Non-phosphorylating alternative respiratory pathways are involved in an increase of wheat resistance to heat stress

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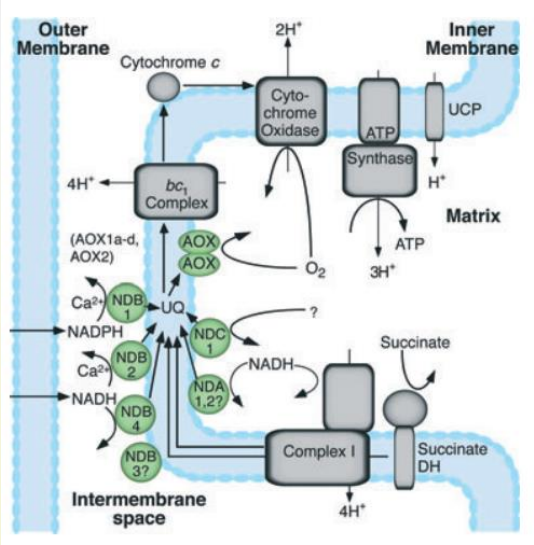
The aim of the study is determined the influence of heat treatments on the heat stress tolerance of spring wheat (*Triticum aestivum L.*) and its relation with the non-phosphorylating alternative pathways, content of ROS in mitochondria, and regulation of the production of ROS by alternative respiratory pathways.



The non-phosphorylating alternative pathways of plant mitochondria are presented by the alternative cyanide-resistant oxidase (AOX) and rotenone-insensitive internal (NDA, NDC) and external (NDB) type II NAD(P)H dehydrogenases (NAD(P)H-DH). The physiological significance of these respiratory pathways is actively studied.

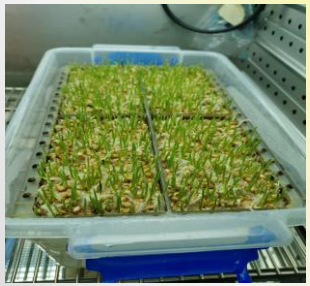
It is known regulation of alternative pathways occurs both at the levels of gene expression and post-translationally and can strongly depends on the physiological state of the plant and growth conditions.

Fig. 1. Oxidative phosphorylation and energy bypasses in plant mitochondria. The non-phosphorylating alternative pathways are denoted in green color. (Rasmusson et al., 2009)

The 4-day-old seedling

Heat influence at 37° C for 6-24 h

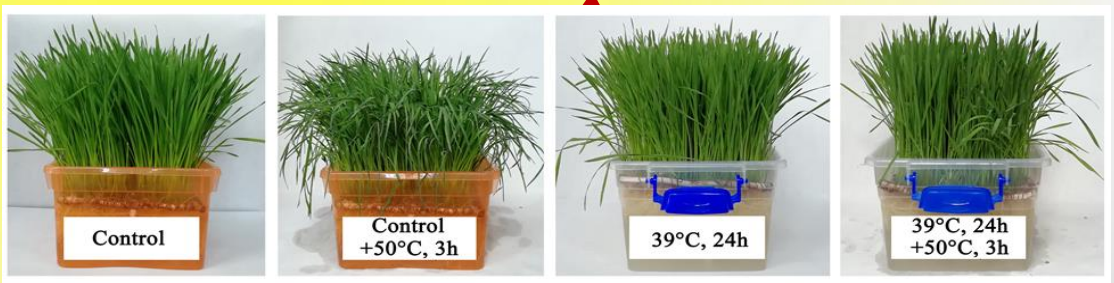
Heat stress at 50 °C for 4 h



The 8-day-old seedling

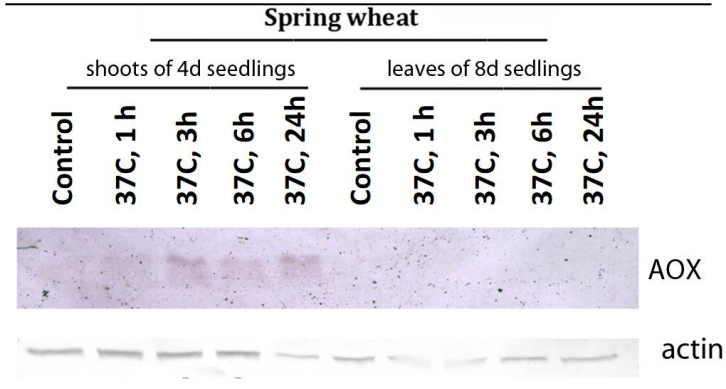
Heat influence at 37 and 39 ° C for 24 h

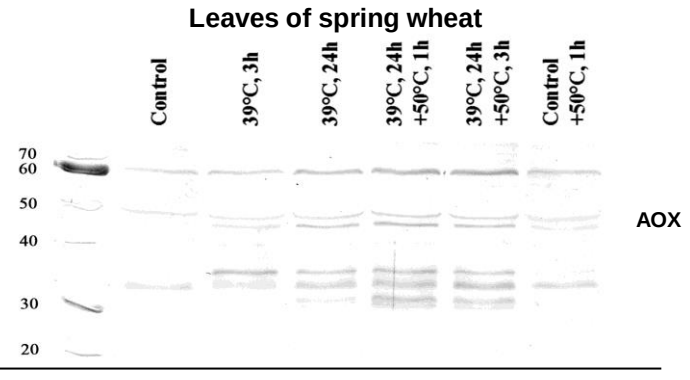
Heat stress at 50 °C for 3 h



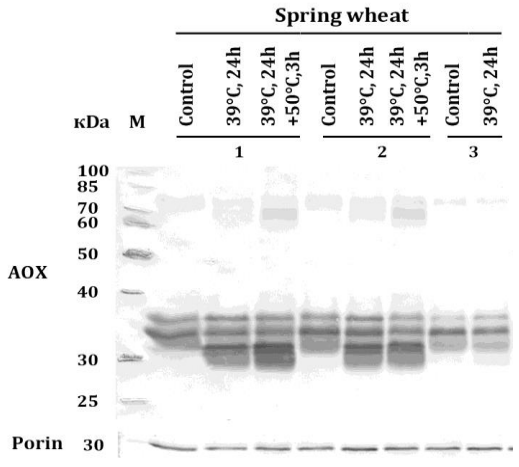
Influence of high temperatures on the content of AOX, NDA, NDB

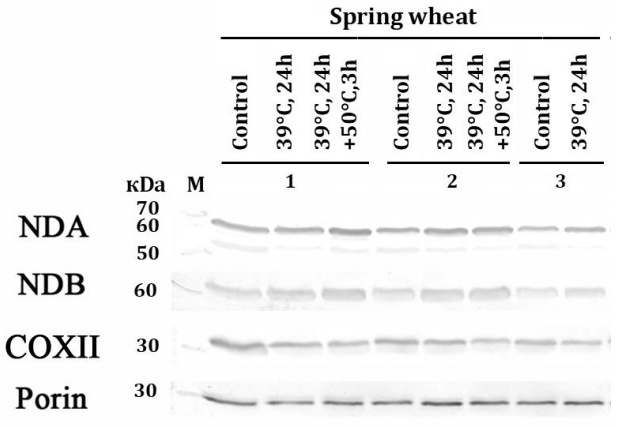
Total protein





Mitochondrial protein from leaves

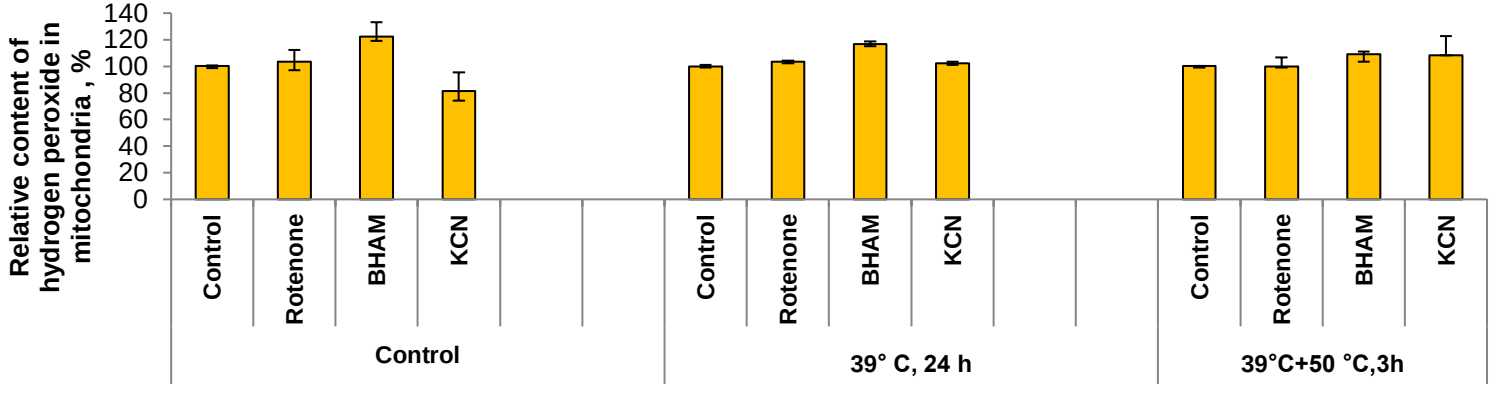




Three active isoforms of the AOX protein have been identified, the content of which depends on the intensity and duration of heat treatments.

The content of AOX protein depends on the age of the seedlings.

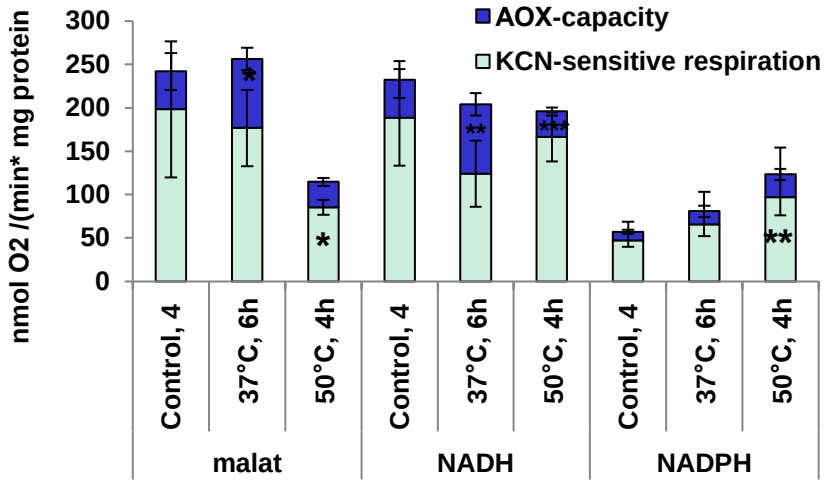
Influence of non-phosphorylating alternative respiratory pathways (AOX and NAD(P)H-DH) at the high temperatures on content of the hydrogen peroxide in mitochondria from leaves of the 8-day-old seedlings



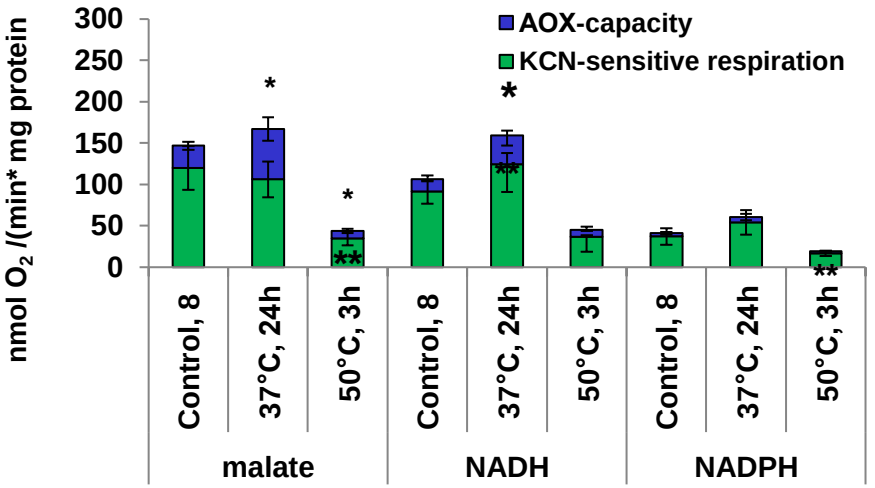
Rotenone is inhibitor of complex I of the respiratory chain;
BHAM is inhibitor of AOX;
KCN is inhibitor of COX

The data are presented as median and percentiles (75th percentile and 25th percentile), n = 3-5

Mitochondria from shoots of 4-day-old-seedlings



Mitochondria from leaves of 8-day-old-seedlings



Effect of heat influence (37 ° C, 6h and 24) and heat stress (50 ° C) on the activity of isolated mitochondria, alternative cyanide-resistant oxidase (AOX) capacity and activity of rotenone-insensitive external type II NADH and NADPH dehydrogenases. The data are presented as median and percentiles (75th percentile and 25th percentile), n = 3. The statistical significance of the differences between medians was determined by ANOVA, Method Dunn's, $P < 0.05$.

The activity of external NADH and NADPH dehydrogenases of type II was assessed by the rate of oxidation of exogenous NADH and NADPH, respectively, in the presence of rotenone (an inhibitor of complex I) and CaCl2 (an activator of NAD(P) H dehydrogenases) without EDTA in an incubation medium. The contribution of AOX was assessed using a BHAM inhibitor in the presence of KCN.

The conditions of seedlings treatments and heat stress tolerance of 4-day-old and 8-day-old seedlings were described in the articles (Borovik and Grabelnych, 2018, Journal of Plant Physiology ; Fedotova et al., 2020, Siberian Journal of Life Sciences and Agriculture)

Conclusion

The data obtained indicates the important role of non-phosphorylating alternative respiratory pathways in adaptation of spring wheat to high temperatures.

The antioxidant function of AOX in leaves was revealed. However, this function is not the main at high temperatures in photosynthetic plants. Moreover, slight increase of peroxide was noted upon inhibition of complex I of the respiratory chain during heat treatments, which may indicate a possible role of NAD(P)H-DH in the formation of ROS.

The activity of non-phosphorylating alternative respiratory pathways in mitochondria at high temperatures depends on the phase of development of spring wheat and is probably tissue-specific, while the functional significance and contribution of the external NAD(P)H dehydrogenases to the development of heat resistance of spring wheat are also likely to differ.

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