Microsporogenesis and drought tolerance of wheat

Yuliya Yanushevska[a], Jolanta Groszyk[a], Anna Nadolska-Orczyk[b] and Wacław Orczyk[a]

[a] Department of Genetic Engineering, Plant Breeding and Acclimatization Institute – National Research Institute, Poland.
[b] Department of Functional Genomics, Plant Breeding and Acclimatization Institute – National Research Institute, Poland.
w.orczyk@ihar.edu.pl

Introduction – the goal of the project

Plant male reproductive development (microsporogenesis and microgametogenesis) is very sensitive to environmental factors. Even mild stresses such as drought or suboptimal temperatures applied during early phases of floret development irreversibly affect microsporogenesis and lead to pollen abortion. In self-pollinating and cleistogamic crops such as wheat and barley this significantly reduces grain number per spike and yield.

The target goal of the project is identification of molecular markers of wheat tolerance to abiotic stress during early phases of spike development.

The project consists of the following steps:

1. Defining plant developmental phases representing the early stages of microsporogenesis.
2. Defining drought timing and drought conditions leading to pollen abortion and reduced number of grains per spike.
3. Identification of wheat cultivars with contrasting reaction (the most tolerant *vs.* the most sensitive) to short drought stress applied during the sensitive stages of spike development.
4. Search for the candidate genes putatively involved in anther response to abiotic stress.

Introduction - plant developmental phases representing early stages of microsporogenesis

In order to identify specific stages of anther development, successive steps of microsporogenesis and microgametogenesis (Fig. 1) were correlated with auricle distance (AD) which was used as a scale to „measure" stages of spike development.

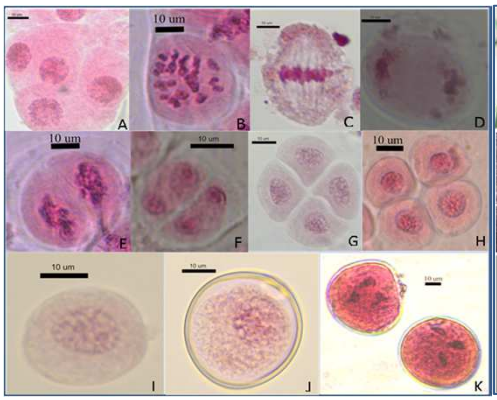


Figure 1. The subsequent steps of microsporogenesis, identified in wheat florets. Sporogenic tissue (A), meiosis (B – F), tetrads (G), young microspores (H – J), pollen grains (K).

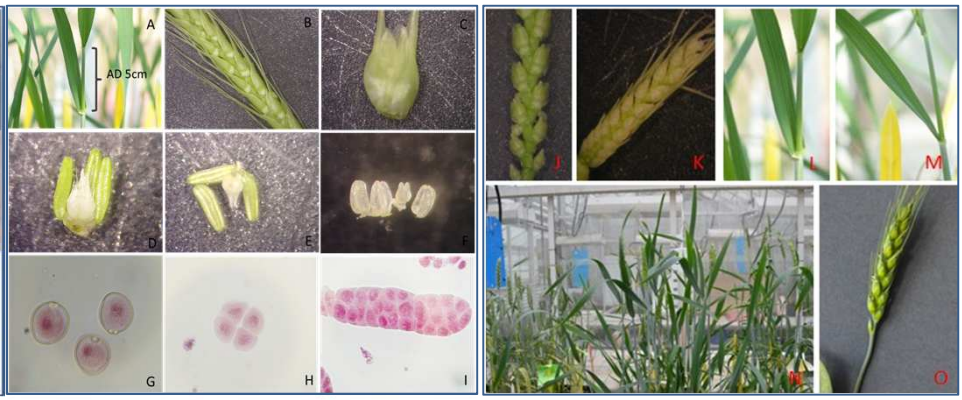


Figure 3. The picture of the wheat plant with AD 5cm (A), spike from the main stem (B), Spikelet collected from the central part of the spike (C), anthers from the 3rd class floret (D), anthers from the 2nd class floret (E), anthers from the 1st class floret (F), young microspores (G), tetrads (H), sporogenic tissue (I).
Spikes collected from different wheat cultivers AD 5cm (J - M). Plants after drought application (N, O).

Results - drought stress

The stages between late meiosis and young microspores are considered to be the most sensitive to abiotic stress (Fig. 4). According to this the drough stress was applied for plants with AD 5cm and was maintained for 5 days. Afterword the growth was continued in normal conditions (Fig. 5).

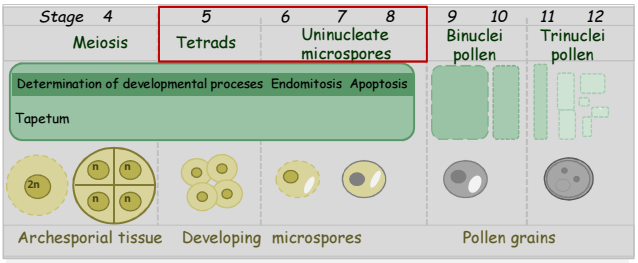


Figure 4. The scheme of the subsequent steps of microsporogenesis.
The most sensitive to abiotic stresses are the stages 5 through 8.

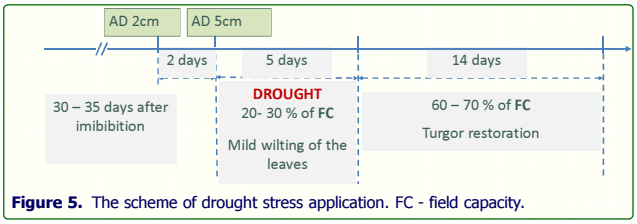


Figure 5. The scheme of drought stress application. FC - field capacity.

Analysis of the pollen viability and grain number per spike (Fig. 6) in plants subjected to drought allowed for selection of wheat cultivars with contrasting reactions for the stress.

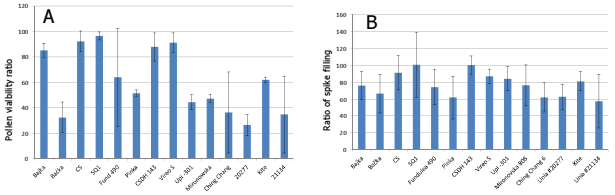
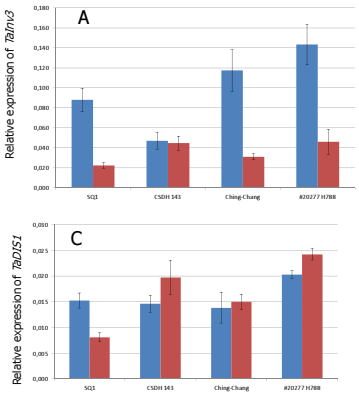


Figure 6. The ratio of pollen viability (A) and grain number per spike (B) in plants grown in normal *vs.* drought stress conditions.

The preliminary analysis of the candidate genes showed that the expression of the *TaInv3* but not *Tainv5* (Fig. 7A and 7B) and the expression of *TaDIS1* (Fig. 7C) a wheat orthologue of rice *OsDIS1* correlated with pollen viability and spike filling ratios of the tested cultivars.



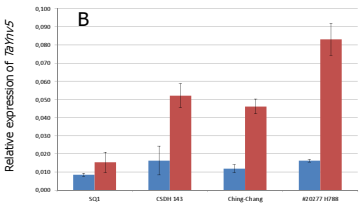


Figure 7. Relative expression of *TaInv3* (A), *Tainv5* (B) and *OsDIS1* (C) in anthers from control and drought stressed plants.

SQ1 and CSDH143 were selected as the most tolerant, Ching-Chang and #20277 H788 as the most sensitive.

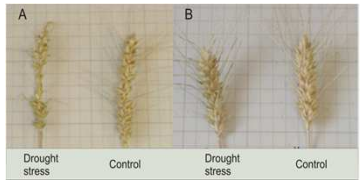


Figure 8. Spikes collected from drought sensitive (A) and tollerant (B) wheat grown in control and stress conditions.

Conclusions

1. Drought stress applied for 5 days during selected stages of microsporogenesis specifically affected the pollen viability and consequently the number of grains per spike.
2. The two genes encoding invertases i.e. apoplastic *TaInv3* and vacuolar *TaInv5* showed contrasting patterns of expressions indicating for a different roles in the reaction for druoght.
3. Repression of *TaInv3* and *TaDIS1* in the two lines which better tolerated stress indicates for a possible role in plant response for drought during early stages of microsporogenesis.

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