

## Acclimation of wheat to drought and frost stresses affects utilization of energy in RNA and protein syntheses

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Acclimation of plants to unfavourable environmental conditions is associated with significant alterations in cellular metabolism, some of them similar both in the cold and the water stress, e.g. promotion of pathways dependent on the availability of reduced pyridine nucleotides and ATP [1-3]. On the other hand, our previous experiments indicated that acclimation of wheat plants to soil drought conditions had practically no effect on ATP level or on the adenylate energy charge [4]. A similar response was observed in crowns of frost-sensitive wheat cultivars during 8-day low temperature treatment [5]. However, low temperature did elevate the ATP level and adenylate energy charge value in frost-resistant cultivars [5]. In the experiments reported here we have tried to quantify the extent of energy-regenerating and energy-consuming processes contributing to the estimated ATP level. Our first approach was to study the ATP expense in protein synthesis since up to 60% of the respiration of mature leaves is ascribed to the cost of protein turnover [6, 7]. Till now, the ATP requirement of protein turnover was calculated on the basis of change in the chemical composition of tissues and the consequent ATP costs.

In our work we have based the evaluation of the respiratory cost of RNA and protein synthesis on the assumption that elimination of an energy-consuming process by a selective inhibitors results in reduction of ATP production

proportionally to the extent of ATP requirement of this pathway [8, 9]. This kind of approach has been applied to animal and human cells [8-14].

The experiments were carried out on two types of wheat cultivars: frost-resistant — *Odessa 16* and frost-sensitive — *San Pastore*. Plants were grown in the climatic chamber as described previously [5]. In the case of drought stress, *San Pastore* cv. was used as described previously [4]. Oxygen uptake was measured manometrically up to 120 min at 25°C. Small segments from the middle portion of fully expanded leaves (drought-acclimated) or crowns of cold-acclimated wheat (2 mm long fragment of seedlings with detached roots and leaves) were used. The segments were incubated in 20 mM Hepes and 10 mM Mes buffer (pH 6.6) containing 0.2 mM CaCl<sub>2</sub>. Respiratory inhibitors, 1 mM KCN and 2.5 mM SHAM (salicylhydroxamic acid), were used for determination of contribution of the cytochrome and the alternative respiratory pathways, respectively. The inhibitor of protein synthesis, 0.5 mM cycloheximide, and the inhibitor of DNA-dependent RNA polymerase, 0.05 mM rifampicin, were applied. The results presented are means for three independent experiments.

The theoretical rate of ATP production was calculated from the rate of oxygen consumption assuming the ADP:O ratio to be 3 and 1 for the cytochrome and the alternative pathways,

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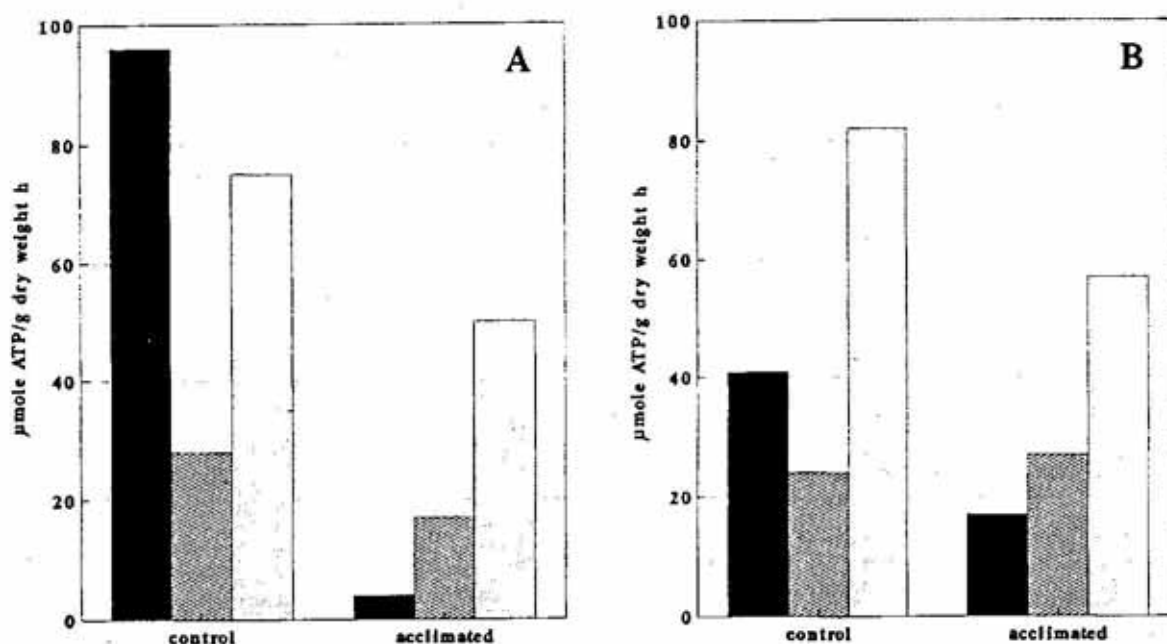


Fig. 1. ATP consumption for RNA (A) and protein (B) syntheses in leaves and crowns of different wheat cultivars.

respectively [15] and also assuming its constancy under the experimental conditions. In this calculation the previously estimated share of either respiratory pathway was taken into account. The results (Table 1) indicate that the theoretical ATP respiratory production in wheat leaves is comparable to that in crowns of both types of cultivars. The acclimation process had a dual effect: it decreased by 18% and 25% the respiratory ATP production in cold-treated crowns in two cultivars but a slight (10%) increase in the drought-acclimated leaves was observed.

To our knowledge, the respiratory energy costs of RNA synthesis has been estimated in plant tissues for the first time (Fig. 1A). Rifampicin, a potent inhibitor of DNA-dependent RNA polymerase, lowered the respiratory  $\text{O}_2$  uptake by about 20% in the drought-nonacclimated wheat leaves and by about 8% and 25% in the crowns of frost-sensitive and frost-resistant cultivars, respectively. Acclimation to either stress lowered ATP consumption for RNA synthesis in all cases, however ATP costs (as compared to non-acclimated plants) were lower as much as by a factor of 24 in the crowns of frost-resistant cultivars but it was only by 40% lower in frost-sensitive cultivars.

Cycloheximide decreased the respiratory  $\text{O}_2$  uptake by about 22% in the drought-nonacclimated wheat leaves and about 6% and 10% in the crowns of frost-sensitive and frost-resistant cultivars, respectively (Fig. 1B). Acclimation lowered the consumption of ATP for the cycloheximide-inhibited protein synthesis in cold-acclimated crowns of frost-resistant cultivars by 60% and in the drought-acclimated wheat leaves by 30% but had no influence on ATP requirement for protein synthesis in the cold-acclimated crowns of frost-sensitive cultivars.

Comparison of the ATP consumption for RNA and cycloheximide-inhibited protein syntheses reveals differences in the response of wheat plants to stresses. In the drought-nonacclimated and acclimated leaves the ATP expense for the two investigated processes is practically the same. However, in crowns of frost-resistant cultivars, ATP requirement for cycloheximide-inhibited protein synthesis is half that of the requirement for RNA synthesis. Acclimation resulted in the opposite ratio, i.e. the requirement for cycloheximide-inhibited protein synthesis was four times higher than ATP consumption for RNA synthesis. The same tendency was observed in the crowns of frost-sensitive cultivars but the shift towards higher ATP expense for protein synthesis was

Table 1

The theoretical respiratory ATP production calculated from the uptake of O<sub>2</sub> ( $\mu\text{mol} \times \text{g}^{-1} \text{ dry weight} \times \text{h}^{-1}$ ) in wheat adapted to frost and drought stresses (n=12)

	Control	Acclimated	Difference
Crowns of frost-sensitive cultivars	375	310	-65*
Crowns of frost-resistant cultivars	390	292	-98*
Leaves	369	408	39*

\*Significant at  $P < 0.01$

less striking. In the crowns of these cultivars ATP consumption for the cycloheximide-inhibited protein synthesis was practically equal whereas under cold stress the ATP consumption for RNA synthesis decreased only by about 30%. The observed differences should be confirmed by measurements of the rate of protein and RNA synthesis.

DNA-dependent RNA synthesis and cycloheximide-inhibited protein synthesis utilize together about 40% of the respiratory ATP in control wheat leaves and 15%–35% in wheat crowns. The value of ATP consumption (about 20%) for leaf protein synthesis is comparable with the evaluation made by Bouma *et al.* [16] for bean leaf.

To sum up, acclimation of plants to unfavourable environment conditions seems to be associated with the decrease in energy expenditure for both RNA and protein syntheses but cold stress restricts to a higher extent the energy consumption for RNA synthesis. A different response to cold and drought of wheat seedlings draws attention to the regulation of RNA and protein synthesis and the interrelation between these processes under environmental stresses.

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