REFLECTION OF ENVIRONMENTAL STRESSES ON THE AMINO ACID COMPOSITION OF WHEAT

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Abstract

The adaptation of wheat to different environmental stresses (drought and cold) was studied. The role of amino acids in this process is well known. Stress induced free amino acid accumulation was compared in tissue cultures and in seedlings. The range of studied species included Chinese Spring, Cappelle Desprez and Cheyenne wheat varieties, as well as disomic chromosome substitution lines of Cappelle Desprez into Chinese Spring and Cheyenne into Chinese Spring.

The profile of free amino acid accumulations caused by stress conditions was found to depend on the stress tolerance of the varieties and the nature of the treatment. The results suggested that there is a link between the drought and frost tolerance of wheat. We have found that chromosomes 5A and 5D were associated with both osmotic and cold stress induced free amino acid accumulation.

Keywords: wheat, environmental stress, amino acids.

Introduction

Wheat is one of the most important cereal crops grown for human consumption. Because of its importance as a staple food, extensive breeding programs are implemented all over the world.

The crop is influenced by several factors, including soil characteristics, possible infections, weather conditions, etc. While some of the factors can be kept under control (for example by the help of proper soil technology), the control of certain other factors is not feasible. Abiotic stresses, such as drought, salinity or extreme ambient temperature are typical factors of this kind.

The most common adaptive mechanisms for plants to overcome abiotic stress conditions are *osmoregulation and cold hardening*.

In higher plants, *osmotic adjustment* refers to the ability of the tissues to lower their internal osmotic potential by accumulating a number of low molecular weight compounds such as sucrose, polyamines and free amino acids in response to water deficits [1]. These compounds are described as compatible solutes.

Cold acclimation (or hardening) is the major adaptive response of plants to low temperatures. Cold acclimation is an inducible process, and this term is used to describe two major functions. According to Guy *one is* the 'adjustment of metabolism and basic cellular functions to the biophysical constraints imposed by low non-freezing temperatures, and *the other* is the induction of freezing tolerance' [2].

In plants, exposure to low temperatures causes the accumulation of low molecular weight compounds with demonstrated cryoprotectant activity. Increased cellular solute concentrations protect biological membranes against freeze-thaw damage by reducing the freeze-induced concentration process of the cytoplasm.

Amino acids represent a group of compatible solutes and also cryoactive substances. The behaviour of amino acids is usually explained partly by a non-specific, collective action and partly by a specific interaction between the solutes present and the membranes.

It has been suggested that there are some similarities between extracellular freezing and desiccation processes (i.e. the removal of water from the cell). Plants develop responses to both stresses that involve common adaptive mechanisms [3].

The aim of our research has been to extend our knowledge on the adaptation of wheat to drought and cold stresses and to find out whether the effect of cold per se is different from the effect of drought stress on free amino acid accumulation in wheat.

Materials and Methods

Osmotic stress study: *Wheat varieties:* Chinese Spring (drought-tolerant) and Cappelle Desprez (drought-sensitive), and their chromosome substitution lines were investigated. Chinese Spring was the recipient and Cappelle Desprez the donor.

Callus induction: immature wheat embryos were cultivated on modified MS (Murashige and Skoog) medium. After propagation half of the calli was transferred onto a Murashige and Skoog medium and MS medium supplemented with 13% mannitol, a non-ionic osmotica. Calli of the parents as well as the disomic substitution lines were cultured on control and treated medium at a temperature of 26°C for 21 days [4].

Free amino acids were extracted from calli after 21 days of osmotic stress by the perchloric acid extraction method. The amino acid content was determined by an automatic amino acid analyser [4].

Cold stress study: *Wheat varieties:* Chinese Spring (frost-sensitive) and Cheyenne (frost-tolerant), and their 5A and 5D chromosome substitution lines were investigated. Chinese Spring was the recipient and Cheyenne the donor variety.

Wheat seedlings were cultivated in wooden boxes containing a 2:1:1 mixture of garden soil:humus:sand and in hydroponics in Hoagland's solution.

The preliminary growth and hardening of the wheat seedlings were carried out in a growth chamber programmed to simulate autumn/winter conditions using a special climate programme. Then the plantlets were subjected to cold stress at +2°C for 20 days. The method used for cold test was developed in the Martonvásár phytotron (Hungary) [5].

Free amino acid determination was carried out in the same way as mentioned above.

Results and Discussion

Differences in response to osmotic stress were found between the Chinese Spring (stress-tolerant) and Cappelle Desprez (stress-sensitive) wheat varieties.

In general, the concentration of *all free amino acids* increased under osmotic stress. The content of acidic amino acids was higher in *Cappelle Desprez* than in *Chinese Spring* (*Fig. 1*). This high concentration of asparagine, glutamic acid and glutamine further increased following mannitol treatment in Cappelle Desprez. This phenomenon was not observed in Chinese Spring. The concentrations of proline and arginine, however, increased significantly in both varieties.

The observed differences between Chinese Spring and Cappelle Desprez calli allowed us to investigate the osmoregulating effects of specific chromosome substitution lines. Each line contains only one pair of Cappelle Desprez chromosome in Chinese Spring background.

The osmotic stress increased the concentration of proline in all of the substitution lines (*Fig. 2*). The content of glutamine and asparagine increased after mannitol treatment. The substitution lines were comparable to the donor Cappelle Desprez.

For the evaluation of the data for all free amino acids, the effects of the three factors (treatment, genomes and chromosomes) were analysed by a statistical program (ANOVA). Naturally, the mannitol increased the amino acid content. Genome A had a strong effect on the amino acid content. Chromosomes 1 through 5 had highly significant effect on the amino acid content, especially in the case of the fifth homologous group.

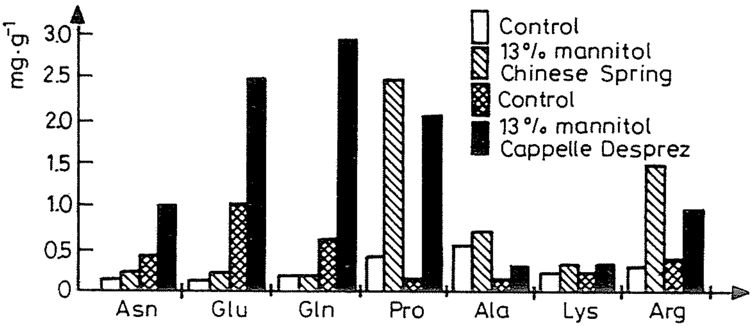

Fig. 1. The effect of 13% mannitol on the amino acid content in the calli of Chinese Spring and Cappelle Desprez after 21 days of treatment

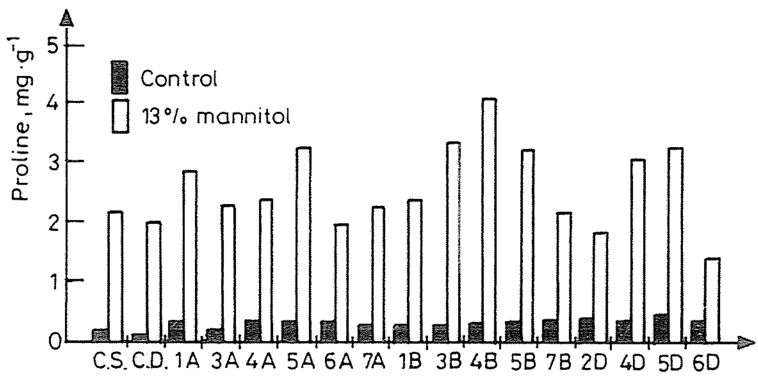

Fig. 2. The effect of 13% mannitol on the proline content in the calli of Chinese Spring and Cappelle Desprez wheat varieties and their chromosome substitution lines after 21 days of treatment

The results of this study suggest that the chromosomes associated with osmotic stress-induced free amino acid accumulation are members of the 5th homologous groups. 5A and 5D carry genes responsible for osmoregulation.

The other series of experiments was carried out to determine the cold-induced free amino acid accumulation in wheat.

Different parts of young wheat plants (*leaf* and *crown means* the base of the shoot, including outer leaf sheaths) were investigated.

The *total free amino acid* content of the recipient Chinese Spring was higher than that of Cheyenne. The crown tissues contained more free amino acids than the leaves in both cases. The accumulation of free amino acids in 5A and 5D substitution lines was similar to those of the parent Chinese Spring during cold treatment.

Comparing the *individual amino acids* contents, glutamine and proline were the most abundant. Only the proline content in Cheyenne proved to be higher than in Chinese Spring (*Fig. 3*). The 5A and 5D Cheyenne chromosomes significantly increased the proline content in the Chinese Spring genetic background, especially in the crowns.

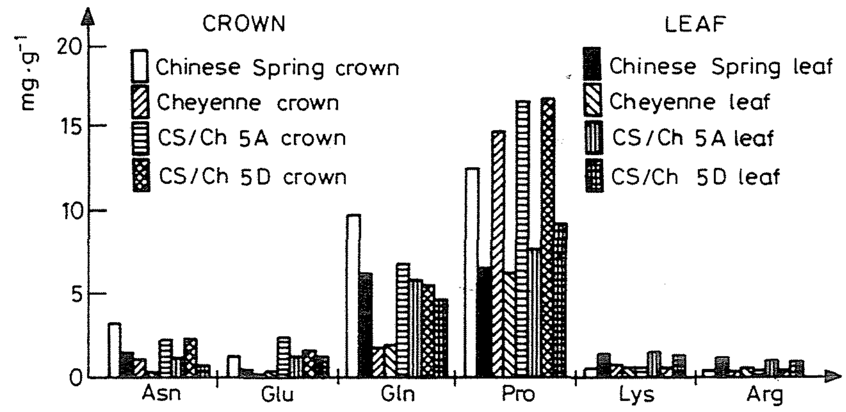

Fig. 3. The effect of cold stress on selected amino acid contents in wheat after 20 days of treatment in soil

This result indicates that chromosomes 5A and 5D carry genes involved in the regulation of the proline content during cold acclimation.

In the *second experiment* free amino acid accumulation was investigated in plants grown in hydroponics (Hoagland's nutrient solution).

The *total free amino acid* content increased in all samples after cold treatment. However, no significant differences were observed between the parent Chinese Spring and Cheyenne varieties, nor between the plant parts (leaf and crown). The total free amino acid levels in 5A and 5D substitution lines were similar to those of the parents.

The *single amino acid* accumulation was different in plant parts (crowns and leaves) (*Fig. 4*). Glutamine, valine, histidine and lysine were the major amino acids detected in *leaves*. While aspartic acid, alanine and leucine took this role in crowns after 20 days of treatment. There was no significant proline accumulation observed in plants grown hydroponics.

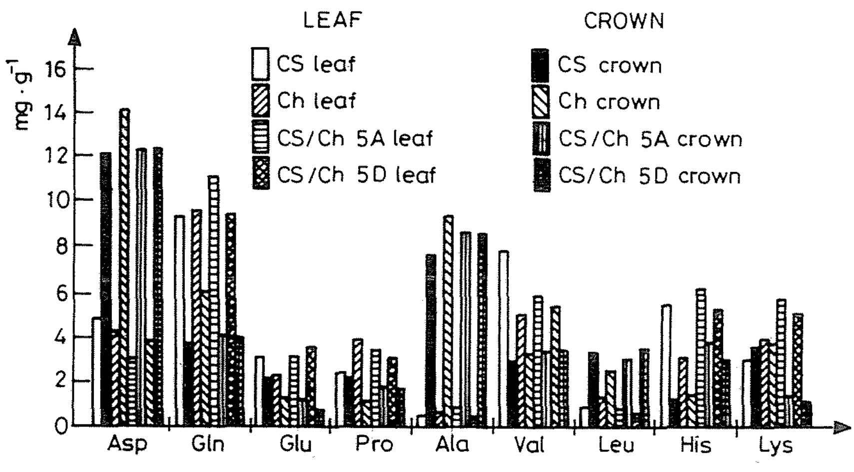

Fig. 4. Changes in free amino acid content after 20 days of treatment in hydroponics

In order to clarify whether the effect of cold *per se* is different from the effect of drought stress, three experiments were carried out:

1. In the first experiment the Chinese Spring variety was grown in soil mixture and exposed to drought stress. For imposing *drought stress* the water content of the soil was reduced to 20% of the soil total water capacity for 20 days.
2. In the second experiment the Chinese Spring variety was grown in soil mixture and exposed to cold stress. During cold stress in the soil water uptake was inhibited, which means cold stress involves 'secondary drought stress'.
3. In the third experiment the Chinese Spring variety was grown in hydroponics and exposed to cold stress.

Only plants grown in soil mixture displayed significant proline accumulation after both drought and the combined cold- and drought-induced stress

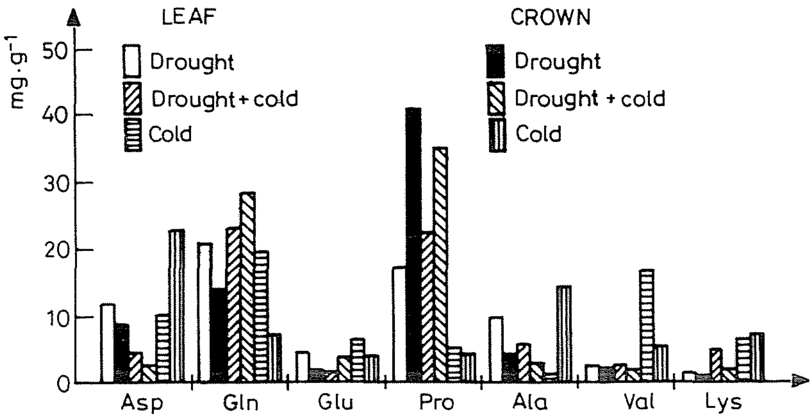

Fig. 5. The percentages of selected amino acids in the total amino acid content in leaf and crown of Chinese Spring after different stresses

(*Fig. 5*). Proline accumulation in the crowns was twice as high as that in the leaves.

On the contrary, for cold-induced changes without drought stress (in hydroponics) Pro accumulation was not significant. The low temperature alone did not affect the proline level.

The relative proportions of free amino acid accumulation induced by cold in hydrophonics were different from the drought- and the combined cold- and drought-induced amino acid accumulation in wheat seedlings.

Conclusions

From the data the following conclusions can be drawn:

1. Our study confirmed the relationship between drought and cold stress. Based on the amino acid content, the cold-induced water deprivation was similar to the effect of water deprivation caused by drought stress.
2. Pro accumulation was associated primarily with water (drought) stress.
3. The accumulation of free amino acids induced by the cold *per se* (in hydroponics) was different from that induced by drought or by a combined exposure to cold and drought.

4. Frost tolerance in wheat was related to the ability of pronounced formation of proline. The chromosomes which carry genes involved in the regulation of Pro are 5A and 5D.

Further research is needed to explain the role of the accumulation of various amino acids under different stress conditions in wheat.

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