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ORIGINAL PAPER

Identification, localization, and characterization of putative *USP* genes in barley

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Abstract The universal stress proteins (*USPs*) play an important role in enhancing survival rate during prolonged exposure to heat shock, nutrient starvation, or stressors from agents that arrest cell growth or damage DNA structures. Searching the HarvEST database of barley resulted in 25 putative *USP* cDNA sequences. Of these, 16 could translate into intact proteins (putative *USPs*). The alignments of multiple amino acid sequences between the putative barley *USPs* with those of *Arabidopsis* and *Methanococcus jannaschii* resulted in a set of common residues involved in ATP-binding. The 16 putative *USP*s in barley and the 21 in *Arabidopsis* were clustered into seven groups, which were distinct from those of *E. coli*. The genes in these different groups have different intron/exon structures. Nine putative *USP* genes of barley were cloned successfully based on their sequence characteristics, and they contain two or three introns each. Two of these introns were present in all the genes, one located between β2 and α2, and the other between β4 and α4. Five sets of primers were successfully developed for these putative *USP* genes. Two of them were mapped on chromosome 1H and the other three were located on three different chromosomes, 2H, 3H and 6H, respectively. Expression analyses were carried out for nine of these putative *USP* genes. The expression for two of them was undetectable within 27 h following exposure to salt stress. Six of the other seven were expressed in both root and leaf, and the remaining one was expressed in root only. The majority of these genes was expressed more in the salt-sensitive variety, Morex, than in the more tolerant variety, Steptoe.

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Introduction

The universal stress protein (*USP*) was originally named, C13.5, based on its migration in a two-dimensional IEF-PAGE gel (Van Bogelen et al. 1990). The *USP* superfamily represents a growing set of small cytoplasmic proteins whose expressions are affected by a wide range of internal or external stresses (Kvint et al. 2003; Nachin et al. 2005). *USP* could enhance the rate of cell survival during prolonged exposure to stress agents, and they might provide a general "stress endurance" strategy (Aravind et al. 2002; Kvint et al. 2003). The ancient and conserved *USP* has been found not only in the genomes of bacteria but also in archaea, fungi, protozoa, and plants (Aravind et al. 2002; Kvint et al. 2003).

The structure of a *USP* from *Methanococcus jannaschii*, *1MJH*, indicates that it binds ATP, although it lacks ATP-binding activity (Zarembinski et al. 1998). These results suggested the biochemical function of *1MJH* to be either an

ATPase or an ATP-binding molecular switch. The structure of another *USP* from *Haemophilus influenzae* (*1JMV*), which shared 68% sequence similarity with the *USP* from *Escherichia coli*, was recently determined (Sousa and McKey 2001). In contrast to *1MJH*, *1JMV* cannot bind ATP, suggesting that two distinctive forms of *USP*s may exist (Kerk et al. 2003).

About one-third of the irrigated land in China has salinity problems with Na^+ as the predominant salt. The total area affected is as high as 1.7×10^8 hectares (Shi 2004). Barley is one of the most important crops and it is highly tolerant to adverse environments such as cold, drought, and salt (McLeod 1982). To further enhance its tolerance or resistance to biotic/abiotic stresses, we embarked on a study to investigate the putative *USP* genes. The amino acid sequences and sequence structures of these genes were analyzed, their linkage map locations determined, and their expressions between two varieties with different levels of resistance to salt stress compared.

Materials and methods

Plant materials and DNA isolation

A doubled haploid (DH) population of barley, produced by a modified *bulbosum* method from a cross between Steptoe and Morex (Chen and Hayes 1989), was used in this study. One hundred and fifty lines were grown in a greenhouse and genomic DNA was extracted from single plants following the CTAB procedure (Sambrook et al. 1989).

Identification of putative *USP* genes from HarvEST database of barley

To identify putative *USP* genes in barley, the HarvEST database of barley was searched. Based on the conserved *USP* domain of *1MJH* from *Methanococcus jannaschii*, sequences identified were further analyzed against the NCBI database and those of non-*USP* genes were removed. The barley genes identified were then compared with those known *USP* genes from *Arabidopsis* and *Escherichia coli*.

Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignments between the putative *USP* genes in barley and those known *USP*s from *Methanococcus jannaschii*, *Haemophilus influenzae*, *Arabidopsi* and *E. coli* were compared by using DNAman 5.2.2 (http://www.lynnon.com) and the results obtained were used for phylogenetic analysis. Phylogenetic trees were constructed using the neighbor-joining (NJ) method based on MEGA version 3.1 (Kumar et al. 2004) with bootstrap support at critical nodes (1,000 replicates). Secondary structures of predicted proteins were analyzed by using Lasergene Version 8.0 (Allex 1999).

Amplification and sequencing of putative *USP* genes in barley

To obtain the genomic sequences of putative *USP* genes in barley, PCR primers were designed using barley EST sequences and tested against two cultivars, Steptoe and Morex. The PCR reaction volume was 25 μl, containing approximately 0.2 μg template DNA, 2.5 U TaKaRa Exo Taq™ polymerase with high fidelity (TaKaRa Bio, Inc., Kyoto, Japan), 0.3 μM each primer (Table 1), 200 μM each of dNTP (TaKaRa Bio, Inc., Japan), 1.5 mM $MgCl_2$, and 1× PCR buffer. PCR amplifications were carried out in a PTC-240 thermocycler (Genetic Technologies, MJ Research, USA). The PCR reaction conditions were 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 50–60°C for 45 s and 72°C for 1.5 min, with a final extension at 72°C for 7 min. Amplified products were directly visualized by gel electrophoresis in 1.5% agarose gels. The PCR products were purified, cloned, and sequenced in both directions by a commercial company (BGI Life Tech Co., Ltd, China).

Linkage mapping of the putative barley *USP* genes

The DNA sequences between the two barley cultivars, Steptoe and Morex, were compared. Based on their differences, PCR primers (Table 2) were designed to allow for the detection of either base substitutions or indels between these two cultivars (GU258509-GU258518). The 150 DH lines were then genotyped with the markers developed, and their map locations were determined by aligning their genotypic profiles with those of existing genotypic data of the mapping population (http://wheat.pw.usda.gov/ggpages/map_shortlist.html). Linkage maps were constructed using Map Manager Version 3.0 (Lincoln et al. 1993) and drawn with the winQTLCart 2.5 (Wang et al. 2007).

Expression analyses of barley *USP* genes by real-time reverse transcription PCR

Barley seeds were washed three times with deionized water and germinated on moistened filter paper in darkness. Germinated seeds were transferred into pots filled with vermiculite in aerated half-strength Hoagland's solution. The plants were grown in a greenhouse. The pH was maintained within the range 5.0–6.5 and adjustments were made when necessary using concentrated sulfuric acid. On day 21 after germination (three-leaf stage), a solution of 300 mM NaCl was added together with $CaCl_2$ to maintain a 10:1 M ratio of Na^+:Ca^{2+}. Leaf (growing point) and root

Table 1 PCR primers designed from conservative regions in the putative *USP* genes

Unigene	Primer	T_m (°C)	Material	Length (bp)
Hv.3739	F: GTCCTTAATTGTTCCTGCGTC	58	Steptoe	1,881
	R: ACGTACACGACACATGCACAG		Morex	1,893
Hv.7364	F: CAACACCTTCGTCCAGAAACTC	57	Steptoe	1,023
	R:AGAGATAACTGAGGAAAACACACTG		Morex	1,032
Hv.11351	F: TTAAAGCCAAAGCAAGCCGT	55	Steptoe	810
	R: TCAAATCGGCAGTAAAATGAAATC		Morex	807
Hv.11741	F: TAACCAAACCAAGCCCTCTAC	55	Steptoe	960
	R: GCTTTTATAGAAACACGGTCG		Morex	961
Hv.23267	F: CGTTCCTCGTAGACAGTTCG	58	Steptoe	1,751
	R: CATGAGTACAGTAGATGGTTGGG		Morex	1,747
Hv.1561	F: ACGACACACAAAAGCATACACG	55	Steptoe	843
	R: TGGGCATCACTGCTAATCTCC		Morex	843
Hv.5327	F: AACCTGTTTGTGTTCATCTG	55	Steptoe	1,119
	R: TTGCTACTACTACTGTTCTTGC		Morex	1,120
Hv.9308	F: TGGTCTCACTCTCACCGCAAG	50	Steptoe	798
	R: AGTAGATGTGGTTCCCTTTGGC		Morex	798
Hv.9303	F: TCGCAGGAGAGAAGCCCAAG	58	Steptoe	1,212
	R:TGAAGGKGTGAACTGTGACATCTG		Morex	1,213

Table 2 Specific primers designed from indels and SNPs in the putative *USP* genes, and the bold letters with underline at the 3′ end means extra mismatched nucleotides

Unigene	Sites maker	Primer	T_m (°C)	Length (bp)
Hv.3739	*BUG1*	F: GTCCTTAATTGTTCCTGCGTC	58	689
		R: CCATATGGCGAACCGAAATC		
Hv.7364	*BUG2*	F: CTCTGGCAGACATGGC**AGC**	60	1,014
		R: AGAGATAACTGAGGAAAACACACTG		
Hv.11351	*BUG3*	F: TTAAAGCCAAAGCAAGC**C**GT	60	361
		R: GCTCGTAGTCGATCCATCAAC		
Hv.11741	*BUG4*	F: AGAAGGGAGGAGGAGGTGATG	62	187
		R: GCTTTTATAGAAACACGGTCG		
Hv.23267	*BUG5*	F: CTTAACCAACTAACCAAGGAGAT	61	271
		R: CATGAGTACAGTAGATGGTTGGG		

(2 cm of the root tips) tissues were harvested from three different plants at 0, 1, 9 and 27 h, respectively, following the salt treatment. Samples were snap frozen in liquid nitrogen and stored at −80°C until RNA extraction.

cDNA was synthesized using TaKaRa reverse transcription reagents following the manufacturer's instructions and was stored at −20°C. Quantitative PCR was carried out using the SYBR green I master mix (TIANGEN BIOTECH) with two replicates. Each 20 μl reaction contained 9 μl SYBR green master mix, 50 ng of cDNA and 100 nM forward and reverse primers. The 18S ribosomal RNA (forward: ATGATAACTCGACGGATCGC; reverse: CTTGGATGTGGTAGCCGTTT) was used as control for RT-PCR experiments (Walia et al. 2007). The data obtained were analyzed with the method of Pfaffi where expression $= C(A-E)/D(F-B)$, C is amplification efficiency of the tested gene, D is amplification efficiency of the reference gene; A and E are C_t values of the tested gene without and with salt stress, respectively, and B and F are the C_t values of the reference gene without and with salt stress, respectively.

Results

Phylogenetic relationship between the putative *USP* genes in barley and those *USP*s in other species

Based on the known *USP* domains of *Methanococcus jannaschii* and *Haemophilus influenzae*, 16 putative *USP*s

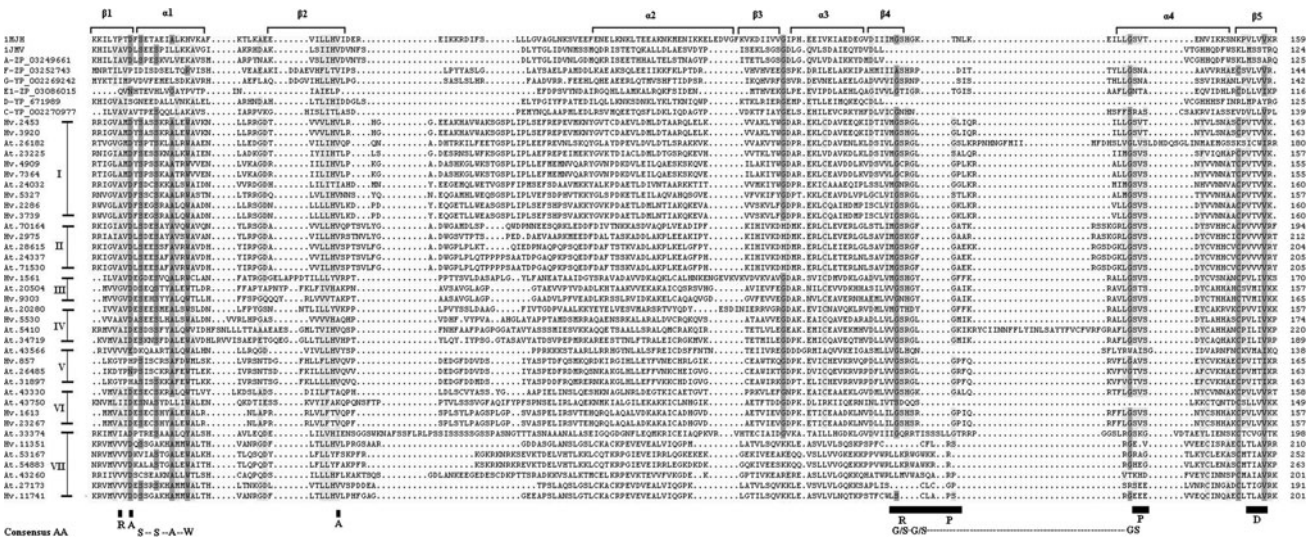


Fig. 1 Multiple sequence alignment (MSA) of thirteen sequence motifs from bacterial and barley. The amino acid sequences of barley USP-like were aligned based on the structures of *1JMV* and *1MJH*. The *black boxes* below the sequence indicate conserved residues contracting ATP (*A* adenine, *R* ribose, *P* triphosphate, *D* dimmer interface)

were identified from the barley ESTs. The alignments of these putative barley *USPs* were compared with those of known *USP*s from other species (Fig. 1). The alignment with the truncated sequences was annotated with features of the secondary structures of the known *USP* gene *1MJH* in *Methanococcus jannaschii*, which consists of five β strands alternating with four α helices (Zarembinski et al. 1998). A number of conserved blocks of hydrophobic sequences were apparent and they corresponded to residues in β1, α1, β2, α3, β4, α4 and β5, respectively. The amino acid identities of α2 and β3 were less than 25%. Among the nine secondary structure elements, there were some differences between plant (*Arabidopsis* and barley) and *E. coli*. For instance, most of the putative *USP*s of *Arabidopsis* and barley contain the 'S-X_2-A-X_2-W' motif at the α1. Similar to that in *1JMV*, the feature of G/S-X_2-G/S-X_9-GS motif was absent in the putative *USP*s in group VII (Fig. 1). Seven groups based on the alignment of the *USP* domains from the *Arabidopsis* and barley were constructed, which were separated from *1JMV*, *1MJH* and the *USP*s of *E. coli* (Fig. 2). As shown, compared with *1JMV* and those *USPs* of *E.coli*, *1MJH* was more closely related to those *USPs* of *Arabidopsis* and barley (Fig. 2).

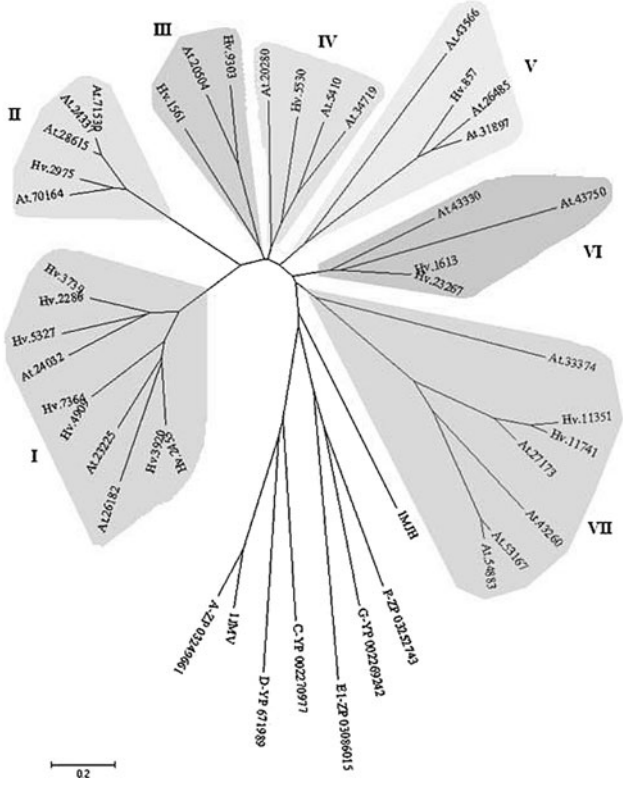


Fig. 2 An unrooted phylogenetic tree of *USP*/*USP*-like from *Arabidopsis*, barley, *E.coli*, *1MJH* and *1JMV*

USP gene structure and phylogenetic classification

The genes in the *USP* family of *Arabidopsis* possessed 1–3 introns (Fig. 3). Two of them, *At.70164* and *At.33374*, possessed one intron each. The *USP* genes in groups III, IV, V, and VI contained three introns each in their coding regions. There was an intron in the 3′ untranslated region of the group II *USP* genes. In these *USP* genes, the first exon was the longest, and the middle one (the second or the third) the shortest. The first exon of groups II and VII *USP* genes occupied at least half of the coding regions. The differences in conserved amino acid residues of the secondary structure elements among the seven groups of *USPs*

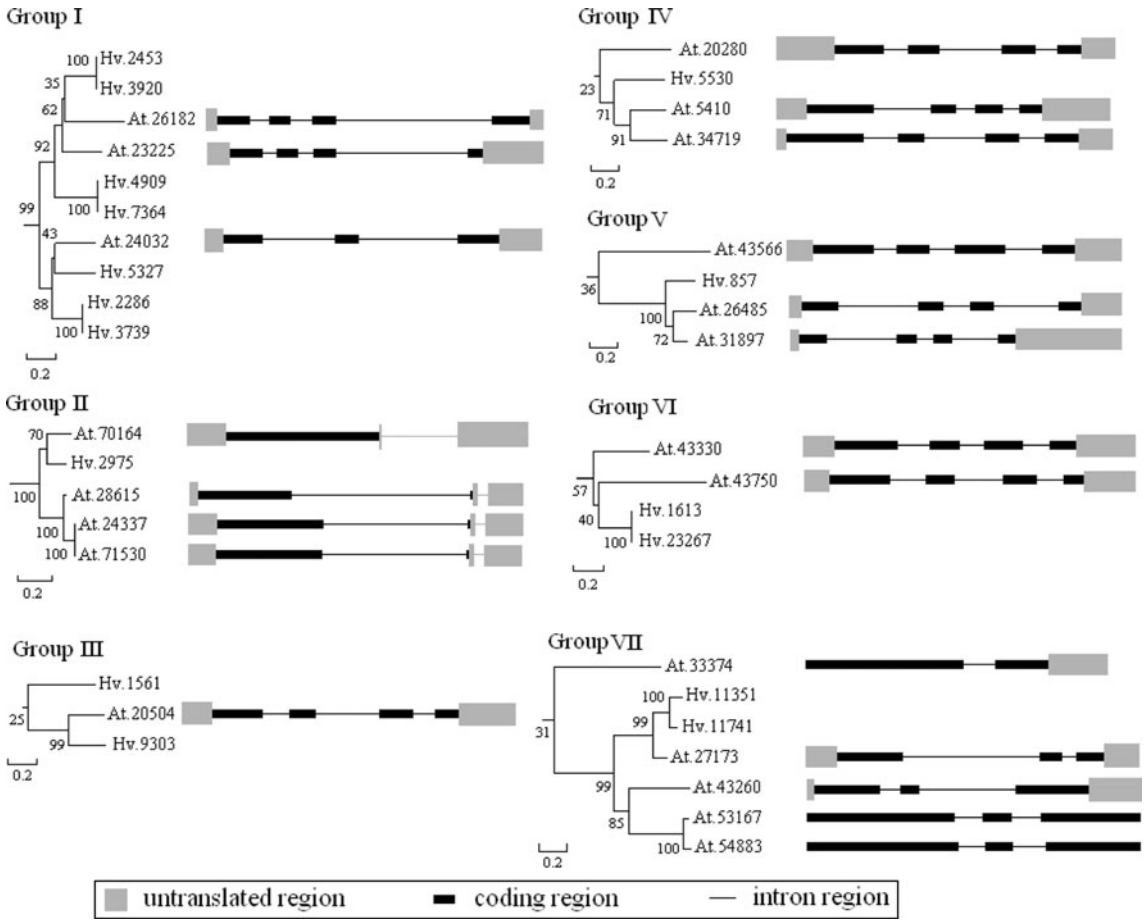


Fig. 3 Phylogenetic relationships among the *Arabidopsis* and barley *USP/USP-like* genes, from group I, group II, group III, group IV, group V, group VI and group VII in the *Arabidopsis* and barley USP/USP-like family. Bootstrap values from 1,000 replicates were used to assess the robustness of the trees

are shown in Table 3. Conserved residues were not detected at the $\beta 2$ for those putative *USP*s in groups I and III, at the $\beta 2$ and $\beta 3$ for those in group IV, at the $\beta 1$ for those in group V, and at the $\beta 3$ and $\beta 4$ for those in groups VI and VII. The seven groups of the putative *USPs* of barley and *Arabidopsis* were clearly separated from *1MJH*, *1JMV* and those of *E. coli* (Fig. 2).

Alignments of amino acid sequences of *USPs*

When comparing the orthologous positions of the putative barley *USPs* with those known ATP-binding residues in the other species a high similarity was found (Fig. 1). Of those sequences known to bind ribose, residue 'G' at position 127 was present in almost all of the sequences. However, the amino acids at positions 11 and 129 of *1MJH* were different among these orthologous sequences. Of the sequences from plants (*Arabidopsis* and barley), 68% had residue 'A' at position 11 and not 'P' as was the case for *1MJH*; 54% of the sequences had residue 'R', and 24.32% had residue 'H' at position 129. Residue 'V' (57%, the same as that of *1MJH*), which was attached to adenine with hydrogen bonds at position 41 was conserved in many of the plant sequences. Residue 'D' at position 13 of *1MJH* (ILYPTD) was also conserved in most of the *E. coli*, *Arabidopsis* and barley amino acid sequences. Residue 'G' at positions 130 (68%, the same as that of *1MJH*) and 132 (76%, different from that of *1MJH*) was conserved in the plant sequences. Residue 'S' at position S141 (65%, the same as that of *1MJH*) was attached to the γ phosphate, whereas residue 'V' at position 142 (81%, the same as that of *1MJH*) and residue 'S' at position 143 (73%, different from that of *1MJH*) were attached to α phosphate. There were no differences in amino acid sequence for residue 'V' at positions 149, 151, or 152 in the dimer interface (D) among *Arabidopsis*, *E. coli*, and barley *USPs*. In addition, residue 'G' at position 140 did not bind to ATP, but it was conserved in most of the *USPs* (>75%).

Among the seven putative *USP* genes with numerous indels between Morex and Steptoe, only three (*Hv.9308*,

Table 3 The conserved residues of *USP*'s secondary structure element in the 7 different groups, and '-'means the variational amino acid

	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
β1	R- -G- - -D	R-I- -AVD	V-VD	A-D		M- -ID	V- -D
α1	S- -S- -A- -W	S-ES- -AV-WAV	S- -AL-W-L	S- -S- -AL-W	A- -W	S- -S- - -L-W-L	A
β2		AV- -LHV			LHV	A- - - - - -FT	L
α2	Y- - - -D- - -L	DFD- -T- -K- -D-A-PL-E	V-KA- - -C	R	L	IC- - -G	C- - - -P
β3	K	KIHIVK	V		E		
α3	K- - -A	ERLCLE-ERL	GDAR- - -C	KE-IC-AV	I- - -V	PK- -I	S
β4	V-G- -GL	S- -IMGS	V- - - -A	D-LV-G	L-VG		
α4	G-V	LGSVSDY-VHHC	A-LGS-SDYC	LGSVS-Y-A	L	C	I/L
β5	V/I–V/I	CPVVVVR	A-C-V-IVK	CP- -IV	C- -LVV	C- -LVV	C- - - -V

Hv.7364 and *Hv.23267*) had different amino acid sequences. However, the amino acid difference of *Hv.23267* between Steptoe and Morex did not result in the change of their secondary structures, but the amino acid differences for the other two genes (*Hv.7364* and *Hv.9308*) did (Fig. 4, 5).

Genomic sequences of the putative *USP* genes from barley

Based on the analysis of the exon/intron structures of the *USP* genes in *Arabidopsis*, conserved regions were identified and used to design primers for detecting putative *USP* genes in barley. A total of nine barley DNA sequences was obtained. Of these, seven (*Hv.3739*, *Hv.7364*, *Hv.23267*, *Hv.5327*, *Hv.9303*, *Hv.1561*, and *Hv.9308*) were gene sequences including all exons and introns, and the remaining two (*Hv.11351* and *Hv.11741*) were partial gene sequences. The details of these sequences (GU258509–GU258526) are shown in Table 1 and Fig. 6.

Of the ones with gene sequences including all exons and introns, five (*Hv.3739*, *Hv.7364*, *Hv.23267*, *Hv.5327* and *Hv.9303*) possessed three introns and four exons each. The other two (*Hv.1561* and *Hv.9308*) lacked intron II (Fig. 6). The sizes of these introns ranged from 75 to 941 bp. Judging from the structure of *1MJH*, the locations of these three introns were between β2 and α2, β4 and α4, and at the tail end of α2, respectively. The types of intron cleavage sites are shown in Table 4.

Of the nine putative *USP* gene sequences, differences were not found for *Hv.1561* and *Hv.5327* between Morex and Steptoe, and the other seven showed numerous insertions and/or deletions. For instance, 'GAGGGA' was absent in *Hv.3739* of Morex but its intron I contained an insertion of 'GCAGCAGCGCCGATTTCG'. Morex had the base

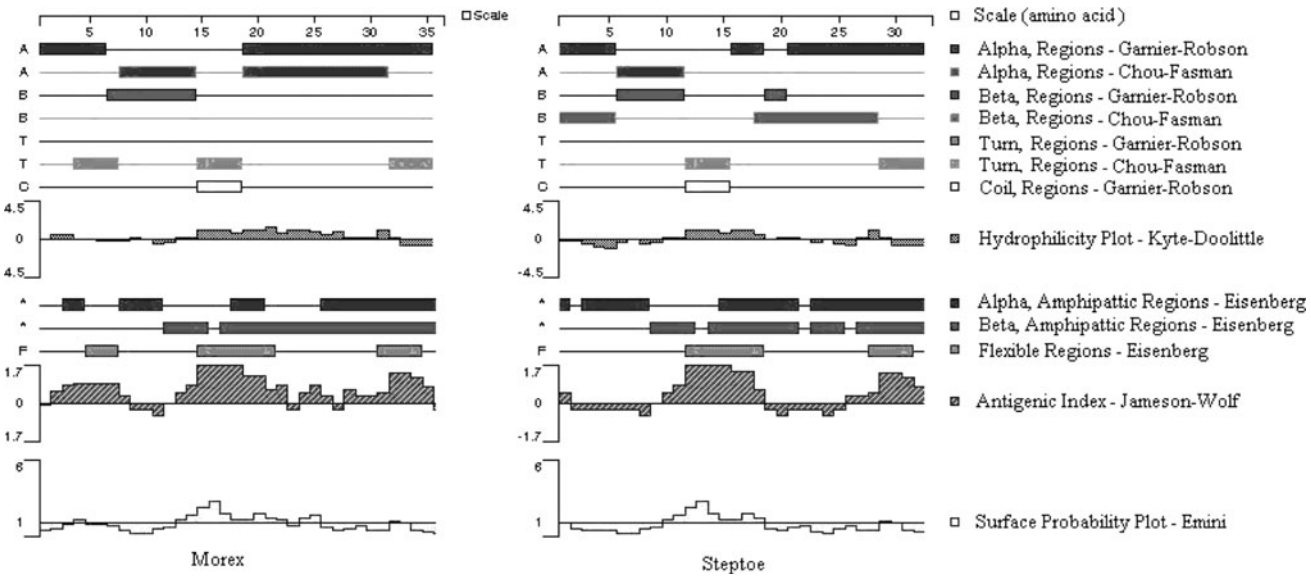


Fig. 4 The different secondary structure regions and characters of *Hv.7364* between Morex and Steptoe based on amino acid sequences

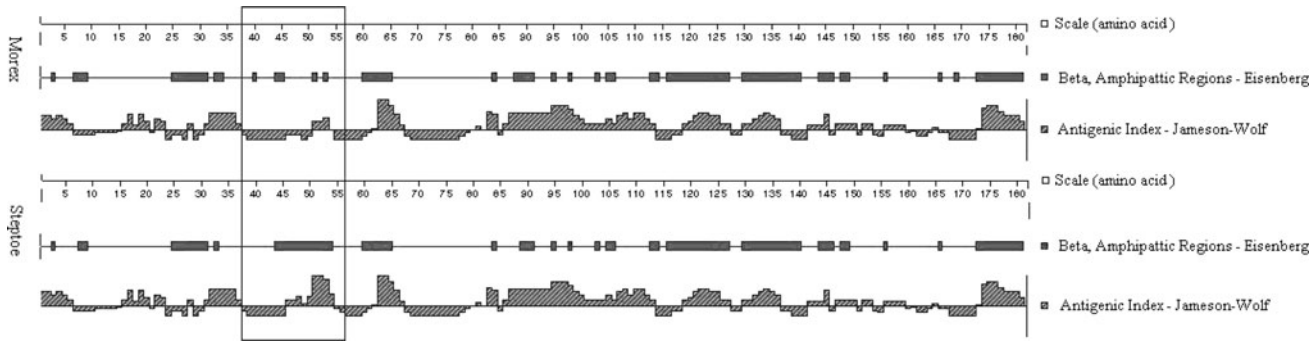


Fig. 5 The different secondary structure regions and characters of *Hv.9308* between Morex and Steptoe based on amino acid sequences, and the differences part was in pane

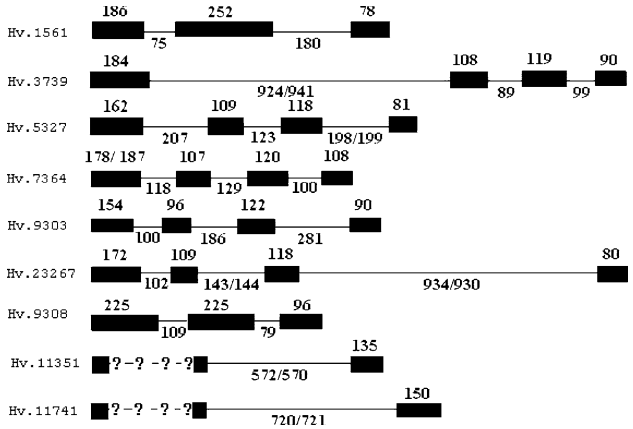


Fig. 6 Exon/intron structures of nine barley putative *USP* genes. Fragment size of Steptoe is the front in two sizes parted by "/", while that of Morex is the latter. *Rectangle* exon, *line* intron, '*?*' unknown number of residues

Table 4 Intron cleavage sites of putative *USP*s in barley

Unigene	Intron I	Intron II	Intron III
Hv.23267	GU-AG	GG-UA	AG-GC
Hv.3739	GU-AG	AG-GG	AG-GC
Hv.7364	GU-AG	GA-AU	AG-GC
Hv.5327	GU-AG	GG-CA	AG-GC
Hv.9303	CA-UU	AG-GU	AG-GC
Hv.1561	GG-AA		AG-GC
Hv.9308	GG-CA		AG-GC
Hv.11351			GG-CA
Hv.11741			GG-CA

sequence 'GGCCGGGAA' in exon I of *Hv.7364*, which was not present in Steptoe (GU258511 and GU258512).

Mapping of the putative *USP* genes in barley

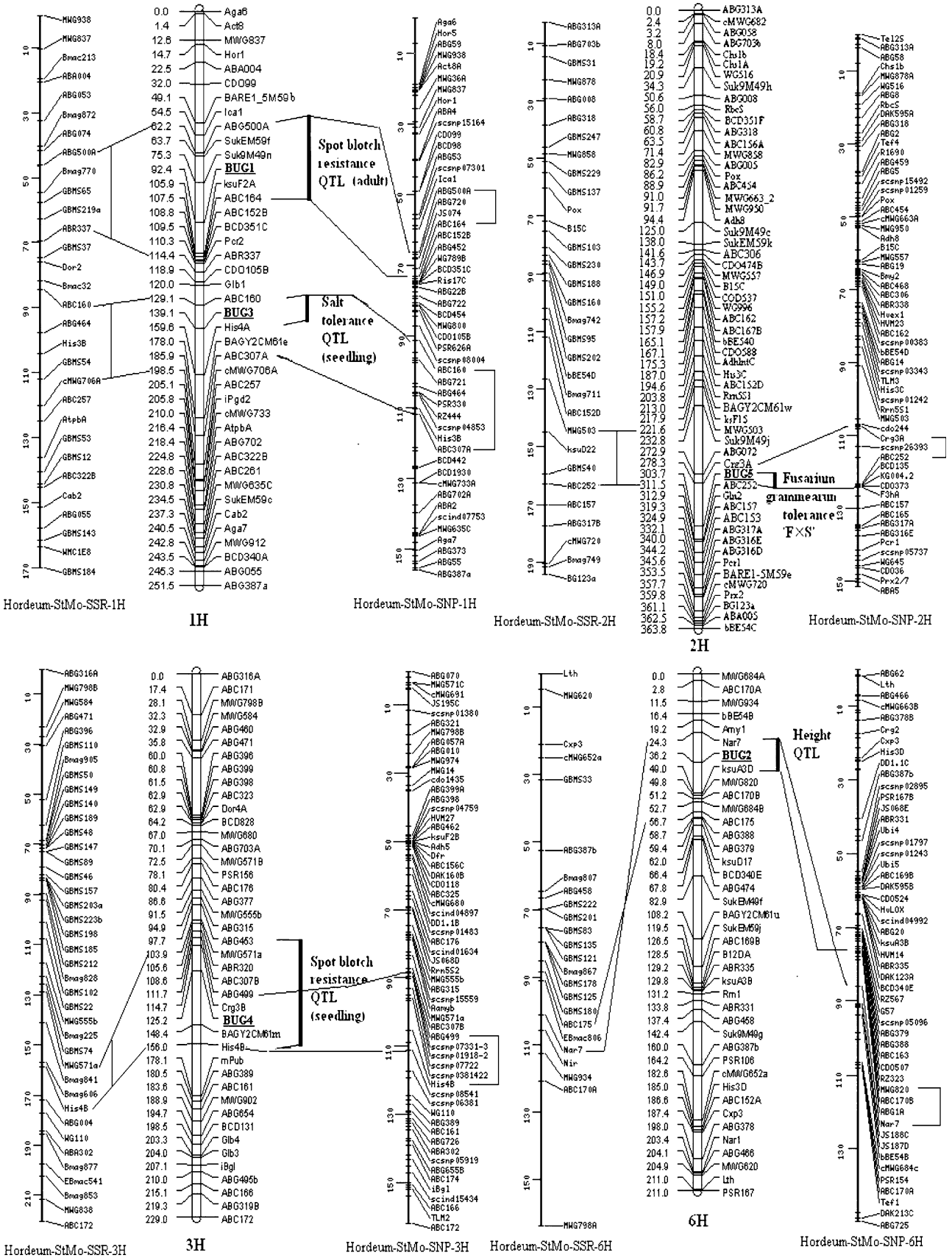
Five sets (designated as *BUG1* to *BUG5*) of locus-specific primers were designed based on the variation of DNA sequences between Steptoe and Morex (Table 2). Two of them were mapped on chromosome 1H, one (*BUG1*) between markers *Suk9M49n* (17.1 cM) and *ksuF2A* (13.5 cM) and the other (*BUG3*) between *ABC160* (10 cM) and *His4A* (20.5 cM). The remaining three were mapped on three different chromosomes: *BUG2* on chromosome 6H between *ksuA3D* (12.8 cM) and *Nar7* (11.9 cM); *BUG4* on chromosome 3H between *Crg3B* (10.5 cM) and *BAGY2CM61 m* (23.2 cM); and *BUG5* on chromosome 2H between *Crg3A* (5.4 cM) and *ABC252* (7.8 cM) (Fig. 7).

Expression of the putative *USP* genes in barley

Eight of the nine putative *USP* genes in barley were analyzed for expression (Fig. 8). *Hv.9308* was the exception for which suitable primers were not obtained. Expressions for three (*Hv.7364*, *Hv.11351*, and *Hv.11741*) of these genes were undetectable in plants not exposed to salt stress. The expressions of the others were detectable although their expression levels were all low. Within 27 h following salt stress, induction was detected in both root and leaf for five (*Hv.3739*, *Hv.5327*, *Hv.23267*, *Hv.7364*, and *Hv.9303*) of the eight genes and in root only for *Hv.1561*. The expression of the remaining two genes, *Hv.11351* and *Hv.11741*, was detectable in neither root nor leaf within 27 h following salt exposure. However, their expression became detectable a week later. There was an up-regulated peak between 0 and 9 h for several of the genes. A second peak of up-regulation was detected at 9 h following salt stress and this was especially the case in leaf. The expressions of these putative *USP* genes were stronger in Morex (salt-sensitive) than in Steptoe (salt-tolerant).

Discussion

Results from this study showed that the putative *USPs* of barley were similar to those of *Arabidopsis*. For instance, only limited differences exist for the conserved ATP-


Hordeum-StMo-SSR-1H
1H
Hordeum-StMo-SNP-1H
Spot blotch resistance QTL (adult)
Salt tolerance QTL (seedling)
BUG1
BUG3
Hordeum-StMo-SSR-2H
2H
Hordeum-StMo-SNP-2H
BUG5
Fusarium graminearum tolerance 'F×S'
Hordeum-StMo-SSR-3H
3H
Hordeum-StMo-SNP-3H
Spot blotch resistance QTL (seedling)
BUG4
Hordeum-StMo-SSR-6H
6H
Hordeum-StMo-SNP-6H
Height QTL
BUG2

◀ **Fig. 7** Map locations of the putative *USP* genes of barley with MAPMAKER (LOD = 3.0), and is compared with SSR and SNP maps with the same mapping population (barley cv Steptoe × barley cv Morex). The genotypes of each putative *USP* gene in this mapping population were compared with mapping data (RFLP, genes and SSAP) The map location of *USP-like* genes is underlined. The dark bar on chromosomes (1H, 3H and 6H) indicate quantitative trait locus (QTL) from barley cv Steptoe × barley cv Morex mapping population, while that on chromosomes 2H indicates a QTL from barley cv Fredrickson × barley cv Stander mapping population

binding residues and their secondary structures. In addition, the number and lengths of exons were also similar. For example, the first exon is the longest one, and the middle one (the second or the third) the shortest. The examination of the alignment data showed that these orthologous sequences of barley and *Arabidopsis* share a higher degree of conservation with the structure of *1MJH* than that of *1JMV*. Similar to that of *1MJH*, most (not those in group VII) of the putative *USPs* of barley and *Arabidopsis* contain a long motif. Residues in this motif seem to interact with the Rib and the phosphate groups of ATP. Similar to that in *1JMV*, the motif in the group VII *USPs* are shorter and they lack the distal binding residues beyond the 'G' at the end of β4, thus they could not bind ATP. The combination of primary amino acid sequence alignment and the analysis of the secondary structures of the putative *USPs* from barley and *Arabidopsis* showed a set of small barley proteins that appear to have descended from a *1MJH*-like ancestor, and they have retained sequence features consistent with a possible ATP-binding function. Conserved hydrophobic residues in β5 apparently appear to mediate this protomer interaction. *1MJH* has two protomers that appear to interact with each other at β5, while *1JMV* has four (Freestone et al. 1997; Sousa and McKey 2001). The shared conserved hydrophobic stretch in β5 and the fact that they are small proteins similar to their bacterial counterparts suggest that the putative *USPs* of barley and *Arabidopsis* may also function through a dimer association via β5.

The *USPs* are vital in the stationary phase of cell growth, and they mediate survival of cells stressed under adverse environments (Nystrom and Neidhardt 1993; Tao et al. 1999). The DNA sequences of Steptoe are different from that of Morex for seven of the nine putative *USP* genes cloned in barley. The amino acid sequences of *Hv.9308*, *Hv.7364*, and *Hv.23267* are also different between the two genotypes. The different amino acids in the putative *USPs* of *Hv.9308* and *Hv.7364* result in different protein structures and characteristics, which may cause the change of their biological functions. These genes with different secondary structures and characteristics may give different tolerance. Morex has a high level of resistance to stem rust and loose smut, and is moderately resistant to spot blotch (Rasmusson and Wilcoxson 1979), whereas Steptoe has superior lodging resistance and is

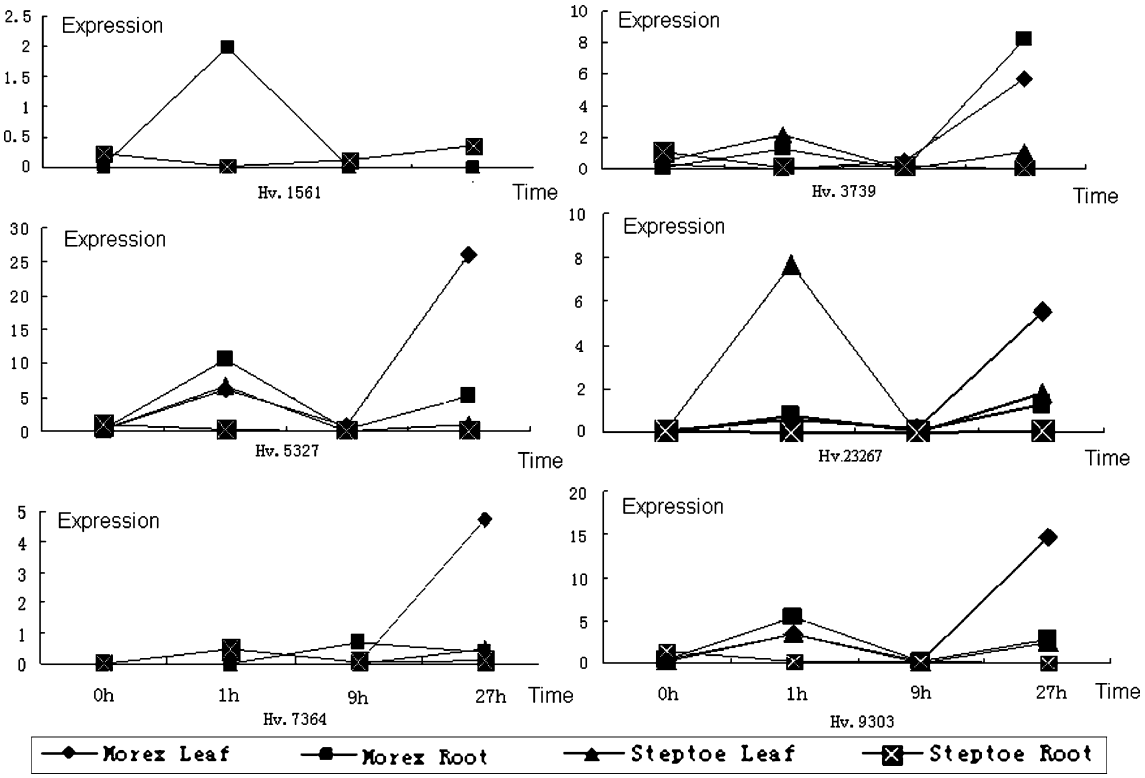


Fig. 8 Expressions of the putative *USP* genes in leaf and root of Morex and Steptoe at the different time points

adapted to both high- and low-rainfall areas (Muir and Nilan 1973).

Five of the nine putative *USP* genes of barley were located on four different chromosomes in this study. Due to the nature of QTL mapping, co-locations between putative *USP* genes and QTL conferring abiotic or biotic tolerance may not represent associations between them. Nevertheless, linkage analysis showed that *Hv.3739* (*BUG1*) falls into a major QTL region conferring adult spot blotch tolerance on chromosome 1H (Bilgic et al. 2005); *Hv.11351* (*BUG3*) was located on chromosome 1H in a similar region where a major QTL for seedling salt tolerance was located (Mano and Takeda 1997); *Hv.11741* (*BUG4*) was located on chromosome 3H where there is a major QTL conferring resistance to seedling spot blotch (Bilgic et al. 2005); *Hv.7364* (*BUG2*) was located on chromosome 6H where there is a major QTL for height (Hayes et al. 1993). In addition, the locations of markers *Crg3A* and *ABC252* on chromosome 2H were similar between the linkage maps derived from 'Fredrickson/Stander' and 'Steptoe/Morex'. In the linage map of 'Fredrickson/Stander' a major QTL conferring *Fusarium graminearum* resistance was flanked by these two markers (Mesfina et al. 2003). Careful analyses of germplasm variation, and consideration of epistatic and genotype × environment interactions could help to clarify the potential role of the putative *USP* genes in abiotic/biotic tolerance in barley and other crops.

The expressions of the putative barley *USP* genes were different at different time points analyzed following salt stress. Among the eight (*Hv.1561*, *Hv.3739*, *Hv.5327*, *Hv.23267*, *Hv.7364*, *Hv.9303*, *Hv.11741*, and *Hv.11351*) putative barley *USP* genes, six (with *Hv.11351* and *Hv.11741* as the exceptions) showed similar speeds of induction. Similar to the expression patterns of the *Arabidopsis USPs* (*At.43260*, *At.26182* etc.,) one of the barley genes was also found to be tissue-specific (leaf/root). These barley genes were more strongly induced in the genotype Morex (salt-sensitive) than in Steptoe (salt-tolerant), suggesting that the increased expression of resistance for putative *USP* genes was not associated with increased defense response. This is similar to what has been reported for disease resistance where increased defense responses are not necessarily always associated with increased expression of resistance (Thatcher et al. 2009). It is possible that the sequence differences between the resistant and susceptible genotypes could lead to differences in processibility of the enzymes involved, thus requiring higher levels of the salt-sensitive *USP* isoforms at the same salt concentration. It is known that ATP serves as the primary energy currency of the cell (Trefil 1992) and that external stress can lead to a reduction in cellular ATP (Francesconi and Mager 1979; Tezara et al. 1999; Zhang et al. 2006; Nandigama et al. 2006; Kawai et al. 2010) and that the biochemical function of the *USP* protein is a factor-dependent ATPase (Zarembinski et al. 1998). Thus, the *USP* isoforms of the sensitive genotype would need to be expressed at higher levels for hydrolysing more ATP to get more energy.

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