CLOSURE REPORT FOR THE RESEARCH GRANT
K-120028

(grant period: 2016.12.01.-2020.11.30.)

Written by:

Imre Majláth
Principal Investigator

Centre for Agricultural Research
Loránd Eötvös Research Network
Martonvásár

Martonvásár, 2022

Table of contents

TASK I

Results of studying the effect of moderate drought on the assimilation of wheat and maize plants

The main project goal was to reveal the optimal level in soil moisture which makes crop plants more tolerant to low temperature. Two durum wheat cultivars were used for the experiments. They have difference in cold- and frost tolerance. Acclimation tests were carried out for 4-week-long periods at 5°C and normal light and low light (250 ± 20 µE; 100 ± 20 µE PFD) (**Figure 1.**). Besides the normally irrigated population, the applied mild water stress was conducted by controlled irrigation and inspected by soil-moisture sensors.

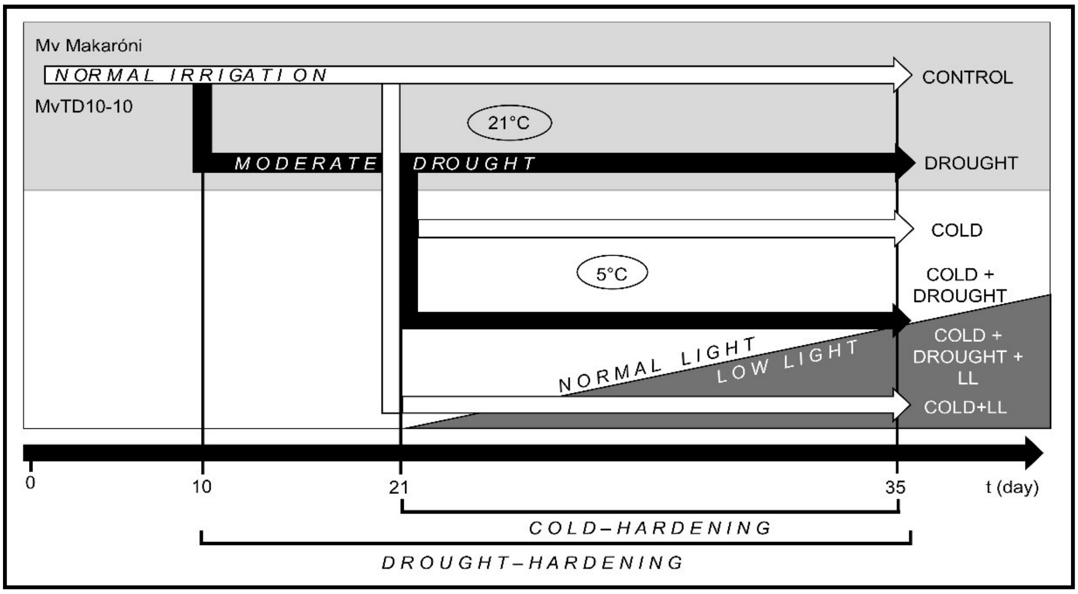


Figure 1. The scheme of the wheat experiments [see Khalil et al. (2021) for further details].

Our key findings was, that Mv Makaróni (cold-tolerant) and MvTd10-10 durum wheat plants grown under moderate water stress and low light intensity exhibited the most optimal photosynthetic performance and sugar remobilisation rates compared to the other abiotic treatment combinations during cold hardening. Temporal differences were found in the actual photosystem II quantum yield during the initial, monotonically increasing phase of chlorophyll fluorescence quenching. It corresponded to the level of cold tolerance of cultivars as followings: the cold-tolerant genotype maintained actual quantum yield higher under mild moderate drought, independently from light conditions and the steady-state level of photosynthesis was reached rapidly at low temperature, as compared to the cold-sensitive genotype.

Our results confirmed the hypothesized positive effect of moderate drought on quantum yield in durum wheat. Protective regulatory mechanisms were also revealed which may be responsible for the changes in light utilization. The photosystems in the chloroplast could be protected by GB in both cultivars. D-sorbit may also have been responsible for the defence of MvTD10-10. Beside photosynthetic light phase, water shortage and normal light resulted in low sucrose content, which serves as a signal for stomatal closure. Additionally, decreasing CO_2 uptake observed led to increased photorespiration and slowing down of glucose formation and facilitated glucose degradation. Oppositely, low light and moderate water deficit positively affected sucrose accumulation, which can serve as an additional source to preserve glucose concentration higher and maintain glycolysis and energy production under chilling in durum wheat. For summary, see **Figure 2.** and Khalil et al. (2021).

Similar, however, not clarified effects of mild drought were found in two investigated bread wheat cultivars, Mv Bodri and Mv21-15. The experiment was the same, which can be seen on **Figure 1**. Similary to durum wheat, all physiological investigations were also carried out, including instrumental metabolomic analysis on sugars, amino acids and organic acids. Changes with biological interest was less emphasized and correlations among metabolites seemed to be contradictionary to drawing further conclusions (**Figure 3.A**).

Positive and more emphasized changes were found in the cold stress tolerenace of maize lines (A654 – cold tolerant line, Cm174 – cold-sensitive line). The most important finding in maize was, that both photosynthetic activity and glutamine biosynthesis were positively affected by moderate drought under chilling (10°C). Changes in physiological variables and metabolites can be seen on **Figure 3.B** (see Task III for more details).

The tabular comparison of responses to moderate drought observed in the investigated wheat and maize genotypes can be seen on **Figure 3A, B.** The most important differences between C3 wheat and C4 maize plants was, that nitrate reduction was facilitated by drought rather in wheat than maize, and oppositely, glutamine synthesis was facilitated in maize and not in wheat cultivars. The photosynthetic performance and was not significantly confirmed in all wheat cultivars. In maize, the CO_2 assimilation and actual quantum yield and non-photochemical quenching processes were clearly facilitated by drought under normal light, which illumination state is more injurious for plant life in the cold, than low light. Thus, our induced drought acclimation treatment was more effective for maize, than wheat.

Overall, our results confirmed that moderate drought may help to retrieve the loss of assimilation at low temperature in wheat and maize. Differences in the level of metabolites responsible for stress-defence and needed by sugar and amino acid assimilation were found, however, they may have not direct role on the differences observed at phenotype level. Nevertheless, we believe, that the physiological state of plants caused by moderate drought is not the same at optimum and low temperature. In other words, a controlled, moderate water stress contributes to maintain assimilation and development both of wheat and maize plants during undesirable, low temperature conditions, contrary to the physiologically optimum temperature, where drought may cause deceleration of metabolism.

RELATED PUBLICATIONS:

Khalil, R ; Tajti, J ; Hamow, KÁ ; Gondor, KO ; Darko, E ; Elsayed, N ; Nagy, Z ; Szalai, G ; Janda, T ; Majláth, I. How does moderate drought affect quantum yield and the regulation of sugar metabolism at low temperature in durum wheat (*Triticum durum* L.)? **PHOTOSYNTHETICA** 59(2):313-326 (2021), DOI: 10.32615/ps.2021.030

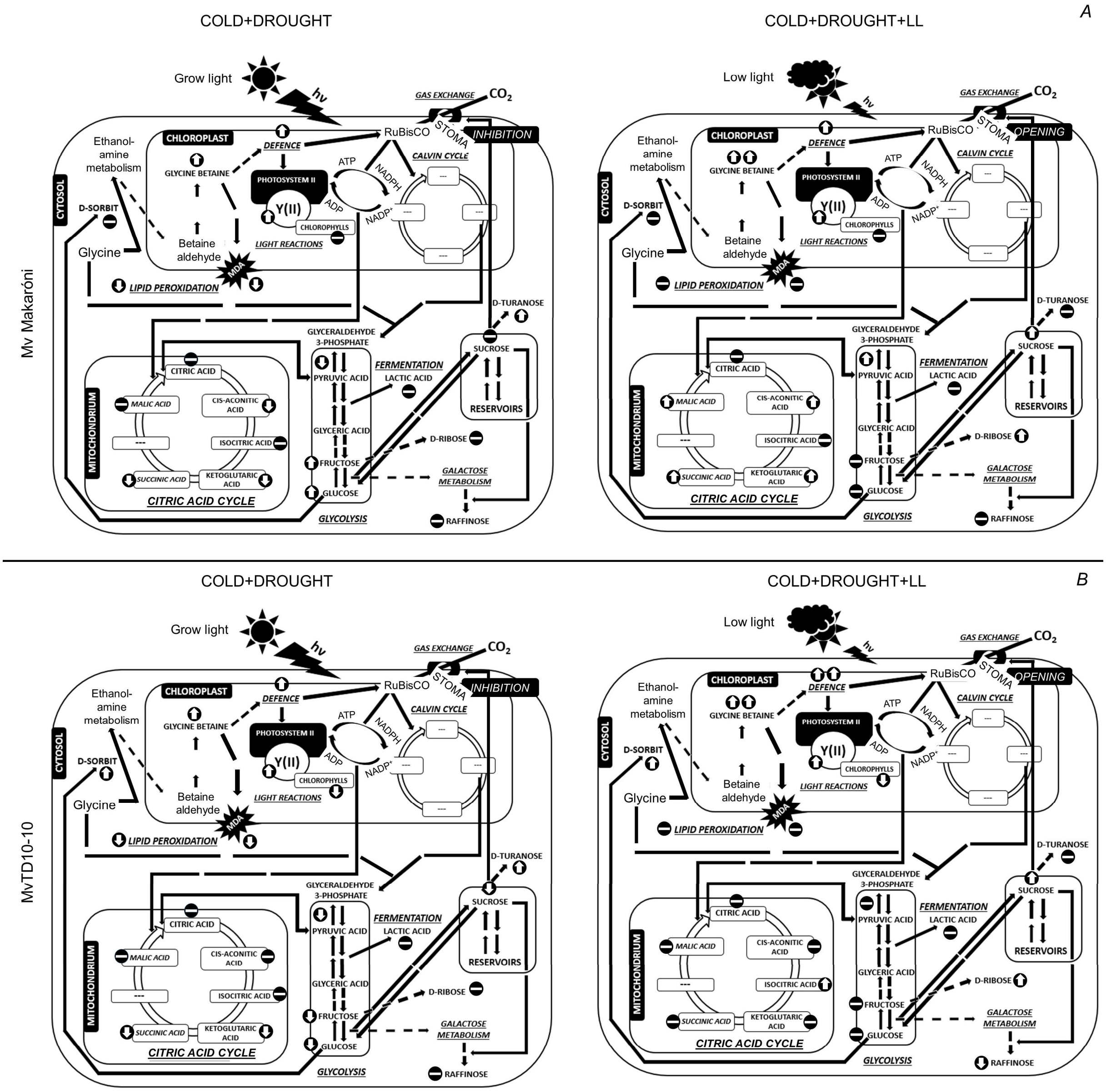
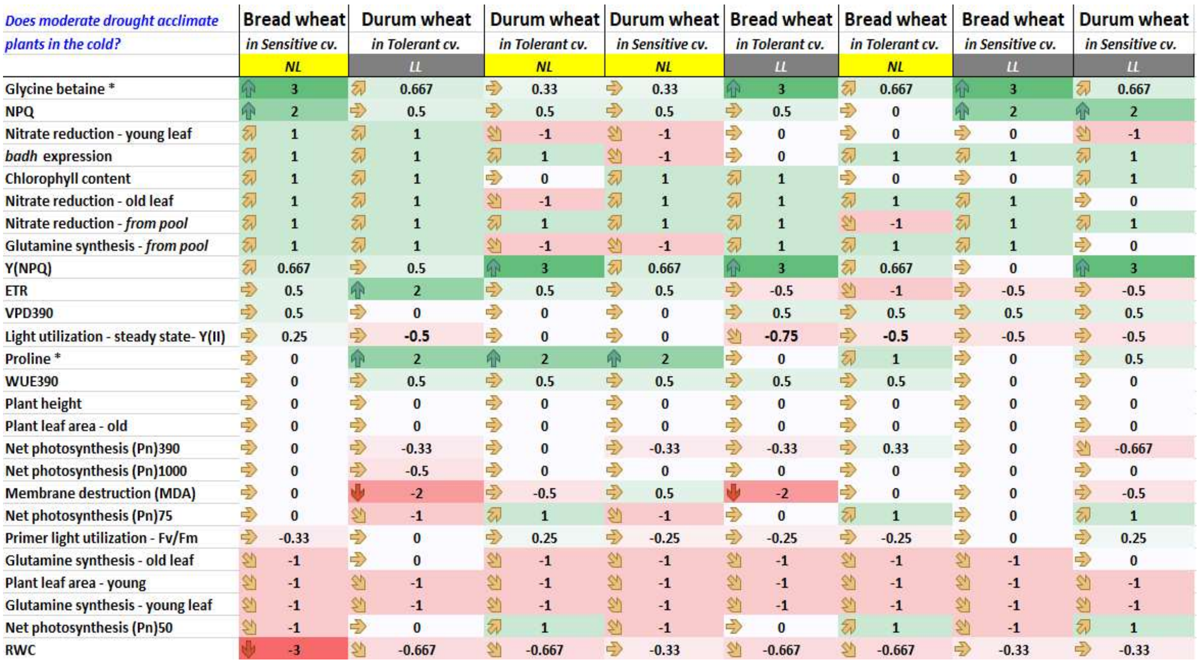


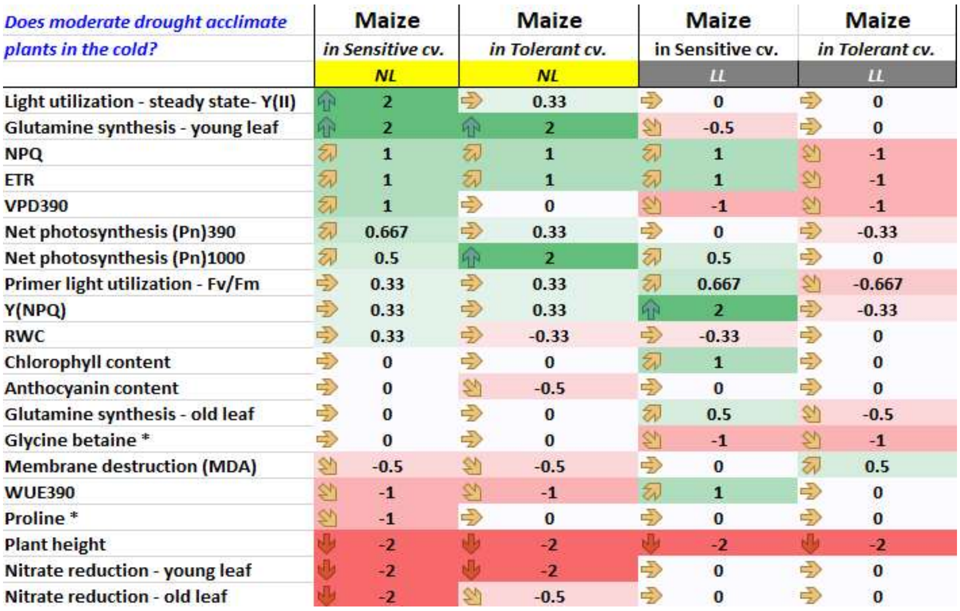
Figure 2. Schematic overview represents the role of moderate drought acclimation on the photosynthetic and sugar metabolic processes at subotimum temperature in the (**A**) cold-tolerant Mv Makaróni and (**B**) cold-sensitive MvTD10-10 durum wheat cultivars. Comparison were made between COLD+NORMALLY IRRIGATED *vs.* COLD+DROUGHT and COLD+LOW LIGHT *vs.* COLD+DROUGHT+LOW LIGHT and all significant changes ($p \leq 0.05$) were indicated by upward and downward arrows which indicate activation and inhibition, respectively. Symbol '-' means compounds did not change and '---' means compounds were not investigated [see Khalil et al. (2021) for more details].

A



Does moderate drought acclimate plants in the cold?	Bread wheat	Durum wheat	Durum wheat	Durum wheat	Bread wheat	Bread wheat	Bread wheat	Durum wheat
	in Sensitive cv.	*in Tolerant cv.*	*in Tolerant cv.*	*in Sensitive cv.*	*in Tolerant cv.*	*in Tolerant cv.*	*in Sensitive cv.*	*in Sensitive cv.*
	NL	*LL*	*NL*	*NL*	*LL*	*NL*	*LL*	*LL*
Glycine betaine *	↑ 3	↗ 0.667	→ 0.33	→ 0.33	↑ 3	↗ 0.667	↑ 3	↗ 0.667
NPQ	↑ 2	→ 0.5	→ 0.5	→ 0.5	→ 0.5	→ 0	↑ 2	↑ 2
Nitrate reduction - young leaf	↗ 1	↗ 1	↘ -1	↘ -1	→ 0	→ 0	→ 0	↘ -1
badh expression	↗ 1	↗ 1	↗ 1	↘ -1	→ 0	↗ 1	↗ 1	↗ 1
Chlorophyll content	↗ 1	↗ 1	→ 0	↗ 1	↗ 1	→ 0	→ 0	↗ 1
Nitrate reduction - old leaf	↗ 1	↗ 1	↘ -1	↗ 1	↗ 1	↗ 1	↗ 1	→ 0
Nitrate reduction - *from pool*	↗ 1	↗ 1	↗ 1	↗ 1	↗ 1	↘ -1	↗ 1	↗ 1
Glutamine synthesis - *from pool*	↗ 1	↗ 1	↘ -1	↘ -1	↗ 1	↗ 1	↗ 1	→ 0
Y(NPQ)	↗ 0.667	→ 0.5	↑ 3	↗ 0.667	↑ 3	↗ 0.667	→ 0	↑ 3
ETR	→ 0.5	↑ 2	→ 0.5	→ 0.5	→ -0.5	↘ -1	→ -0.5	→ -0.5
VPD390	→ 0.5	→ 0	→ 0	→ 0	→ 0.5	→ 0.5	→ 0.5	→ 0.5
Light utilization - steady state- Y(II)	→ 0.25	→ -0.5	→ 0	→ 0	↘ -0.75	→ -0.5	→ -0.5	→ -0.5
Proline *	→ 0	↑ 2	↑ 2	↑ 2	→ 0	↗ 1	→ 0	→ 0.5
WUE390	→ 0	→ 0.5	→ 0.5	→ 0.5	→ 0.5	→ 0.5	→ 0	→ 0
Plant height	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0
Plant leaf area - old	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0
Net photosynthesis (Pn)390	→ 0	→ -0.33	→ 0	→ -0.33	→ -0.33	→ 0.33	→ 0	↘ -0.667
Net photosynthesis (Pn)1000	→ 0	→ -0.5	→ 0	→ 0	→ 0	→ 0	→ 0	→ 0
Membrane destruction (MDA)	→ 0	↓ -2	→ -0.5	→ 0.5	↓ -2	→ 0	→ 0	→ -0.5
Net photosynthesis (Pn)75	→ 0	↘ -1	↗ 1	↘ -1	→ 0	↗ 1	→ 0	↗ 1
Primer light utilization - Fv/Fm	→ -0.33	→ 0	→ 0.25	→ -0.25	→ -0.25	→ -0.25	→ 0	→ 0.25
Glutamine synthesis - old leaf	↘ -1	→ 0	↘ -1	↘ -1	↘ -1	↘ -1	↘ -1	→ 0
Plant leaf area - young	↘ -1	↘ -1	↘ -1	↘ -1	↘ -1	↘ -1	↘ -1	↘ -1
Glutamine synthesis - young leaf	↘ -1	↘ -1	↘ -1	↘ -1	↘ -1	↘ -1	↘ -1	↘ -1
Net photosynthesis (Pn)50	↘ -1	→ 0	↗ 1	↘ -1	→ 0	↗ 1	↘ -1	↗ 1
RWC	↓ -3	↘ -0.667	↘ -0.667	→ -0.33	↘ -0.667	↘ -0.667	→ -0.33	→ -0.33

B



Does moderate drought acclimate plants in the cold?	Maize	Maize	Maize	Maize
	in Sensitive cv.	*in Tolerant cv.*	in Sensitive cv.	*in Tolerant cv.*
	NL	*NL*	*LL*	*LL*
Light utilization - steady state- Y(II)	↑ 2	→ 0.33	→ 0	→ 0
Glutamine synthesis - young leaf	↑ 2	↑ 2	↘ -0.5	→ 0
NPQ	↗ 1	↗ 1	↗ 1	↘ -1
ETR	↗ 1	↗ 1	↗ 1	↘ -1
VPD390	↗ 1	→ 0	↘ -1	↘ -1
Net photosynthesis (Pn)390	↗ 0.667	→ 0.33	→ 0	→ -0.33
Net photosynthesis (Pn)1000	↗ 0.5	↑ 2	↗ 0.5	→ 0
Primer light utilization - Fv/Fm	→ 0.33	→ 0.33	↗ 0.667	↘ -0.667
Y(NPQ)	→ 0.33	→ 0.33	↑ 2	→ -0.33
RWC	→ 0.33	→ -0.33	→ -0.33	→ 0
Chlorophyll content	→ 0	→ 0	↗ 1	→ 0
Anthocyanin content	→ 0	↘ -0.5	→ 0	→ 0
Glutamine synthesis - old leaf	→ 0	→ 0	↗ 0.5	↘ -0.5
Glycine betaine *	→ 0	→ 0	↘ -1	↘ -1
Membrane destruction (MDA)	↘ -0.5	↘ -0.5	→ 0	↗ 0.5
WUE390	↘ -1	↘ -1	↗ 1	→ 0
Proline *	↘ -1	→ 0	→ 0	→ 0
Plant height	↓ -2	↓ -2	↓ -2	↓ -2
Nitrate reduction - young leaf	↓ -2	↓ -2	→ 0	→ 0
Nitrate reduction - old leaf	↓ -2	↘ -0.5	→ 0	→ 0

Figure 3. Summarising changes of biological variables under moderate drought at low temperature in wheat (**A**) and maize (**B**) genotypes. **NL**: normal light; **LL**: low light; positive rates and upward green arrows: strong elevation or increase; yellow arrows and white cells and zero: slight or no change; negative rates and downward red arrows: strong drop or decrease. + and – numeric values mean the ratio of positive response of moderate drought compared to the responses of all experiments (*not published figure*).

TASK II

Results of studying the effect of artificial reactive aldehyde response on the low temperature tolerance of wheat and maize plants

In the frame of this topic of the project, we aimed to reveal how defence response be induced by methylglyoxal (MG). We conclude that exogenous MG in appropriate concentration enhances the photosynthetic performance of wheat plants. The MG spray causes transient oxidative stress in the cell which triggers alarm state in the early developmental stage of wheat plants. This includes enzymatic scavenge mechanisms, maintains photosynthetic pigments, the synthesis of D-sorbitol and increase fatty acid desaturases which maintain membrane integrity. The priming effect of 10 mM MG prepares wheat to survive subzero temperature without prior cold-hardening. The MG-induced defence state enhances the frost-hardiness in the tolerant cultivars, but do not develop tolerance in the frost-sensitive plants. Eventually, MG spraying can be considered as a powerful and non-GMO tool to enhance crop survival under adverse temperature conditions (**Figure 4.**) [see in Majláth et al. (2020)].

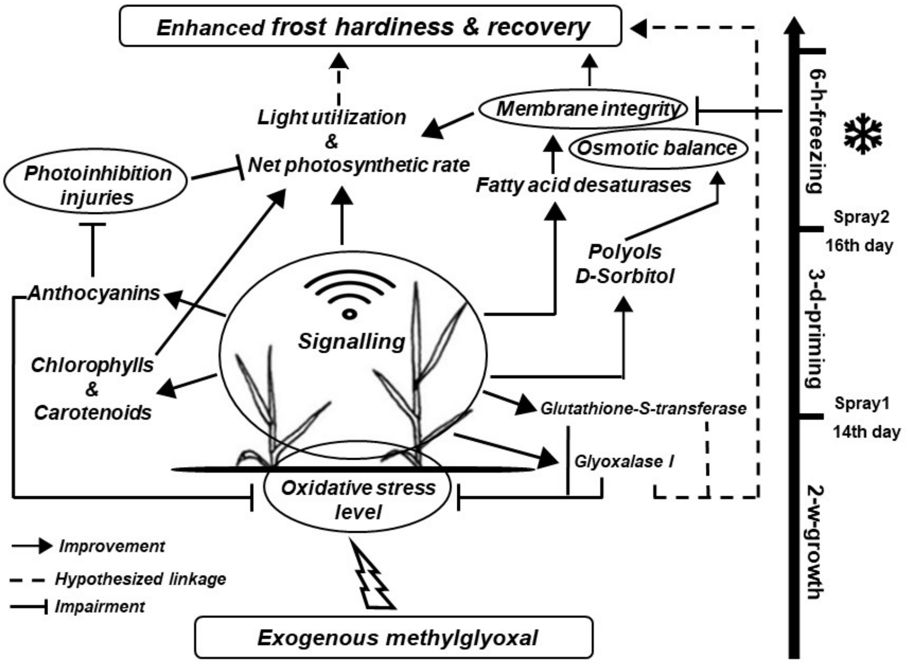


Figure 4. The effect of exogenous methylglyoxal on the frost tolerance of wheat [see Majláth et al. (2020) for more details].

In contrast to wheat, maize is more sensitive to cold injury, especially during germination. We have found promisful preliminary results on the germination and early growth in maize during chilling. Namely, germination of maize at 13°C is very limited and it fails in the case of most genotypes. We found, that soaking maize seeds in 10 mM (and maybe to 25 mM) MG significantly improved the germination and growth and photosynthesis rates during germination in the cold (**Figure 5.**) [see in Majláth et al. (2021)]. As we realized the novelty of our findings at this less-well-known field, we undertake genome sequencing and hormone and flavonoid profiling analyses to see, what happened at molecular level. These investigations indicated MG-induced changes in photosystem antenna proteins, pigments, late embryogenesis abundant proteins, abscisic acid catabolism, chaperons, members of the phenylpropanoid biosynthetic pathway. The MG-response of maize cultivars (A654 and Cm174) were somewhat different, but we recorded higher endogenous hydrogen peroxide (H_2O_2) and lower nitric oxide (NO) level in at least one of the treated genotypes. These ROS signals initiated changes in the hormonal, metabolic and gene expression status. Decreased auxin transport, but increased abscisic acid degradation and cytokinin and jasmonic acid synthesis, as well as an altered carbohydrate metabolism and transport could have promoted germination of MG-pretreated seeds. While LEA accumulation could have protected against osmotic stress and catalase expression and production of many antioxidants, like para-hydroxybenzoic acid and anthocyanins may have balanced the oxidative environment for maize germination. Our results showed that MG priming could be an effective way to promote cold germination. The facilitation was more pronounced in the less-tolerant genotype.

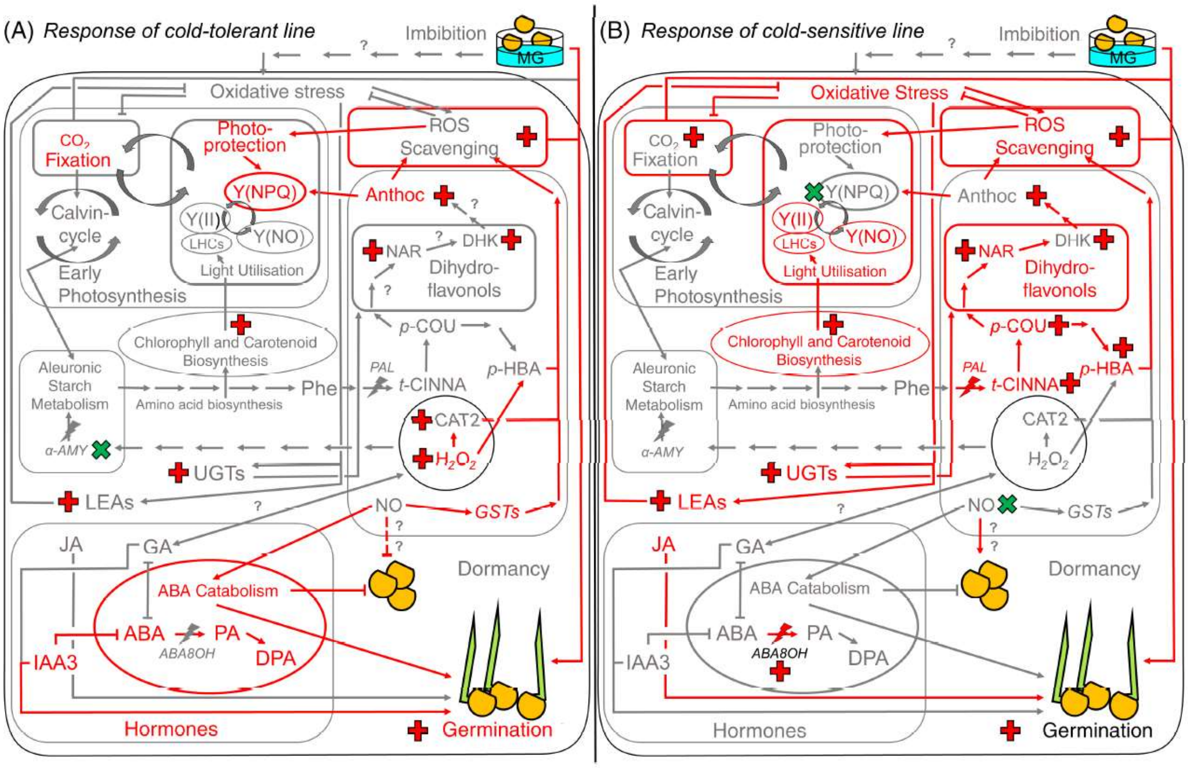


Figure 5. Overview of the significantly activated physiological processes (red lines) under MG pretreatment during the cold germination of (**A**) cold-tolerant A654 and (**B**) cold-sensitive Cm174 maize varieties. Terms in italics mean variables which were confirmed as differentially expressed gene only. Terms with red font show higher abundance in the given genotype. Cross symbols indicate the MG-induced activation/accumulation (red) or inhibition/loss (green) of a biological variable. Flash marks mean enzyme catalysis. List of abbreviations: α-amy: α-Amylase, ABA8OH: Abscisic acid 8'-hydroxylases, ANTHOC: total anthocyanins, CAT2: Catalase isoenzyme 2, DHK: Dihydrokaempferol, DPA: Dihydrophaseic acid, GST: Glutathione-S-transferase, JA: Jasmonic acid, LEA: Late Embryogenesis Abundant Protein, LHC: Light harvesting complex, NAR: Naringenin, PA: Phaseic acid, PAL: Phenylalanine ammonia lyase, Phe: Phenylalanine, UGT: UDP-Glycosyltransferase (see more in Majláth et al., 2021).

Overall, our researches derived from TASK II confirmed the mechanisms of the reactive aldehyde (i.e. MG)-induced defence, which adequately acted against mild freezing and chilling temperatures. The physiological background of these phenomena was clarified in both wheat and maize. We have proven that exogenous MG treatment can be safely added to wheat plants as preparatory treatment without detrimental effects but inducing some of the stress-protective mechanisms, which contribute to frost-hardiness.

Additionally, an MSc thesis was written from Topic II by Altafur Rahman (SZIE, Agricultural Biotechnology, supported by the Stipendium Hungaricum Grant), entitled 'THE EFFECT OF THE

REACTIVE ALDEHYDE METHYLGLYOXAL ON THE EARLY DEVELOPMENT OF MAIZE UNDER CHILLING' and defended in 2020 (marked for good) (**Figure 6**). He also presented some results on a poster entitled 'METHYLGLYOXAL STIMULATES STRESS SIGNALING AND GERMINATION OF MAIZE AT SUBOPTIMUM TEMPERATURE' (authors: Altafur Rahman, Imre Majláth and Magda Pál). It was presented online on the 7th International Istanbul Scientific Research Congress of in 18-19., December, 2021 (https://en.internationalistanbulcongress.com/). The current NRDI grant number was displayed on the poster.

SZENT ISTVÁN
EGYETEM

SZENT ISTVÁN UNIVERSITY
FACULTY OF HORTICULTURAL SCIENCE
BUDAPEST

ATK

THE EFFECT OF THE REACTIVE ALDEHYDE METHYLGLYOXAL ON THE EARLY DEVELOPMENT OF MAIZE UNDER CHILLING

Student: **Altafur Rahman**

Supervisor: **Dr. Imre Majláth (ATK MGI)**
Co-Supervisor, **Dr. Csaba Éva (ATK MGI)**
Supervisor from the university: **Prof. Attila Hegedűs**
Department of Genetics and Plant Breeding, Szent István University,

Figure 6. The title slide of Altafur Rahman's MSc Thesis which was successfully defended in June, 2020 and based on the topic of Task II of this research project.

RELATED PUBLICATIONS:

Majláth, I ; Éva, Cs ; Tajti, J ; Khalil, R ; Elsayed, N ; Darko, E ; Szalai, G ; Janda, T. Exogenous methylglyoxal enhances the reactive aldehyde detoxification capability and frost-hardiness of wheat. **PLANT PHYSIOLOGY AND BIOCHEMISTRY** 149:75-85 (2020), DOI: 10.1016/j.plaphy.2020.02.003

Majláth, I ; Éva, Cs ; Hamow, KÁ ; Kun, J ; Pál, M ; Rahman, A ; Palla, B ; Nagy, Z ; Gyenesei, A ; Szalai, G ; Janda, T. Methylglyoxal induces stress signaling and promotes the germination of maize at low temperature. **PHYSIOLOGIA PLANTARUM**, e13609 (2022), DOI: 10.1111/ppl.13609

TASK III

Results of studying the effect of moderate drought on the nitrogen assimilation of maize

Related to the main topic of Task I, our main questions was that, how moderate drought affect the amino acid assimilation in the cold in maize. We highlighted to study the relationship between the changes of glutamine biosynthesis, amino acid and sugar levels in maize. Two maize cultivars, A654 and Cm174, were used for the experiments. Maize cultivar pair has difference in cold-tolerance. The experiment schedule was similar to the wheat experiments, which can be seen on **Figure 1**. Although, some modifications of settings were made for growth (25°C) and hardening (10°C) temperatures, as well as in light intensities: normal (450 ± 20 μE PFD) and low (150 ± 30 μE PFD) light. Besides the normally irrigated population, the mild water stress was conducted by controlled watering and inspected daily by soil-moisture sensors.

Moderate drought improved the net photosynthesis rate both at low and normal and elevated atmospheric CO_2 concentrations. Regarding light utilization rate of photosynthesis, the whole-quencing kinetics of actual photosystem II quantum yield showed, that maize plant were able to utilize absorbed light under normal (stronger) light than low light in a better manner during moderate drought and cold conditions. It is important to emphasize, that positive effect of mild drought observed under normal light has great of importance, because normal light and irrigation at low temperature stronger injures cellular metabolism due to the greater ROS accumulation.

Our results confirmed that moderate drought has efficiently mitigated the noxiuos effects of chilling temperature in maize. The activity of the key enzyme of mnitrogen assimilation, glutamine synthetase which basically considered to be drought sensitive, was less-affected or slightly increased. The effective quantum yield has increased at both genotypes under mild drought. The accumulation in anthocyanins was decelerated at NL and did not changed at LL. This may reflected that photoinhibition was mitigated or compensated via other defence routes. The level of carbon dioxide fixation under drought was almost four-fold in A654 and two-fold in Cm174 at NL than LL, and two-fold higher in A654 than Cm174. The multivariate comparison showed that distinction of the polar metabolite profiles was more pronounced in Cm174 under drought. In other words, the response of the cold sensitive variety was more pronounced. Malic acid, alphaketo glutaric acid and glutamic acid are intermediers between nitrogen fixation and sugar metabolism. The level of these organic acids, the glucose degradation and the asparagine and alanine biosynthesis routes were activated under drought and NL in A654. In Cm174, oppositely, the accumulation of glucose was manifold higher. GABA levels clearly indicated, that LL and mild drought helped to maintain GABA level which, may have contributed to the integrity of sugar metabolism under cold. The most of the amino acid assimilation processes, however, were repressed. Proline, as an important osmolyte, has not significantly changed. Nevertheless, proline level may have indicated that drought pretreatment was not severe. The level of glycine betaine, however, mostly decreased in a correlation to the general drop in glycine concentrations. Finally, LL has down-regulated more biosynthetic processes in A654, than in Cm174.

The major changes observed in the investigated physiological and biochemical variables were summarized and they can be seen on **Figure 7**. which is the summary figure of the related paper, since we have prepared and submitted a manuscript (with six figures) to the Journal of Plant Biology (Springer) on 22nd April, 2022. The study is under review now.

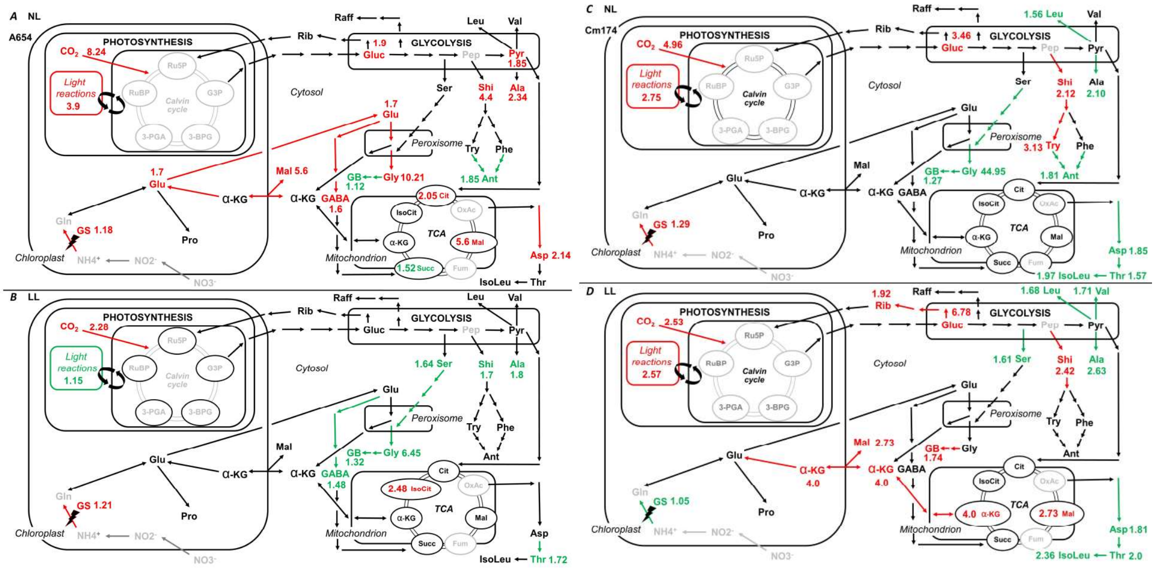


Figure 7. Overview of changes in photosynthesis, carbohydrate catabolism and biosynthesis of amino acids in the A654 cold-tolerant maize variety under normal light (NL) (A) and low light (LL) (B) and Cm174 cold-sensitive maize variety under normal light (NL) (C) and low light (LL) (D). Black font means metabolites were quantitatively determined, grey were not determined, red increase and green decrease. Numbers mean fold-change (FC) of metabolites when drought pretreated plants were compared to irrigated plants [(COLD+DROUGHT vs. COLD and COLD+DROUGHT+LL vs. COLD+LL (FC threshold > |1.5| except for GS activity, where less then |1.5| was allowed)]. Dark flash symbol means enzym catalysis. Abbreviations for this figure: Ala: L-alanine, Ant: anthocyanins, Asp: L-aspartic acid, Cit: citric acid, Fum: fumaric acid, GABA: γ-aminobutyric acid, GB: glycine betaine, Gln: glutamine, GS: glutamine synthetase, Glu: glutamic acid, Gluc: glucose, Gly: glycine, G3P: 3-phosphoglyceric acid, IsoCit: isocitric acid, IsoLeu: L-isoleucine, Leu: L-leucine, Mal: malic acid, OxAc: oxaloacetic acid, Pep: phosphoenolpyruvic acid, Phe: L- phenylalanine, Pyr: pyruvic acid, Pro: L-proline, Raff: raffinose, Rib: D-ribose, RuBP: ribulose 1,5-bisphosphate, Ru5P: ribulose 5-phosphate, Ser: L-serine, Shi: shikimic acid, Succ: succinic acid, Thr: L-threonine, Try: L-tryptophan, Val: Valine, 3-BPG: 1,3-bisphosphoglyceric acid, 3-PGA: glyceraldehyde 3-phosphate, α-KG: ketoglutaric acid

RELATED PUBLICATIONS:

Kamirán Á. Hamow, Edit Németh, Orsolya K. Gondor, Csaba Éva, Krisztina Balla, Magda Pál, Tibor Janda, Imre Majláth. DROUGHT IS A LESSER EVIL THAN COLD FOR PHOTOSYNTHESIS AND AMINO ACID METABOLISM OF MAIZE. **JOURNAL OF PLANT BIOLOGY**, manuscript number: JOPB-D-22-00107 – ***under review***

Minor changes of the project and their justification

1) The planned proteomic investigations for enzymes (the question is How was happened?) of nitrogen assimilation (nitrate reductase, glutamine synthetase) and invertase did not get promisful results. After few attempts, we started to focus on the quantitative changes of the end products (the question is How many/much was accumulated?), amino acids, using instrumental analitical chemistry procedures. The knowledge of sugars and amino acid helped us to establish more precise biochemical routes. Additionally, the catalytic activity two key nitrogen assimilation enzymes, nitrate reductase and glutamine synthetase, were measured in at least three independent experiments in all wheat and maize genotypes. Changes of these enzyme activities were summarized on **Figure 3**.

2) The osmolyte compound N, N, N-trimethyl-glycine, i.e. glycine betaine (GB) has versatile roles in plant metabolism. GB content was determined in more than three experiments. In contrast to our early preliminary results, GB accumulation was found to be very contradictionary and its role on the defence against cold under various combinations of light and water status is questionable. Regarding this fact, we have decided, that we do not to perfom deeper analyses for the GB metabolism.

3) The induction of systemic reactive aldehyde response was done using methylglyoxal instead of the originally planned glutaraldehyde. We have made this change based on the fact, that methylglyoxal naturally occuers in plant cells and the effect of exogenously added methylglyoxal was less-well known on plant cold-tolerance at the start of the project (as compared to a glutaraldehyde). We guessed more novelty for expected results which then was proven by regarding our two published papers in this topic.