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Regulation of Corm Dormancy

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ABSTRACT

Our work focused on the dormancy - related changes in heat tolerance in gladiolus cormels, on dormancy related changes in two pathways essential for the mobilization of reserves during the break of dormancy and on the mode of regulation of these processes by ABA and by cytokinins.

We found that glucose transport was increased by 50% during the break of dormancy and that this increase was inhibited by ABA.

We estimated the in vivo rate of PEP carboxylase activity in dormant and in non-dormant cormels. This activity is regulated by ABA, cytokinins and low temperature. It accounts for the diversion of 20% of the glycolytic carbon flux into anaplerotic pathways which supply carbon skeletons for protein biosynthesis.

We analysed differences between dormant and non dormant cormels in the heat shock response which correlated with the higher thermoresistance of dormant cormels.

The differential response of the above phenomena to ABA and to cytokinins were analysed.

Evaluation of the research achievements and benefit to agriculture.

Previous studies indicated that cytokinins and abscisic acid are the hormones involved in the regulation of dormancy in cormels. A search for early metabolic changes in the cormels during the break of dormancy has revealed that the in vivo activity of PEP carboxylase increases early during cold storage, which is the common horticultural practice for dormancy breaking. This activity can be modulated by BA and by ABA within a response time of three hours. PEP carboxylase combines the fixed CO_2 with phosphoenol pyruvic acid, thus diverting PEP from the glycolytic pathway into partly anabolic pathways which supply carbon skeletons for the formation of amino acids, and eventually for protein biosynthesis during germination.

Our research focused on three basic areas which appeared essential for the breaking of dormancy.

1. The role of PEP carboxylase. An estimate of the contribution of PEP carboxylase to the mobilization of reserves into amino acid producing pathways could be only achieved by quantifying its in vivo activity in relation to changes in dormancy. This was carried out by equilibration of cormels and cormel slices with $^{14}\text{CO}_2$ in atmospheres enriched with CO_2 . Activities of $65 \text{ nmol g}^{-1}\text{h}^{-1}$ in dormant and $110 \text{ nmol g}^{-1}\text{h}^{-1}$ in non-dormant cormels were measured. These values are equal to 5% of the respirational CO_2 output in cormels. Since every molecule of CO_2 fixed diverts ^{one} ~~the~~ molecule of PEP (=3 carbon atoms) from the glycolytic pathway this means that an equivalent of 20% of the glycolytic carbon flux is diverted by ^{PEP} ~~PEP~~ carboxylase into partly anabolic pathways, indicating an important role for this enzyme in the supply of carbon skeletons for protein synthesis.

The induction of PEP carboxylase at an early stage during the break of dormancy and its rapid response to BA and to ABA fit well with such a role. Moreover, the increase in PEP carboxylase activity during cold storage could be correlated with the increase in germination capacity of different cormel stocks and used as a quality control test for the estimation of dormancy.

2. The Regulation of Glucose Uptake. Cormels are starch-storing organs and the transport of sugar must constitute one of their major cellular activities, particularly during the mobilization of reserves in anticipation of germination. The transport of glucose was characterized by its sugar specificity, energy and pH dependence and by its kinetic behaviour. Uptake was monitored in cormel slices using the non metabolised analog of glucose, 3-O-methyl glucose, which enabled us to isolate the uptake step from ensuing metabolism. An increase of 50% in the capacity of sugar transport was measured during the break of dormancy. ABA reduced glucose uptake in non dormant cormels to the level found in dormant ones, and cytokinins did not effect this process.

3. Dormancy-Related Changes in Heat Tolerance. Dormant cormels can tolerate more extreme temperatures than non dormant ones. This has a particularly important implication on the commercial handling of cormels, since the common practice for treating cormels against Fusarium and other fungul diseases is a hot water treatment for 30 minutes at 57°C. We explored the biochemical basis of heat tolerance in cormels in relation to dormancy with the aim of defining cellular regulative mechanisms which could be utilized for the induction of heat tolerance.

We found that like in other plant systems which were described so far, exposure of dormant cormels to heat stress brings to the rapid induction of a set of newly synthesised proteins termed heat shock proteins, and to a decline in the rate of synthesis of most other cellular proteins. In non dormant cormels this response was different. The new heat shock proteins were induced at a lower intensity, one of these proteins was not induced, and there was a weaker inhibition of the synthesis of non - stress proteins. Taken together these results suggest that the heat shock response of cormels is reduced during the break of dormancy.

A common characteristic of the heat shock response is that a gradual exposure of plant material to elevated temperatures induces a more pronounced response than an abrupt transfer from ordinary to high stress conditions, and increases heat resistance. Our experiments indicated that the induction step is more heat sensitive than the following step of actual synthesis of the heat shock proteins. Cormels that were exposed gradually to stress temperatures synthesised more species of heat shock proteins than cormels that were transferred directly from regular to stress conditions. ABA induced the synthesis of heat shock proteins in non dormant cormels under stress conditions.

4. An Evaluation of The Antagonistic Relation Between Cytokinins and ABA in the Regulation of Dormancy. A comparison of the responses of the above phenomena to cytokinins and to ABA enabled us to have a better understanding of the antagonistic relations between these two hormones in the regulation of dormancy.

(i) Cytokinins induce and ABA inhibits germination and the activity of PEP carboxylase. (ii) ABA affects the transport of glucose, and cytokinins do

not, (iii) Both ABA and cytokinins promote cormel respiration, (iv) Cytokinins induce ethylene production in cormels. This does not occur during normal cormel germination, (v) ABA and cytokinins affect the heat-shock response in different ways.

Taken together these observations exclude the possibility that the two hormones compete on a common site of action in the control of cormel dormancy.

Possibly the most important outcome of this project was that we were able to learn the biochemical basis of some of the more essential steps during the break of dormancy, relate them to the function of the whole plant and explore their hormonal regulation. This was rarely done in a corm crop before. We have also developed the tools to study the economically important heat shock response in corms and the possibility to use biochemical methods in a quality control test of the corms ability to germinate.

Mode of Cooperation

The Cooperation between the American and Israeli partners in this project consisted of joint planning and evaluation of the results.

L.R. visited Israel 3 times during the work on this project.

C.G. visited the US once.