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# Possible chromosomal location of genes determining the osmoregulation of wheat

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**Summary.** Stress-induced free amino acid accumulation in the presence of 0.7 M mannitol has been compared in tissue cultures of moderately stress-tolerant 'Chinese Spring' and stress-sensitive 'Cappelle Desprez' cultivars and in disomic chromosome substitution lines of 'Cappelle Desprez' into 'Chinese Spring'. The profile of amino acid accumulation was different in the two parents. The amino acid concentration of the substitution lines belonging to the A, B and D genomes, respectively, altered characteristically under stress condition. The 'Cappelle Desprez' chromosomes associated with non-ionic osmotic stress-induced free amino acid accumulation were 5A and 5D.

**Key words:** Chromosome substitutions – Drought tolerance – Osmotic adjustment – Tissue culture – Wheat

## Introduction

In higher plants, osmotic adjustment refers to the lowering of osmotic potential caused by the net accumulation of solutes in response to water deficits or salinity. It is considered as a major component of drought tolerance mechanisms in plants, counteracting water stresses induced by changes in the soil evaporative environment (Barlow et al. 1980). Positive correlations have been found between yield and osmotic adjustment (Blum et al. 1983), and genetic variation for osmotic adjustment within wheat genotypes has also been observed (Morgan 1977; Fisher and Sanchez 1979). Measurements of osmotic adjustment in random $F_4$ wheat lines that were derived from a cross between a high and low osmoregulating variety yielded two main groups and a smaller intermediate group (Morgan 1983). These data indicated that osmotic adjustment may be attributable to single-gene control.

Osmotic adjustment has been observed in tissue cultures subjected to osmotic stress by non-penetrating or less-readily penetrating osmoticum, e.g., polyethylene glycol, dextran, and mannitol (Heyser and Nabors 1981; Harms and Oertli 1985; Galiba and Erdei 1986). Based on changes in amino acid composition, putrescine content and protein metabolism, Galiba et al. (1989) suggested that the response of wheat callus cultures to non-ionic osmotic conditions may be genotype-dependent and related to the stress resistance of the cultivars. This assumption was supported in a more recent study where the effects of both osmotic and salt stresses on wheat calli were evaluated (Trivedi et al. 1991). It was concluded that the growth parameters, total N and P, and changes in $Na^+$ and $K^+$ concentrations can be considered as factors of adaptive value under osmotic- and/or salt-stress conditions. The results suggested that callus cultures may give genotype-dependent responses under osmotic- and salt-stress conditions.

Differences in responses to non-ionic osmotic stress were also found between 'Chinese Spring' (moderately stress-tolerant) and 'Cappelle Desprez' (stress-sensitive) cultivars (Galiba et al. 1989).

These differences prompted us to investigate the osmoregulating effects of specific chromosome substitution lines derived from crosses of donor 'Cappelle Desprez' to recipient 'Chinese Spring'. These lines were the basis of this study and provided the opportunity to obtain additional information about chromosomes that

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are involved in osmoregulation in wheat, and how they influence amino acid biosynthesis.

## Materials and methods

*Plant materials*

*Triticum aestivum L.* cultivars 'Chinese spring' (recipient) and 'Cappelle Desprez' (donor) and their various chromosome substitution lines (kindly provided by Professor C. N. Law, Cambridge, UK) were studied. The entire substitution set was used, except for 2A and 2B which were not available. For callus-induction, immature embryos were placed on a modified MS medium (Sears and Deckard 1982) containing 2 mg/l of 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.8% Bacto Agar. After 4 weeks, the calli were subcultured on the same medium for propagation. After 8 weeks, the calli were transferred onto media containing 0 (control), or 0.7 M (13%) mannitol (treated). The experiments were performed in triplicate, in Petri dishes (10 cm in diameter) containing ten calli each on 25 ml of autoclaved culture medium. Calli were grown at a temperature of 26 °C with 16 h daily illumination of 20 $Wm^{-2}$. Calli were examined for free amino acid levels after 21 days of culture.

*Amino acid analysis*

Free amino acids were extracted from calli by the perchloric acid (PCA) extraction method (Galiba et al. 1989). Amino acid content was estimated by the ninhydrin method, using an automatic amino acid analyser (Labor MIM, Hungary).

*Statistical analysis*

The data were analysed by the STATGRAPHICS statistical package, using the t-test and ANOVA functions to assess significant differences ($P < 0.05$) between means.

## Results and discussion

In principle, the concentration of all free amino acids increased under osmotic stress. Recently, we observed significant differences between 'Chinese Spring' and 'Cappelle Desprez' calli (Galiba et al. 1989). These former results showed that the content of acidic amino acids in 'Cappelle Desprez' was higher than in 'Chinese Spring'. Additionally, after mannitol treatment, Asn, Glu, and Gln were greatly increased in 'Cappelle Desprez' but not in 'Chinese Spring'. Proline and arginine showed the greatest change in the wheat calli. This was also apparent from the data presented in Fig. 1. The osmotic stress significantly increased the concentration of proline and asparagine in all of the substitution lines. After osmotic stress, significantly higher proline accumulation was detected in substitution lines 1A, 5A, 3B, 4B, 5B, 2D and 5D (Fig. 1b). The content of glutamine was similar to asparagine in the substitution lines, which was comparable to the donor 'Cappelle Desprez'

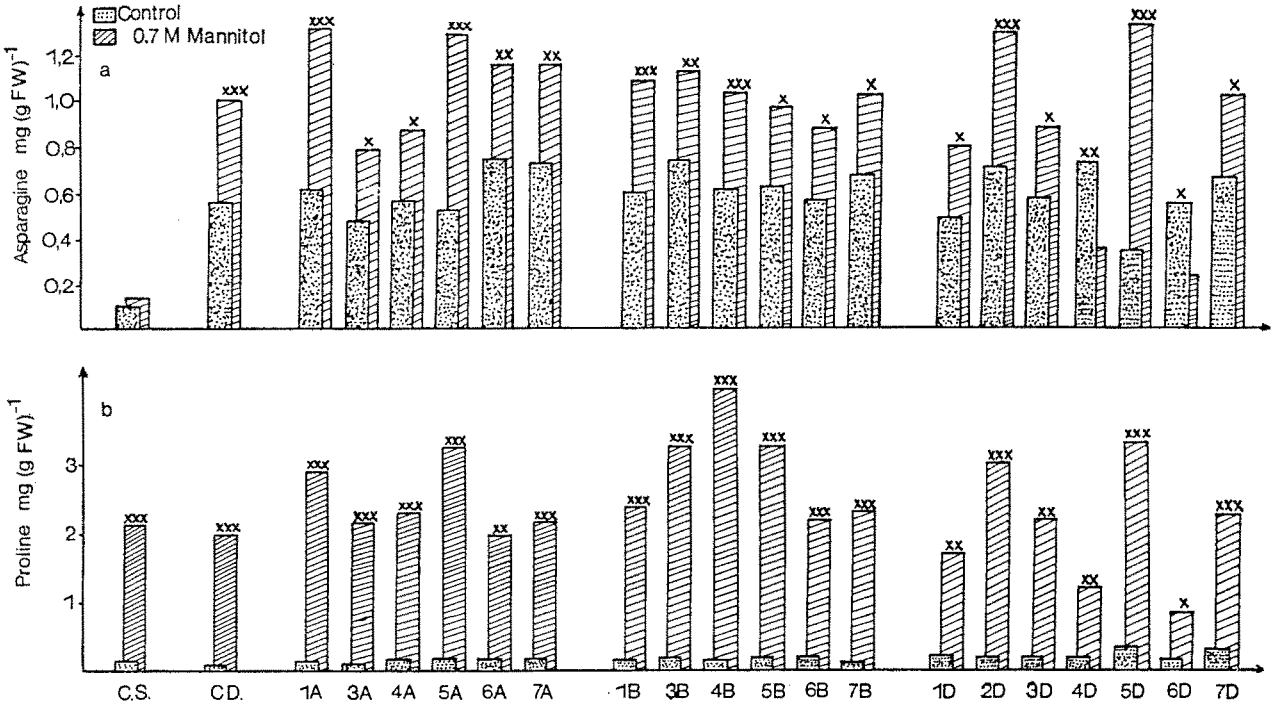


**Fig. 1.** The effect of 0.7 M mannitol on asparagine (**a**) and proline (**b**) content in the calli of 'Chinese Spring' (*CS*) and 'Cappelle Desprez' (*CD*) wheat varieties, and their chromosome substitution lines, after 21 days of treatment. The differences from the control were significant at the $P < 0.05$ (x), $P < 0.01$ (xx), and $P < 0.001$ (xxx) levels. *1–7*, chromosomes; *A, B, D*, genomes

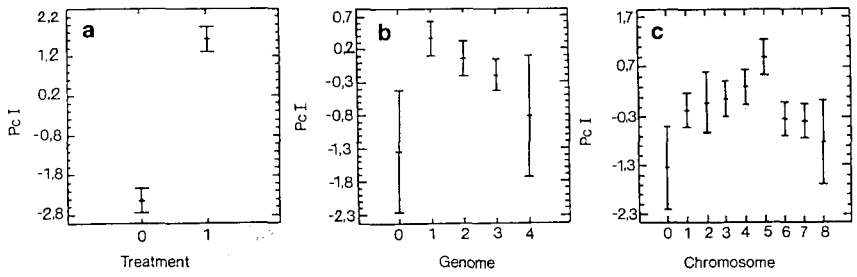


**Fig. 2.** Effect of (**a**) treatments (0.7 M mannitol), (**b**) genomes (A, B, D), and (**c**) chromosomes (*1–7* homoeologous groups), on the first principal component (representing 27% of the variance) calculated from the amino acid change. *Pc I*, first principal component; F proportion of treatment = 391 ($P < 0.001$); F proportion of genome = 2.76 ($P = 0{,}034$); F proportion of chromosome = 318 ($P < 0.0039$); **a:** *0*, control; *1*, 0.7 M mannitol; **b:** *0*, 'Chinese Spring'; *1*, A genome; *2*, B genome; *3*, D genome; *4*, 'Cappelle Desprez'; **c:** *0*, 'Chinese Spring'; *1–7* homoeologous groups; *8*, 'Cappelle Desprez'

(Fig. 1a). The value of Asn and Gln in substitution lines 1A, 5A, 2D and 5D was significantly greater than in 'Cappelle Desprez' after mannitol treatment.

For the evaluation of the data of all free amino acids, the effects of the three factors (treatment, genomes, and chromosomes) were analysed by ANOVA. The use of the principal component analysis suggested that the first three principal components estimated 58% of the variance of the 19 amino acids investigated. The first principal component contained the effect of Pro and Arg while the second one contained the effect of Asn and Gln. Treatment, genomes, and chromosomes (homoeolog groups) caused significant changes in amino acid composition (Fig. 2). Naturally, the osmoticum increased the amino acid content (Fig. 2a). Genome A had a highly significant affect while genome D had only a moderate affect on amino acid content (Fig. 2b). Chromosomes 1 through 5 had a highly significant affect on amino acid content, especially in the case of the 5th homoeolog group. Chromosomes 6 and 7 had only a moderate affect (Fig. 2c). Similar results were obtained analysing the second principal component (data not presented).

The results of the present study suggest that the members of the 5th homoeolog group, namely chromosomes 5A, 5B and 5D, carry genes responsible for osmoregulation.

In wheat, the 5A and 5D chromosomes are involved in the control of frost tolerance (Sutka 1981). More recently, it was demonstrated that a gene for frost resistance was located on the long arm of chromosome 5A (Sutka and Snape 1989). Several studies indicate that drought stress can induce increased frost tolerance in some plants, including cabbage (Cox and Levitt 1976), wheat and rye (Clouter and Siminovitch 1982). It has been suggested that the link between drought and frost tolerance in plants lies in the ability of resistant varieties to tolerate dehydration (Steponkus and Lynch 1989; Thomashow 1990).

The protective effect of proline against a range of stress conditions, especially drought (Paleg et al. 1984; Corcuera et al. 1989) and freezing (Withers and King 1979), has already been demonstrated. Significant positive correlations between proline content and frost tolerance have also been found (Dörffling et al. 1990; Tantau and Dörffling 1991). We have observed positive correlations between the accumulation rate of proline during cold hardening and the presence of the 5A and 5D chromosomes of cv 'Cheyenne' (frost tolerant) that were substituted in a 'Chinese Spring' (sensitive) genetic backround (Galiba, unpublished). It is reasonable to hypothesize that genes which have roles in drought tolerance (osmoregulation) may also have roles in freezing tolerance. Support for this hypothesis comes from the recent finding that the mRNA levels of four alfalfa cold-resistance (*cor*) genes increased in drought-stressed plants (Thomashow 1990).

With regard to osmotic adaptation, the A genome of 'Cappelle Desprez' substituted into 'Chinese Spring' seems to be important. According to our results, the genes controlling osmoregulation are primarily located on chromosomes 5A and 5D although the contribution of other chromosomes, e.g., 1A and 2D, cannot be ignored.

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