

# EXPRESSION ANALYSIS OF *DRG1*: A DROUGHT-RELATED GENE IN DURUM WHEAT

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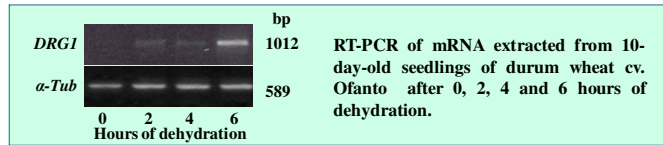
Water deficit is one of the major adverse environmental factor to which crop plants are subjected during the course of their growing season, and it is one of the most important factors limiting plant growth, development, and crop productivity. Plants have developed different strategies to cope with this stress, involving changes in the pattern of gene expression due to activation of stress-specific genes and to a general re-programming of genetic activity in the cell. The results of these mechanisms are in general developmental, physiological and biochemical changes with adaptation to the changing environment. In particular cellular response to water stress depends on the degree of water deficit, on the length of the stress and can differ in the various plant species. Furthermore variations are related to the organ, to the cell type and to the developmental stage of the plant. Response to drought stress can be studied in a top-down approach by identifying genes that modify their expression in response to water shortage. Some of these genes have been already identified and classified, and in the recent years many reports have been focused on transcriptional analysis of these stress genes by microarray (Seki *et al.*, 2001, Ozturk *et al.*, 2002).

A regulator gene encoding a putative drought-induced transcription factor was identified in barley and named *SRG6* (Malatrasi *et al.* 2002). The deduced amino acid sequence has similarity to an *Arabidopsis* hypothetical protein and to a human and mouse DNA-binding protein. A cDNA homologous to this gene was identified in bread wheat and named *TaSRG6* (Tong *et al.*, 2007).

We have identified a cDNA homologous to these stress-responsive genes in durum wheat. A semi-quantitative RT-PCR analysis was performed on RNA extracted from 10-day-old seedlings dehydrated for 2, 4 and 6 hours (drought treatment) using specific primers designed on the barley *SRG6* gene (AJ300144). The primers designed on the *Triticum aestivum*  $\alpha$ -tubulin gene (U76558) were used as control. The PCR product was cloned, sequenced and named *DRG1* (AJ62289). The full length cDNA is 1101 bp long and the deduced amino acid sequence is 98% identical to wheat *TaSRG6*, and 92% identical to barley *SRG6*.

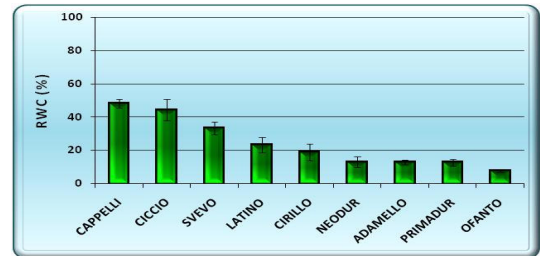
SRG6	MADHAADQE	PPVLLERAAR	ATRGKRITKL	VEEVEVDEA	FWGQDALKED	EEDDNYQEEQ
TaSRG6	MADHAADDEE	PPVLLDHAAR	ATRGKRITKL	VEEVEVDEA	FWGQDALKED	EEDDNYQEEQ
DRG1	MADHAASEEE	PPILLDRAAR	ATRGKRITKL	VEEVEVDEA	FWGQDALKED	EEDDNYQEEQ
SRG6	DAGDVFDSD	DEDEVSRLLL	RPICSIGEPQ	PDDDPKEVVS	ERLPIKRLV	FPGKTMKMK
TaSRG6	DAGDVFDSD	DEDE-----	-----PQ	PDDDPKEVVS	ERLPIKRLV	FPGKTMKMK
DRG1	DAGDVFDSD	DEDE-----	-----PQ	PDDDPKEVVS	ERLPIKRLV	FPGKTMKMK
SRG6	AKKKKKKRF	IKLEDD-ID	DEAPDKTTSS	KQSDVPDWE	SEKTRKSTR	TSVIVRQER
TaSRG6	AKKKKKKRV	IKLEDDDEGID	DKNDPKTTSS	KQSDVPDWE	SEKTRKSTR	TSVIVRQER
DRG1	AKKKKKKRF	IKLEDDDEGID	DKNDPKTTSS	KQSDVPDWE	SEKTRKSTR	TSVIVRQER
SRG6	EATRAEKQAT	AKPIKRRKEG	EERKVTQEM	LLEAAETEIM	NMRNLERVA	REEVEKKAIV
TaSRG6	EATRAEKQAT	AKPIKRRKEG	EERKVTQEM	LLEAAETEIM	NMRNLERVA	REEVEKKAIV
DRG1	EATRAEKQAT	AKPIKRRKEG	EERKVTQEM	LLEAAETEIM	NMRNLERVA	REEVEKKAIV
SRG6	VQKAVYEGPT	LRFHSDGEGS	RLEFINGASF	GSELCTTSTP	YPEKSVCVVT	GLPAKYRDPK
TaSRG6	VQKAVYEGPT	LRFHSDGEGS	RLEFINGASF	GSELCTTSTP	YPEKSVCVVT	GLPAKYRDPK
DRG1	VQKAVYEGPT	LRFHSDGEGS	RLEFINGASF	GSELCTTSTP	YPEKSVCVVT	GLPAKYRDPK
SRG6	TGLPYATMAA	FKIIRERFLK	EEDPKRRPDM	SNMGELFESV	AGEHSTPKKK	RIEGRSPISV
TaSRG6	TGLPYATMAA	FKIIRERFLK	EEDPKRRPDM	SNMGELFESV	AGEHSTPKKK	RIEGRSPISV
DRG1	TGLPYATMAA	FKIIRERFLK	EEDPKRRPDM	SNMGELFESV	AGEHSTPKKK	RIEGRSPISV
SRG6	DLRHGGRFR	IPALDVMDED				
TaSRG6	DLRHGGRFR	IPALDVMDED				
DRG1	DLRHGGRFR	IPALDVMDED				

Comparison of the deduced amino acid sequence of barley *SRG6*, bread wheat *TaSRG6* and wheat *DRG1* proteins.



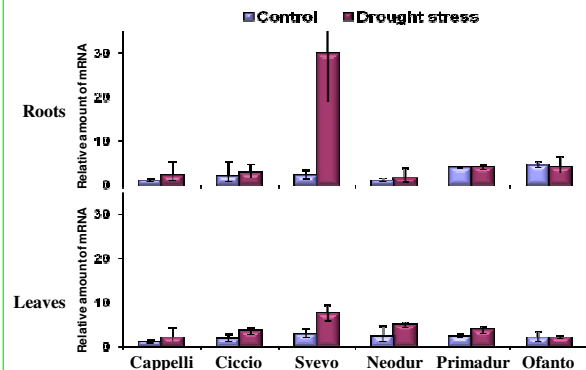
In order to identify differences in the response to water stress of the different genotypes, a physiological test was performed. Measurement of RWC (Relative Water Content) was used as a quick screening method for selection of genotypes most contrasting in their response to water stress. The selection was based on the response of plants to a long-term stress (24 hours dehydration). RWC was calculated according to Barrs and Weatherley (1962). WRL (Water Loss Rate) and free proline content tests were also performed (data not shown).

There is a significant variability among the genotypes. The most contrasting *T. durum* genotypes (Cappelli, Ciccio and Svevo with the highest values of RWC; Neodur, Primadur and Ofanto with the lowest values of RWC) were chosen and utilised to analyse *DRG1* expression.

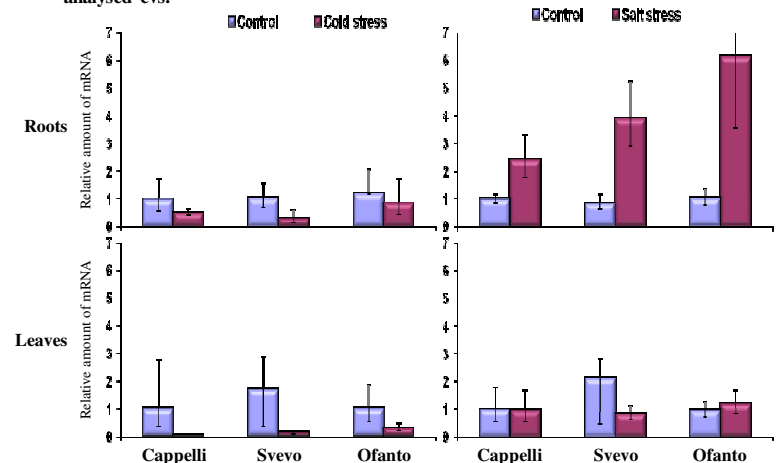


Expression analysis of *DRG1* gene was performed by quantitative (Real-Time) PCR on 10-day-old seedlings subjected to 8 hours of dehydration of the durum wheat cvs. characterised as more sensitive (Neodur, Primadur and Ofanto) or less sensitive (Cappelli, Ciccio and Svevo) to drought stress. The possible *DRG1* induction by other abiotic stresses was analysed on leaves and roots of seedlings subjected to different stress conditions, such as cold (4 °C) and salt (NaCl 250 mM) stress for 24 hours in cvs. Cappelli, Svevo and Ofanto.

Drought stress induces *DRG1* transcript accumulation in both roots and leaves of all the cvs. analysed except for cv. Ofanto in which *DRG1* is inhibited by drought stress. Particularly interesting is the cv. Svevo in which accumulation of *DRG1* transcript is very evident in roots.



Cold stress inhibits *DRG1* expression in roots and leaves of all cvs. On the contrary, salt stress induces *DRG1* transcript accumulation in roots but not in leaves of the analysed cvs.



Drought stress induces *DRG1* gene expression in almost all the analysed *T. durum* cvs. This induction is particularly evident in the cv. Svevo. *DRG1* expression is also enhanced in roots after salt treatment. On the contrary, *DRG1* expression seems to be inhibited by cold stress.

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