EXECUTIVE SUMMARY

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Characterization of the gene complex regulating the response of *soybean roots,* leaves and meristems to heat, cold, water and salt stress

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Using three different PCR reactions, the effect of annealing temperatures (TA) on PCR amplification of cDNAs, obtained from the roots of soybean *(G./wine* max (L) Mere ea, Acme) grown under heat, cold, water stress, and control conditions were compared. Non-specific PCR products could be minimized by optimizing the annealing temperature using a two step PCR programme (The TA at 45°C for 3 cycles, then increasing the TA to 60°C for 27 cycles).

Heat, cold, and water stress subtraction libraries were constructed in a pBluescript or pLJCl8 vector with the PCR amplified cDNAs using subtractive hybridization. One hundred and eighteen clones were obtained from these libraries. Of these, 35 white clones from the heat stress library, 52 white clones from *the cold stress* library, and 31 white clones from the water stress library were selected. Southern blotting analysis indicated that ,3 clones from the heat stress subtraction library contained inserts (H86, H87, and HS13). These clones hybridized to the heat stress oDNA library. Five clones in the cold stress subtraction library (the inserts CS1, C83, CS6, CS16, and CS18) could be hybridized to the cold stress eDNA library. Two clones from the water stress subtraction library contained inserts which *could be* hybridized to the water stress CDNA library.

Eighty thousand plaques from the *heat stress* cDNA library, 100,000 plaques from the cold stress cDNA library, and 60,000 plaques from water stress eDNA library were screened using the subtraction library inserts HS13, CS18, and WS38 as probes. Four clones 081 8-13, CS18-14, CS18-15, and CS18-16 from the cold stress soybean root eDNA library have been confirmed to have inserts that could hybridize to the CS18 probe obtained from the cold stress subtraction library. Sequence analysis of insert 0518 revealed that the insert cDNA had a 76% homology with the sequences of the corresponding portion of glucose dehydrogenase from *Thermoplasma acidophllum* and 62.0% homology with a genomic DNA of *Arabidopsis.* Multi-enzyme digestion analysis indicated that insert CS1814 and CS18-15 have a *Xba* I and Xho I fragment of approximately 3,500 by in length corresponding to the insert 0818 which probably encodes for glucose dehydrogenase, induced by low temperature in soybean roots.