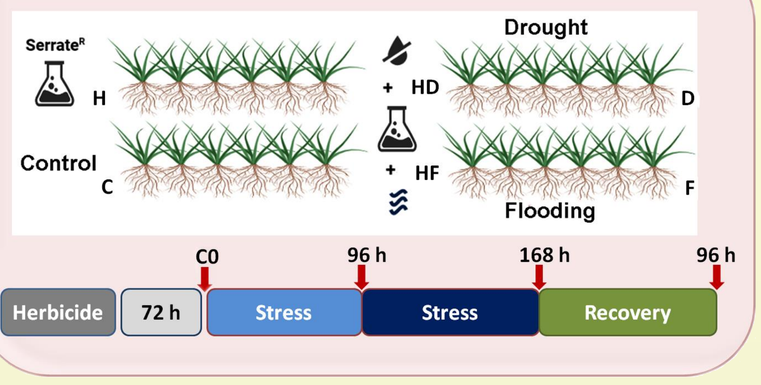
Winter wheat and triticale exert differential cross-adaptation towards drought and flooding after pretreatment with a selective herbicide

Irina I. Vaseva, Ana Blagoeva, Margarita Petrakova, Dessislava Todorova

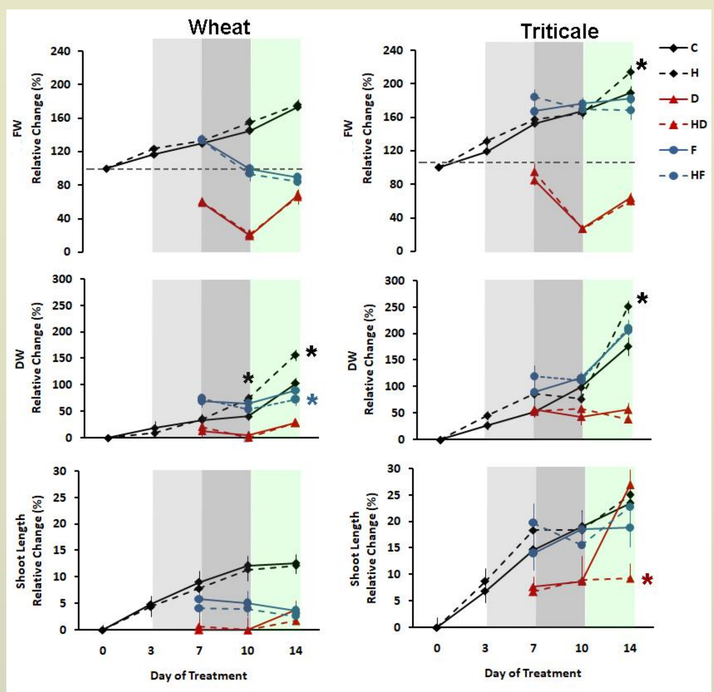
Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

Abstract: The widely used agrochemicals that do not exert negative effects on crops and target selectively weeds could influence plant resilience towards unfavorable conditions. The cross-adaptation of wheat (*Triticum aestivum* L.) and triticale (× *Triticosecale* Wittm.) exposed to drought and flooding was evaluated after treatment with a selective herbicide (Serrate®, Syngenta). Biometrical measurements and histochemical staining were employed for the comparative analyses of stressed and recovered plants. The ambivalent effects of the herbicide on the two studied crops were particularly distinct in waterlogged plants with a significant reduction of wheat growth and a better performance of triticale individuals exposed to the same combined treatment. The comparative transcript profiling of ROS scavenging enzymes (superoxide dismutase, peroxidase, glutathione reductase, and catalase) of stressed and recovered plants revealed crop-specific variations resulting from the unfavorable water regimes in combination with herbicide treatment. The short-term dehydration was relatively well tolerated by the hybrid and this aligned with the considerable upregulation of the genes for L- Proline biosynthesis. Its drought resilience was diminished by herbicide application as evidenced by increased ROS accumulation after prolonged water deprivation. The obtained data suggest that commonly used weed-managing products could be beneficial for plant resilience under certain conditions but their use may also have negative consequences depending on the crop and stress type.

Experimental Design



Growth measurements



∗indicates statistically significant difference between the experimental groups grown under the same conditions with or without herbicide treatment ($p < 0.05$)

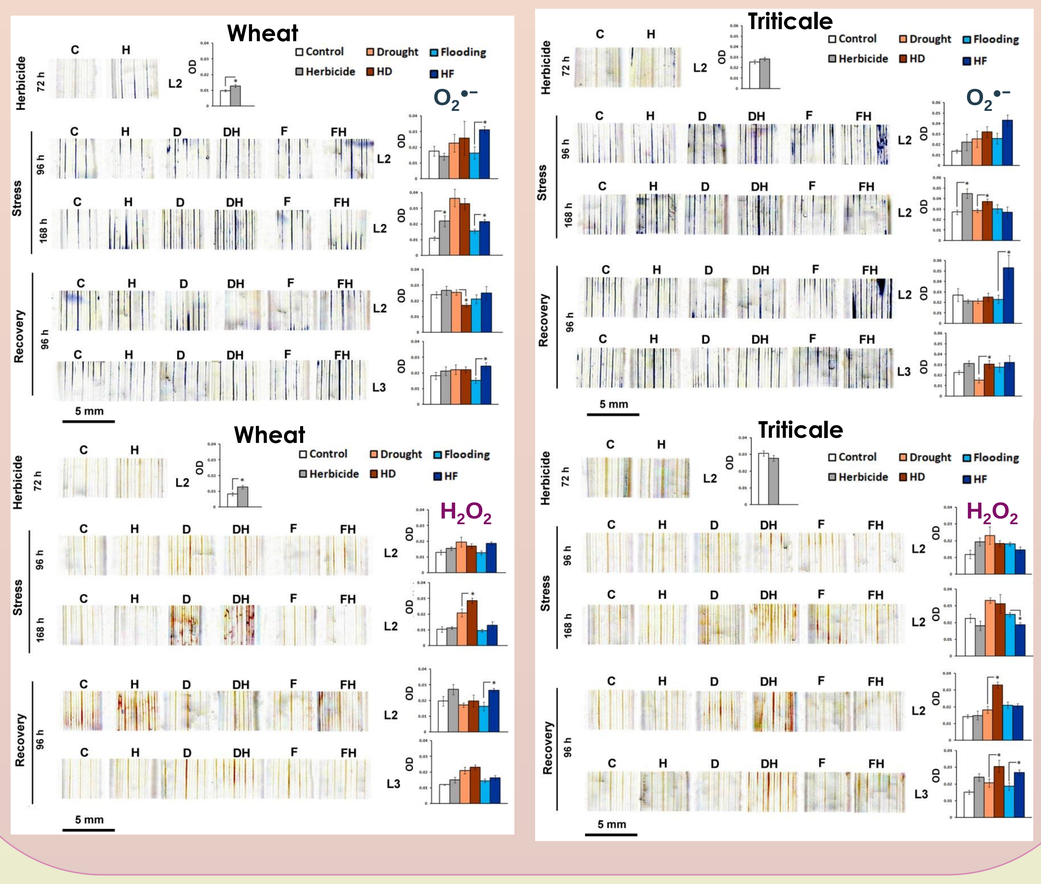
$O_2^{\bullet-}$

- Standard herbicide dose provokes transiently higher $O_2^{\bullet-}$ only in wheat.
- $O_2^{\bullet-}$ accumulation in drought-stressed wheat accelerates with the progress of water limitation.
- Slightly higher $O_2^{\bullet-}$ is detected in triticale which is subjected to the combined effects of herbicide and drought.
- Herbicide application increases the accumulation of superoxide anion in flooded wheat and the effect persists in the younger leaves (L3) of the recovered individuals.
- Triticale F and HF groups do not exhibit elevated $O_2^{\bullet-}$ which corresponds to their better physiological status compared to the flooded wheat plants. The accelerated senescence of triticale L2 coincides with the higher $O_2^{\bullet-}$ levels detected therein.

H_2O_2

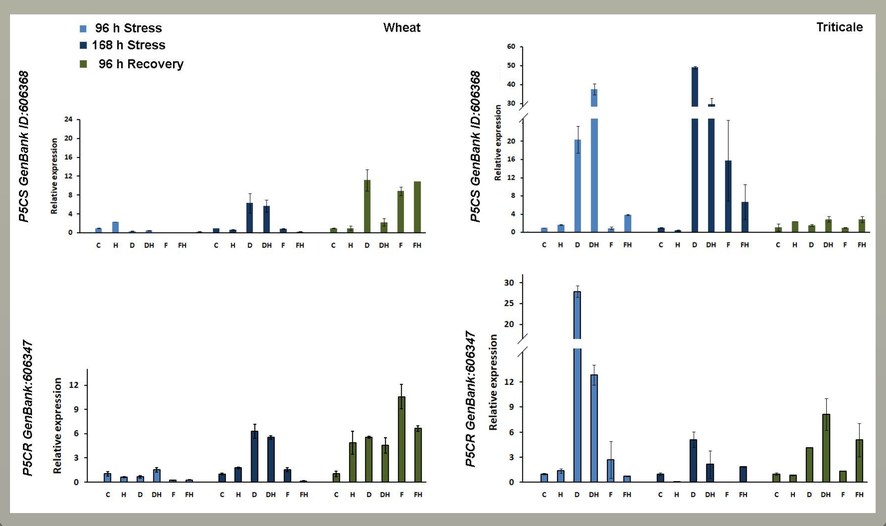
- Standard herbicide dose provokes transiently higher H_2O_2 content only in wheat.
- Higher hydrogen peroxide levels are detected both in wheat and triticale leaves after prolonged dehydration.
- Herbicide treatment resulted in additionally increased H_2O_2 content only in *T. aestivum* individuals subjected to extended water limiting conditions.
- After recovery the levels of hydrogen peroxide in triticale HD samples derived from the older (L2) and the last fully expanded younger leaves (L3) remained higher than the controls reflecting the enhanced stress effects of the combined treatment.
- H_2O_2 levels in both crops are less affected during waterlogging.
- Elevated H_2O_2 in L2 (wheat) and L3 (triticale) have been detected after recovery.

Histochemical staining of ROS



- Wheat and triticale plants that received the recommended Serrate® dose and were grown under optimal conditions showed statistically significant increase of dry weight (DW). This phenomenon has been previously documented in other crops.
- Overall, the measured growth parameters of triticale plants (FW, DW and shoot length) demonstrate its relatively better performance at unfavorable water regimes (both excessive or limited water availability).
- The application of the selective herbicide diminish the ability of drought-stressed triticale to recover its elongation growth upon recovery but does not show an additional negative effect on the flooded individuals.
- Flooded wheat plants that received herbicide dose (HF) accumulated less dry matter compared to the F experimental group.

L- Proline biosynthetic pathway – gene expression

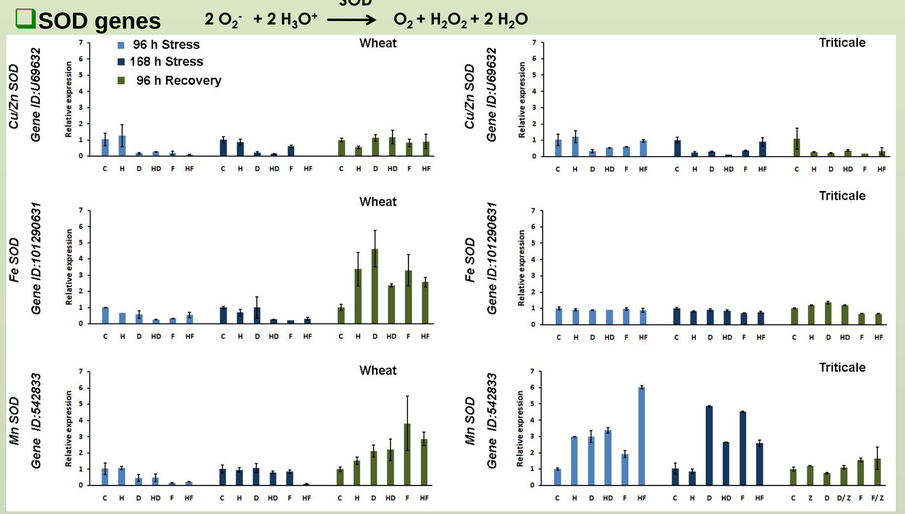


- *P5CS1* – encodes rate limiting enzyme delta-1-pyrroline-5-carboxylate synthase (two catalytic domains EC: 2.7.2.11 ad EC: 1.2.1.41) .
- *P5CR* – encodes pyrroline-5-carboxylate reductase operating at the final step of L-Pro biosynthesis (EC 1.5.1.2).
- Drought-stressed triticale (D and HD) has considerably higher *P5CS* and *P5CR* content already at 96 h of stress.
- The treatment with standard dose herbicide did not affect significantly the levels of *P5CS* and *P5CR* expression in drought-stressed wheat while at 168 h of stress period reduced transcript levels of both genes in HD triticale samples were registered .
- *P5CS* gene was upregulated in the leaves of waterlogged triticale unlike the low levels of its expression registered in the same treatment group of wheat plants (W and HW).
- *P5CS* transcripts in all triticale samples recovering from drought flooding are close to the control . In contrast, wheat plants still exhibited high expression of *P5CS* gene except in the HD group.
- During the recovery period, *P5CR* gene remained upregulated in most wheat and triticale samples.

ROS scavenging system – gene expression

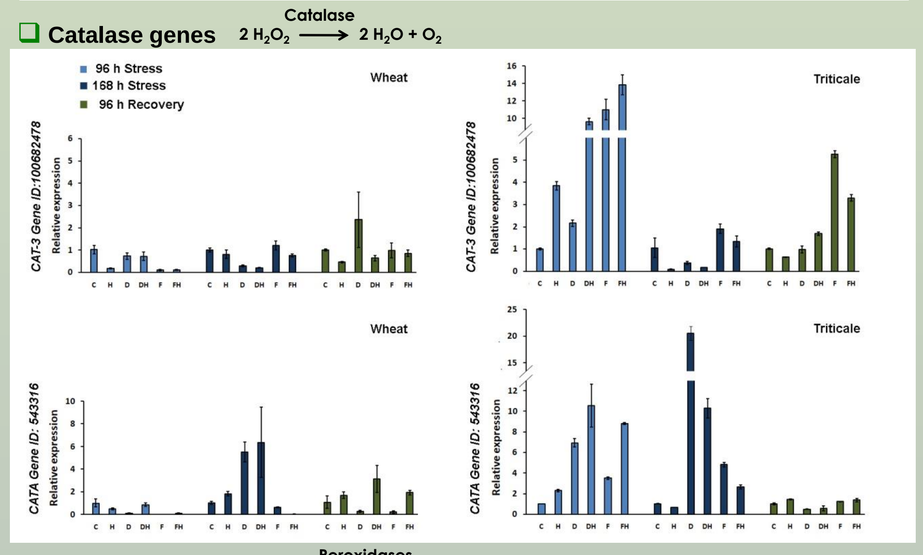
❑ SOD genes

$$2\,O_2^{-} + 2\,H_3O^{+} \xrightarrow{\text{SOD}} O_2 + H_2O_2 + 2\,H_2O$$

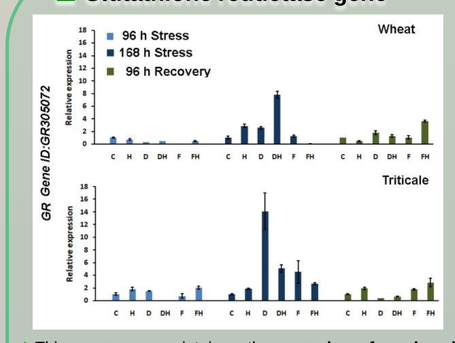


❑ Catalase genes

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$



❑ Glutathione reductase gene



- This enzyme maintains the **supply of reduced glutathione – a key player in the cellular control of ROS**.
- The hybrid crop triticale accumulates considerably higher transcript amounts under drought.
- The pre-stress herbicide treatment had divergent effect on *GR* expression levels in dehydrated wheat and triticale which is apparent at 168 h of drought: wheat HD group has higher GR transcript level than D group; the opposite is observed in the respective triticale individuals.
- The transcript abundance in flooded triticale (F) exhibited a moderate increase and remained relatively stable in HF-treated plants unlike the trend in the same experimental group of wheat where GR expression was strongly inhibited.

❑ SOD genes

$$2\,O_2^{-} + 2\,H_3O^{+} \xrightarrow{\text{SOD}} O_2 + H_2O_2 + 2\,H_2O$$

- **MnSOD** functions in the mitochondrial matrix and peroxisomes; higher transcript abundance is detected in triticale during the stress period.
- **Fe-SOD** isoforms operate mostly in chloroplasts; the hybrid exhibits stable transcript levels in all experimental groups while in wheat the gene expression was downregulated in HD, W, and HW samples.
- **Cu/Zn-SOD** is active in cytoplasm; the differences in *Cu/Zn-SOD* gene expression profiles of wheat and triticale samples are not as distinct as the ones identified for the other two SOD genes except the observed strong inhibition in HW wheat group.

❑ Catalase genes

$$2\,H_2O_2 \xrightarrow{\text{Catalase}} 2\,H_2O + O_2$$

- The abundance of catalase transcripts (***CAT-3*** and ***CATA***) in the leaves of triticale subjected to drought and flooding is considerably higher than the one measured in wheat suggesting elevated synthesis of the enzyme for timely detoxification of the excessive amounts of hydrogen peroxide.
- Catalase gene expression is stimulated in the control triticale plants which received standard herbicide dose (H) with particularly distinct upregulation of *CAT-3* transcripts .

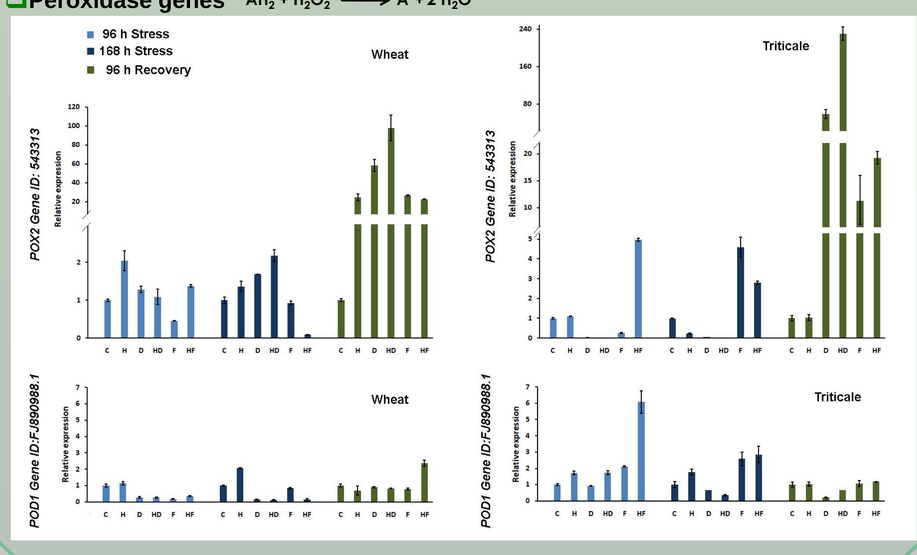
❑ Peroxidase genes

$$AH_2 + H_2O_2 \xrightarrow{\text{Peroxidases}} A + 2\,H_2O$$

- The expression of **ascorbate peroxidase (*POD1*)** and **guaiacol peroxidase genes (*POX2*)** are evaluated.
- A sustained higher abundance of *POD1* and *POX2* transcripts is measured in flooded triticale plants coinciding with the relatively better performance of the hybrid under this type of stress.
- *POX2* transcripts have stable levels in wheat D and HD plants while they are undetectable in the respective triticale samples .
- Very strong induction of *POX2* expression after recovery is registered in the samples derived from both crops with herbicide treatment acting as a synergistic element in the upregulation of this gene.

❑ Peroxidase genes

$$AH_2 + H_2O_2 \xrightarrow{\text{Peroxidases}} A + 2\,H_2O$$



Conclusions

Commonly applied weed-managing products could be beneficial for improved plant performance under certain unfavorable conditions but their use may have also negative consequences that depend on the crop and stress type. We identified some elements from the antioxidant defense system that might be related to the divergent capacity of winter wheat and the hybrid crop triticale to tolerate adverse water supply after the application of a conventional dose of a selective herbicide. Flooded triticale showed considerably higher expression of catalase and peroxidase coding genes compared to wheat which correlates to its better performance under excessive water regime. The hybrid crop had more stable transcript levels of glutathione reductase gene which could also contribute to its better capacity to detoxify ROS through glutathione defense system. We conclude that the administration of selective herbicides on winter wheat fields that are prone to flooding should be considered after carefully evaluating the risks and benefits as the herbicide treatment could enhance the negative consequences of excessive water stress. In contrast, the hybrid crop triticale tolerates the combination of herbicide application with subsequent flooding events but its drought resilience seemed to be negatively influenced by the treatment with the tested agrochemical product.

Acknowledgement: This research was funded by THE BULGARIAN NATIONAL SCIENCE FUND, grant number KP-06-H36/3-30.09.2020.