

# Biochemical and molecular markers for genotype's susceptibility to oxidative stress

Tsveta Vladimirova Angelova

Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences,  
2 Gagarin Street, 1113 Sofia, Bulgaria

PhD Thesis

Sofia, 2018

In the recent years drought and UV-B becoming more harmful to worldwide agro ecosystems. During the evolution plants have developed different adaptive mechanisms to cope with drought and UV-B irradiation. Some data illustrate that plants response to UV-B irradiation, or drought highly depends on genotype, plant tissue, developmental stage and/or interactions among environmental factors. The mechanisms of sensitivity/tolerance of crop plants to drought and UV-B are still not completely known. Therefore, clarification of these mechanisms is needed for their better understanding.

Generally, the main approach for assessment of stress response and adaptive potential of cells/organisms to abiotic stress includes mutant forms that differ in their sensitivity/resistance to oxidative stress, genotypes from contrasting habitats or transgenic plants. In the present work we propose another approach. Here we hypothesize that genetically closely related genotypes would have approximately similar stress response and adaptive potential to inducers of oxidative stress. To check our hypothesis a complex of biochemical/molecular markers were used.

The **aim** of the present study was to analyze and to compare: 1) biochemical/molecular response of three genetically closely related genotypes common bean (*Phaseolus vulgaris* L) to drought induced oxidative stress; 2) the adaptive potential of three genetically closely related genotypes, ten days after the removal of single and combined treatment with drought and UV-B. To elaborate a complex of biochemical and molecular markers for the evaluation of genotype's susceptibility to oxidative stress induced by single and combined drought and UV-B light. As a model system, three local genotypes of *P. vulgaris* L. were used: Dobrudjanski 2, Dobrudjanski 7 and Dobrudjanski ran. Previously, based on molecular analyses it was found that they are genetically closely related. Two experimental schemes were used: 1) PEG treatment on the third-leaf phase; 2) single and combined PEG and UV-B treatment at cotyledon phase. Several commonly used biochemical and molecular parameters as malondialdehyde (MDA), total peroxides (Pox), proline (Pro), reducing sugars (RS), photosynthetic pigments (total chlorophylls ( $a+b$ ), chlorophyll *a*, chlorophyll *b*, total carotenoids, chlorophyll  $a/b$  ratio), heat shock proteins (HSP70B), superoxide dismutase (SOD) and catalase (CAT) activity were assessed. Measurements were carried out immediately after the treatment and after ten days recovery time at physiological conditions.

One-way ANOVA with Tukey multiple comparison posttests, Two-way ANOVA with Bonferroni multiple comparison posttest (GraphPad Prism 6.04 software, San Diego, USA), ANOVA (EXCEL 2007) and Analysis ToolPak were applied to assess differences among samples. To identify the most reliable marker/s statistical data were summarized with Boolean algebra. Further possible combinations were analyzed using Boolean operators AND, OR, XOR. Our results indicate that drought simulated by polyethylene glycol (PEG) resulted in an increase of all tested parameters (MDA, Pox, Pro, RS, total chlorophylls ( $a+b$ ), chlorophyll *a*, chlorophyll *b*, total carotenoids, HSP70B). Pro and HSP70B were the only parameters strongly enhanced after PEG treatments depending on the genotype. There is no change in chlorophyll  $a/b$  ratio just after the treatment and after ten days recovery time. Different genotype response

was established concerning content of Pro, SOD activity, CAT activity and HSP70B, even ten days after single and combined treatments. On the other hand, genotypes response was similar (no effect of the genotype) ten days after the removal of single and combined, PEG and UV-B treatment measured as MDA, Pox, RS, total chlorophylls, chlorophyll *a*, chlorophyll *b* and total carotenoids. Despite the fact that genotypes are genetically closely related they differ in their adaptive potential and the main protective strategies (Pro, HSP70B, SOD and CAT activity). Pro accumulation probably is one of protective mechanisms for Dobrudjanski 7 and HSP70B for Dobrudjanski 2 and Dobrudjanski ran.

#### **Confirmatory conclusions:**

1. It was found that mild/moderate stress induced by both PEG concentrations of 8% and 16% does not cause serious membrane damage in investigated genotypes.
2. It was found that Pro and HSP70B are reliable markers for the evaluation of the magnitude of biochemical/molecular response to oxidative stress even in genetically closely related genotypes.
3. Simulated in lab conditions drought can increase the levels of Pox in three genotypes, but this biochemical parameter does not clearly distinguish the response of three closely related genotypes. This parameter would be informative only in cases when genotypes differ distinctly in their susceptibility to drought stress.
4. Overproduction of Pro and HSP70B could be used as reliable markers for assessment of adaptive potential even in genetically closely related genotypes.
5. It was established that genetically closely related genotypes used by us differ in their adaptive potential and protective mechanisms (Dobrudjanski 2- HSP70B, SOD activity; Dobrudjanski 7-Pro, SOD activity; Dobrudjanski ran-HSP70B, SOD and CAT activity).
6. The most pronounced stressful effect of UV-B was obtained in comparison with those of simulated drought stress using a complex of markers.
7. Supporting data were found that mild to moderate drought stress could increase the adaptive potential to another type of stress factor.
8. The role of the genotype was confirmed-the mild/moderate drought stress can provoke increased adaptive potential of genotypes involving different mechanisms-Dobrudjanski 2 and Dobrudjanski ran-overproduction of HSP70B; Dobrudjanski 7-overproduction of Pro.

#### **Original conclusions:**

1. Mutual use of experimental data and statistical/mathematical (ANOVA/Excel/Analysis ToolPak /Excel/Boolean Algebra) methods could be recommended as a good approach for the evaluation and comparison of the adaptive potential of genetically closely related genotypes.
2. New information revealing the contribution of HSP70B in recovery processes after the removal of stress factor (s) was obtained using experimental scheme 2.

#### **Contributions:**

1. A new approach was elaborated for the assessment of stress response and adaptive potential of cells/organisms based on genetically closely related genotypes.
2. Both Pro and HSP70B could be recommended as reliable markers to distinguish stress response and adaptive potential of genetically closely related genotypes.
3. Complex of biochemical/molecular markers (Pro, HSP70B, SOD activity, CAT activity) in a combination with mathematical methods could be recommended in order to obtain reliable information concerning adaptive potential of genotypes.