Impact of drought and high temperature on the expression of selected housekeeping genes in potato leaves

Anna Pawłowska[1], Dorota Michałowska[1], Anna Bilska-Kos[2], Dominika Boguszewska-Mańkowska[3], Krzysztof Treder[1]
[1]Division in Bonin, [2]Institute Headquarters in Radzików, [3]Division in Jadwisin

INTRODUCTION

Both high temperature and drought significantly affect gene expression in plants. However, even with slightly different genotypes, plants may differ in their response to stress. Potato cultivars provide an excellent model for studying such differences due to their clonal nature. The research aimed to select reference genes, i.e., whose expression level will not change in potato leaves under drought and high temperature. House-Keeping Genes (HKG) are most often used as references. HKG is theoretically expressed at the same level in every tissue type, regardless of external factors (biotic and abiotic). It is necessary to investigate which HKGs retain similar expression levels for every kind of stress and tissue. We use selected HKGs in subsequent studies to determine quantitative changes in the expression of potential marker genes for drought and high-temperature tolerance.

MATERIAL AND RESEARCH METHODS

Two potato cultivars were selected that differ significantly in their response to drought. The pair consisted of the drought-tolerant cv Gwiazda, and cv Oberon, which is very sensitive to water shortage. In vitro, plantlets were propagated from accessions stored at the Potato Gene Bank (IHAR-PIB, Division in Bonin) to prepare plants for experiments. The resulting *in vitro* plants were removed from tubes and planted in pots filled with peat-sand substrate supplemented with fertilizer. They were next transferred to the phytotron and grown for 14 days at the optimal conditions (21°C, humidity 80%, 16 hours of daylight, 8 hours of the night). After this acclimatization period, plants were subjected to soil drought and high-temperature stresses. The research was carried out in four combinations: (1) watered plants growing in optimal temperature conditions (21°C, control), (2) plants not watered, growing in optimal temperature conditions (drought), (3) plants watered and subjected to high temperature (38°C), and (4) plants not watered and subjected to high temperature. Plant material was collected the next day (after 12 h) and after 3, 6, 9, and 12 days from the third or fourth floor. Total RNA was isolated from the samples using the MagMAX™ Plant RNA Isolation Kit (ThermoFisher Scientific) on a robot KingFisher DuoPrime (ThermoFisher Scientific). The RNA quality and concentration were determined spectrophotometrically on an Epoch microplate spectrophotometer. RNA samples were transcribed into cDNA using the AccuScript HighFidlty 1st Strand cDNA Synthesis Kit (Agilent) by adding 300 ng of RNA to each reverse transcription reaction. The obtained cDNA was a template for real-time PCR reactions performed on a CFX96 Touch thermal cycler (BioRad) using the FastStart SYBR Green Master kit (Roche). The expression levels of ten mRNA-coding HKG genes were examined using primers published by other teams. The stress-responsive gene, used in this experiment as a positive stress control was RAB18. The expression level of this gene was measured in drought and high-temperature conditions using the newly designed primers and EF1α as a reference.

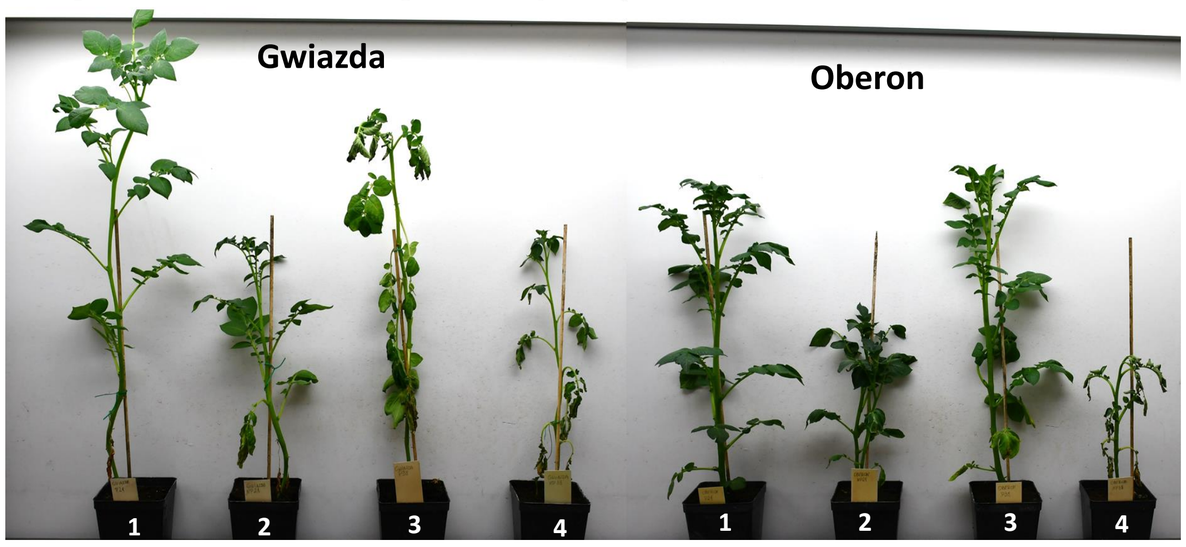

Fig.1. Plants of both cultivars after three days of the treatments. Watered plants at optimal temperaturę – 1, drought-stressed plants at optimal temperature – 2, watered plants at high temperature – 3, drought-stressed plants at high temperature - 4.

RESULTS

The range of Cq values for individual genes in control and stress conditions for both cultivars was very variable. The lowest variability was observed for elongation factor 1α (EF1α), adenyl phosphoribosyltransferase (APRT) and 18S RNA. The Cullin 3A gene (CUL3a) had the most variable range of Cq values. The range of this variability and its maximum and minimum values practically coincided with those observed for the stress-induced gene - rab18 (Fig. 2).

The RefFinder program was used to analyze the genes stability. This analysis indicated the genes encoding EF1α and APRT mRNA as the most stable (Fig. 3).

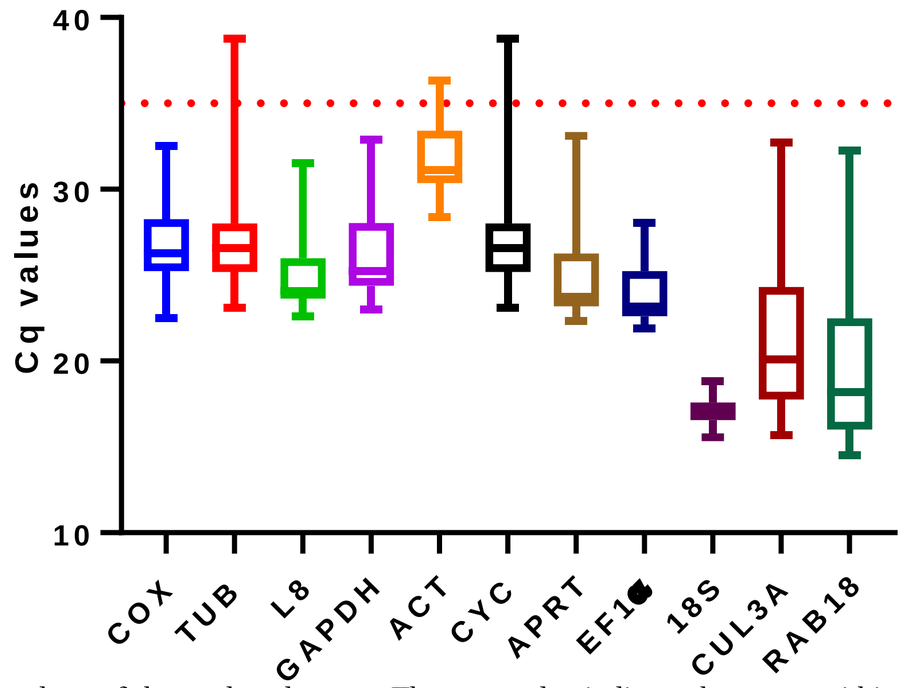

Fig.2. Cq values of the analyzed genes. The rectangles indicate the range within 95% of the Cq value for the given gene, the line in the bar represents the mean, and the error bars indicate the minimum and maximum values.

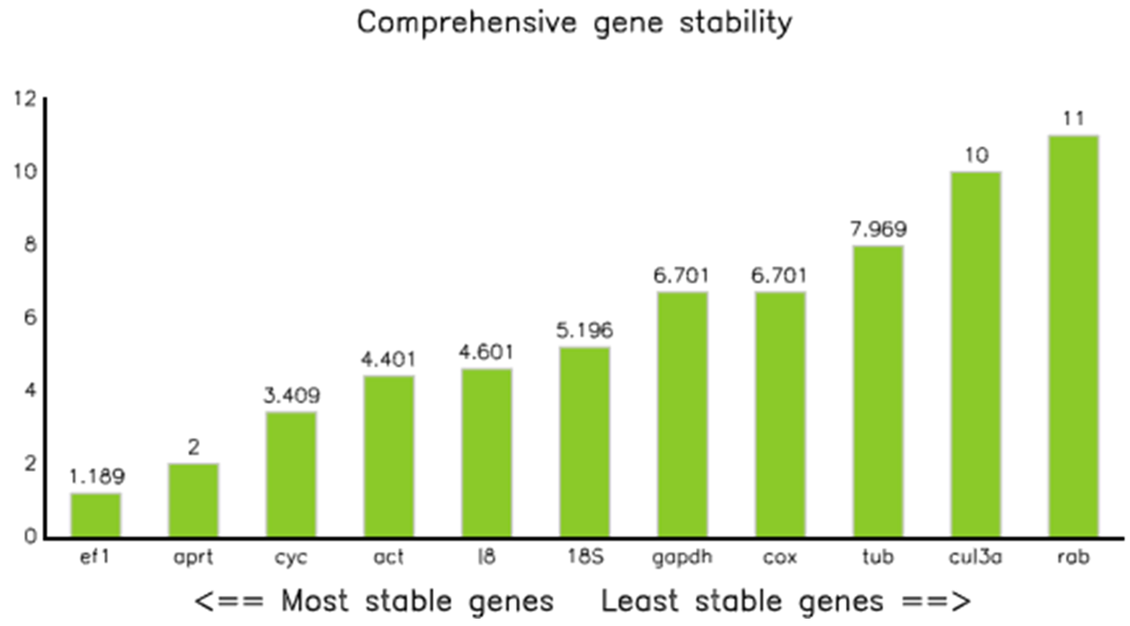

Fig.3. RefFinder comprehensive ranking of RGs according to employed algorithms for stability assessment. http://www.ciidirsinaloa.com.mx/RefFinder-master/

The RAB18 gene was selected as a positive control of the stress response because its expression increased under drought (Pieczynski et al. 2018. Plant Biotechnol J. 2018 Feb;16(2):603-614. doi: 10.1111/pbi.12800.). Here, we confirm this observation. In our experiment, RAB18, as expected, was strongly induced by drought (Fig. 4). Compared to the optimal temperature conditions, high temperature eliminated this response in cv. Gwiazda, and significantly reduced the RAB18 induction level in cv. Oberon. In the drought-tolerant Gwiazda, RAB18 expression was induced immediately after stress start, while the Oberon variety expressed this gene significantly later. The delayed response of the stress-sensitive cultivar compared to the resistant one fits into the generally accepted mechanism explaining differences in tolerance of different genotypes of plant species to stress by the differences in the response rate.

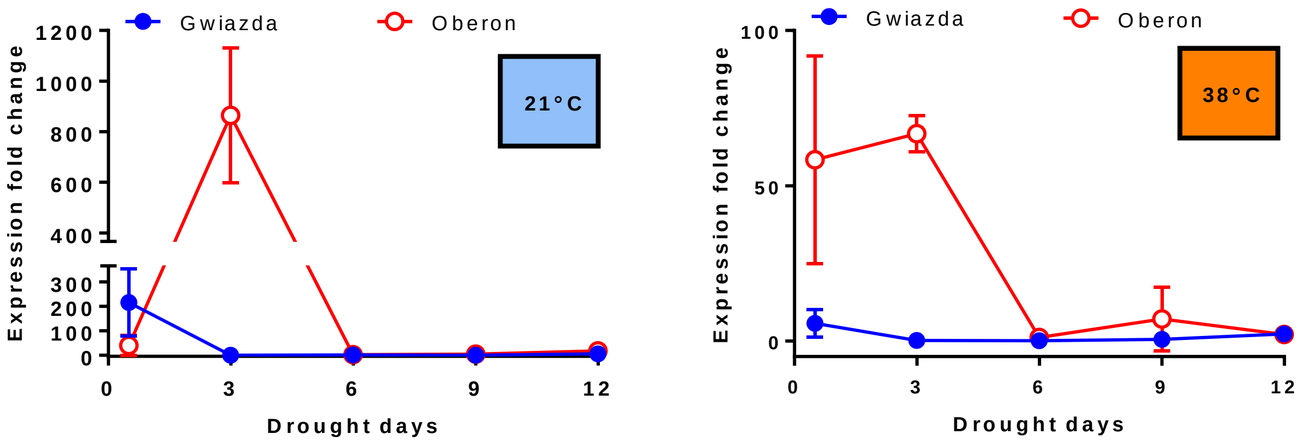

Fig.4. Impact of drought at optimal and high temperaturę on the xpression of RAB18 relative to reference gene EF1α

SUMMARY

- The optimal reference genes for normalizing the expression level of genes induced by drought and high-temperature stresses are the eF1α and APRT genes.
- Drought stress induces the expression of the RAB18 gene to a significantly greater extent than thermal stress in cv Oberon and eliminates induction in cv Gwiazda.
- The expression level of the RAB18 gene is generally higher in the cv Oberon, but its peak occurs later than in cv Gwiazda.

Anna Pawłowska, a.pawlowska@ihar.edu.pl
Plant Breeding and Acclimatization Institute – National Research Institute (IHAR-PIB)
https://ihar.edu.pl/pl/instytut/struktura/bonin
www.ziemniak-bonin.pl