

## Update section

### Sequence

# Nucleotide sequence of an osmotin cDNA from the *Nicotiana tabacum* cv. White Burley generated by the polymerase chain reaction

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Salt stress-induced changes in gene expression have been observed in a number of plant species, in both whole plants and cultured cells. In tobacco and tomato salt adaptation is associated with the accumulation of a 26 kDa protein, which has been termed osmotin in tobacco [1, 2, 3]. Highly homologous cDNA clones encoding osmotin or an osmotin-like protein have been isolated and sequenced from libraries constructed from the roots of salt-adapted tomato (*Lycopersicon esculentum* VFNT) plants, and from salt-adapted suspension cultures of tobacco (*Nicotiana tabacum* Wisconsin-38) [3, 4].

The present communication describes the nucleotide sequence of a PCR-amplified osmotin cDNA from salt-adapted suspension cultures of the tobacco cultivar White Burley, using primers derived from published sequence from the Wisconsin-38 cultivar [4]. The cDNA was cloned in both orientations into the phagemid vector pTZ (Pharmacia) and the sequence determined by the dideoxynucleotide chain termination method [5], each position being determined on average three times. The coding region is 738 bp, 6 bp longer

than that of Wisconsin-38, and the predicted molecular mass of the encoded preprotein is 26697 Da. Assuming a signal processing site identical to the Wisconsin-38 osmotin [6], the molecular mass of the mature protein would be 24283 Da.

The main differences between the White Burley sequence, shown in Fig. 1, and the Wisconsin-38 sequence [4] can be summarised as follows: (a) nucleotide substitutions occur at 11 positions, 4 of them resulting in no change in amino acid. The remaining substitutions result in 5 changes in amino acid sequence, 3 of them being conservative changes; (b) there are 7 nucleotide insertions and 1 deletion, resulting in 2 additional amino acids; (c) there is a region of considerable variation from nucleotide positions 446–465 where 2 nucleotide substitutions followed by an insertion and a compensatory deletion result in changes in 7 consecutive amino acids.

Although the physiological function of osmotin is unknown, we are currently investigating its role in salt adaptation by introducing the gene into a different cellular environment.

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M G N L R S S F V F F L L A L V T Y T Y
ATGGGCAACTTGAGATCTTTGTTTCTTCCTCCTTGCCTTGGTGACTTATACTTAT
10      20      30      40      50      60

A A T I E V R N N C P Y T V W A A S T P
GCTGCCATATCGAGGTCCGAAACAACGTCCGTACACCGTTGGCGGGCTGCACACCC
↑      70      80      90      100     110     120

I G G G R R L D R G Q T W V I N A P R G
ATAGGCGGTGGCCGGCTCTCGATCGAGGCCAAACTTGGGTGATCAATGCGCCACGAGGT
130     140     150     160     170     180

T N M A R V W G R T N C N F N A A G R G
ACTAATATGGCAGTGTATGGGGCCCTACTAATTTGTAACCTTCAATGCTGCTAGGGGT
190     200     210     220     230     240

T C Q T G D C G G V L Q C T G W G K P P
ACGTGCCAAACCGGTGATCTGGTGGAGTCCACAGTGCACCGGTGGTAAACACCA
250     260     270     280     290     300

N T L A E Y A L D Q F S G L D F W D I S
AACACCTTGGCTGAATACGCTTTGGACCAATTCAGTGGTTAGATTTTGGGACATTTCT
310     320     330     340     350     360

L V D G F N I P M T F A P T N P S G G K
TTAGTTGATGGATTCAACATTCCGATGACTTTCCCGGACTAACCCCTAGTGGAGGGAAA
370     380     390     400     410     420

C H A I H C T A N I R R M S R E L R V P
TGCCATGCAATCCATTTGTACGGCTAATATACGGCGAATGTCCCGCAACTTAGGGTTCCC
430     440     450     460     470     480

G G C N N P C T T F G G Q Q Y C C T Q G
GGAGGATGTAATAACCCCTGTACTACATTCGGAGGACAACAATATTTGACACAAGGA
490     500     510     520     530     540

P C G P T F F S K F F K Q R C P D A Y S
CCTTGGTCCCTACATTTTCTCAAATTTTCAAACAAGATGCCCTGATGCCTATAGC
550     560     570     580     590     600

Y P Q D D P T S T F T C P G G S T N Y R
TACCCACAAGATGATCCTACTAGCACTTTTACTTGCCCTGGTGGTAGTACAAATTATAGG
610     620     630     640     650     660

V I F C P N G Q A H P N F P L E M P G S
GTTATCTTTTTCCTAATGGTCAAGCTCACCAAAATTTCCCTTGGAAATGCTGGAAGT
670     680     690     700     710     720

D E V A K *
GATGAAGTGGCTAAGTAG.
730

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Fig. 1. Nucleotide sequence and derived amino acid sequence of an osmotin cDNA clone from tobacco (*Nicotiana tabacum* cv. White Burley). The arrow indicates the likely cleavage site for the removal of the signal sequence. Variations with the Wisconsin-38 sequence are indicated: nucleotide substitutions are underlined once, insertions are underlined twice and the deletion point is indicated by ^. Amino acid substitutions are boxed and insertions are circled.

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