACAAACAGTGTTT  960

961  TGAGTTTTTCGTCTGTTTATTACACTGTATAATCTTGTAAGTTTCCTGTGGTTAAACCCT  1020

1021 GTAGTACGCTTTACGGTATGGTCCTCGCGTAAAAAAAAAAAAAAAAA  1068
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Fig. 2. Nucleotide and deduced amino acid sequence of wheat p*Tawsp* 23.

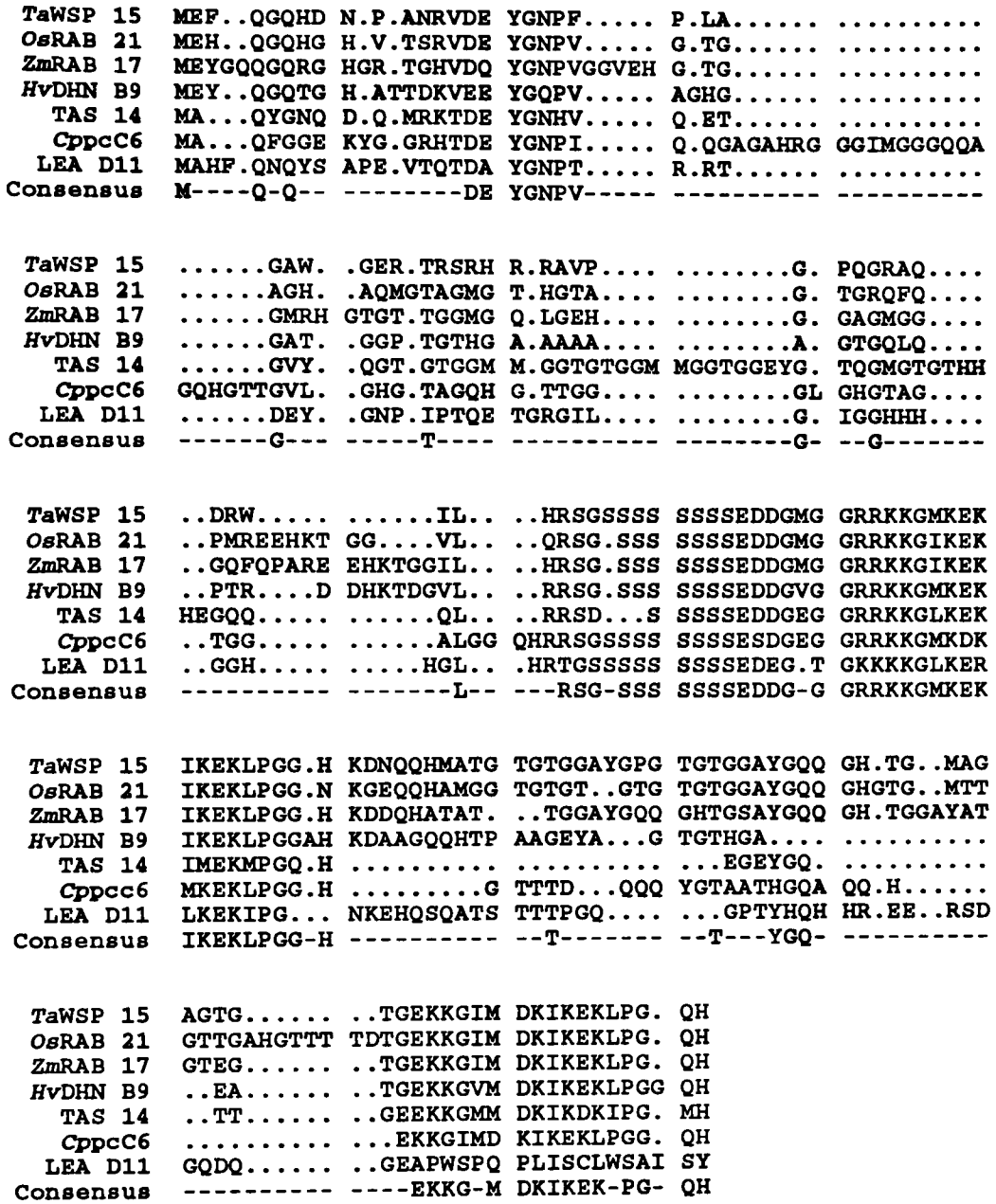

Fig. 3. Amino acid sequence comparison of reported RAB and DHN proteins from wheat (*Ta*WSP 15), rice (*Os*RAB 21), maize (*Zm*RAB 17), barley (*Hv*DHN B9), tomato (TAS 14), resurrection plant (*Cp*pcC6), and cotton (LEA D11).

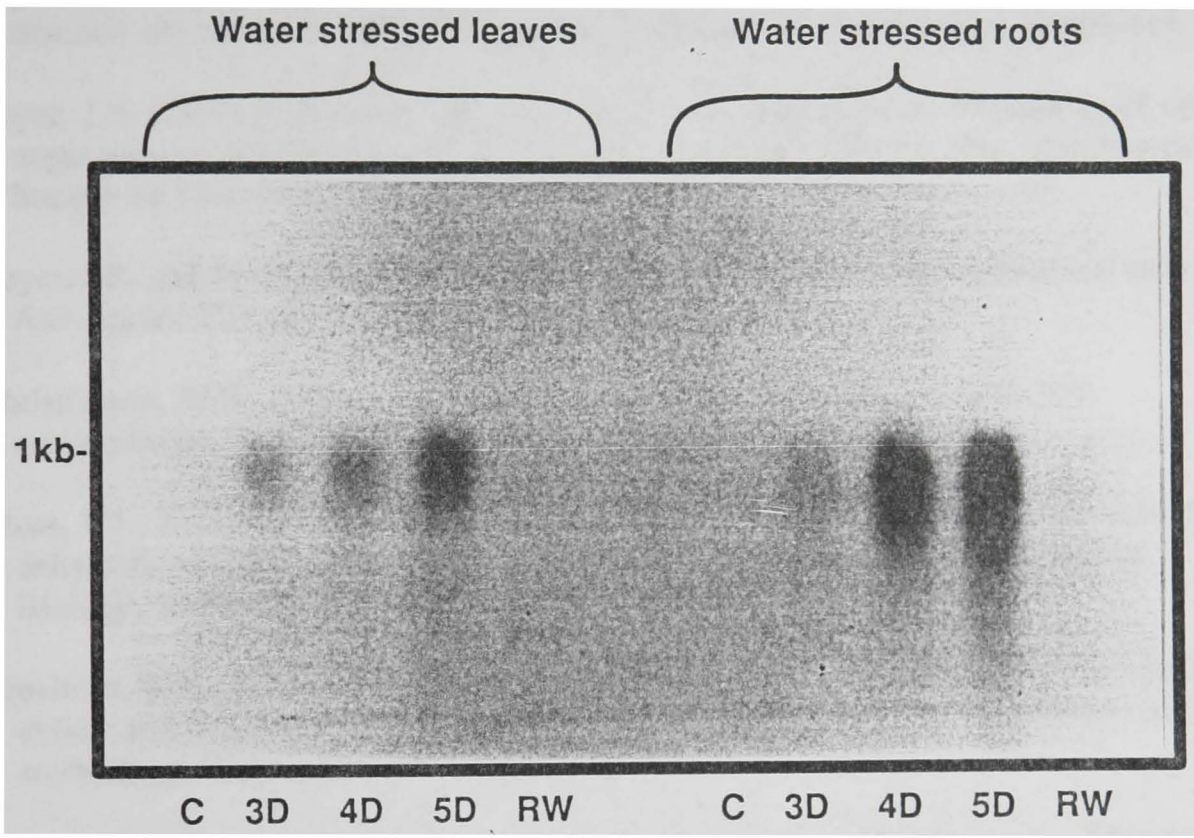

Fig. 4. Northern blot analysis of total RNA (control (C), 3-day stressed (3D), 4-day stressed (4D), 5-day stressed (5D) and 24 hours after rewatering (RW)) from water stressed wheat leaves and roots using p*Tawsp* 15 cDNA as a probe.

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