EVALUATION OF MEMBRANE STABILITY OF LEAF CELLS OF CYNODON (CYNODON DACTYLON) AND FOUR WHEAT GENOTYPES AFTER EXPOSURE TO ETHANOL

Petar Petrov[1], Ivan Dimitrov[2]

[1]Institute of Plant Physiology and Genetics – Bulgarian Academy of Sciences
e-mail: pivanovp@abv.bg
[2]Space Research and Technology Institute – Bulgarian Academy of Sciences
e-mail: IDimitrov@mail.space.bas.bg

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Abstract: *The stability of leaf cell membranes is one of the factors on which the resistance of agricultural crops to the abiotic factors of the environment depends. Ethanol, as a very good solvent of lipids and highly damaging to cell membranes, was used to test the membrane stability of leaf cells of cynodon (Cynodon dactylon) and four genotypes of wheat. The membranes of the cynodon leaf cells are the most resistant. A concentration of 15% ethanol was chosen as the most suitable for testing the wheat genotypes. Of the wheat genotypes, the leaf cells of Katya and Enola have the greatest membrane stability. The rates of damage to leaf cell membranes are constant in the cynodon and the Katya genotype, while in the other genotypes they increase exponentially.*

ОЦЕНКА НА СТАБИЛНОСТТА НА МЕМБРАНИТЕ НА ЛИСТНИТЕ КЛЕТКИ НА ТРОСКОТ (CYNODON DACTYLON) И ЧЕТИРИ ГЕНОТИПА НА ПШЕНИЦА СЛЕД ТРЕТИРАНЕ С ЕТАНОЛ

Петър Петров[1], Иван Димитров[2]

[1]Институт по физиология на растенията и генетика – Българска академия на науките
e-mail: pivanovp@abv.bg
[2]Институт за космически изследвания и технологии – Българска академия на науките
e-mail: IDimitrov@mail.space.bas.bg

Ключови думи: *пшеница, сухоустойчивост, студоустойчивост, стабилност на мембраната, йонна пропускливост, устойчивост на етанол, индекс на увреждане на листата*

Резюме: *Стабилността на мембраните на листните клетки е един от факторите, от които зависи устойчивостта на селскостопанските култури към абиотичните фактори на околната среда. Етанолът, като много добър разтворител на липиди и силно увреждащ клетъчните мембрани, беше използван за тестване на мембранната стабилност на листните клетки на троскот (Cynodon dactylon) и четири генотипа на пшеница. Най-устойчиви са мембраните на листните клетки на троскота. Концентрация от 15% етанол е избрана като най-подходяща за тестване на генотипите на пшеница. От генотипите на пшеницата листните клетки на Катя и Енола имат най-голяма устойчивост на мембраната. Темповете на увреждане на листните клетъчни мембрани са праволинейни при троскота и генотипа Катя, докато при останалите генотипи нарастват експоненциално.*

Introduction

Climatic changes require the selection of wheat genotypes resistant to the abiotic factors of the environment. The stability of cell membranes of leaf cells is key to the tolerance of wheat genotypes to abiotic stresses. Electrolyte leakage method provides an assessment of the stability of cell membranes, i.e. the greater the electrolyte leakage, the more damaged the cell membranes. This method helps in the selection of more cold tolerant (Yazdi-Samadi et al., 2006), more heat tolerant (Dias et al., 2010)

and more drought tolerant (Bajji, Mohammed et al., 2002; Ahmadizadeh et al., 2011; Petrov et al., 2018) wheat genotypes. The method is also used to assess salinity tolerance of wheat (Basra et al., 2005).

The organization of cell membranes is very complex. Lipids are a major component of membranes. Ingólfsson et al., 2014 proposed a model of 63 different types of lipids. The lipid bilayer environment surrounding membrane proteins strongly influences their structure and functions (Tero et al., 2017). The dynamically structured mosaic model proposed by Vereb et al. (2003) suggested a dynamic rearrangement of lipids and proteins through Brownian motion. According to Bhat et al. (2005) biological membranes are not a simple homogeneous layer of proteins and lipids, but rather are organized into discrete regions that can be characterized by different lipid and protein contents. These membrane microdomains are called "lipid rafts". Rafts in membranes are defined by the physical properties of the lipid bilayer and function by selectively partitioning membrane lipids and proteins into membrane domains with specific phase behavior and lipid packing (Harder, 2003).

Lipid solvents could damage the structure of cell membranes and disrupt their functioning. Ethanol, as a very good lipid solvent, has a toxic effect on membranes. Studies of the resistance of cell membranes to ethanol have been carried out in animal and human cells. The fluidity of cell membranes is important for their functioning and stability. One of the effects that ethanol induces on membranes is to alter their fluidity by altering the fluidity of membrane lipids. Alcohol treatment induces changes in rat synaptic membrane fluidity (Zerouga et al., 1992). Goral et al. (2008) suggested that ethanol may have dose-dependent effects on cell membrane fluidity in human cells. No studies are known to assess the tolerance of plant cell membranes to ethanol. Testing the membrane stability of agricultural crop cells with ethanol solutions, by conductometry with a simplified protocol, is performed in a short time as an express test applicable in breeding practice.

Materials and methods

Plant material and growing conditions

The cynodon leaves were plucked from plants growing under natural conditions. Leaves of wheat genotypes were plucked from plants grown for 21 days under controlled conditions. The four Bulgarian wheat (Triticumaestivum L.) varieties were used, of which 1 old historic varieties and 3 modern releases. The old varietie Slomer represent relatively homogeneous selection made within landraces, or early breeding releases. This germplasm is not cultivated anymore and is nearly extinct. Seed samples have been kindly provided by Dr. Andreas Börner, German Federal Genebank, Leibniz Institute of Plant Genetics. Seeds were soaked for 4 h in tap water and planted in 1-kg pots with alluvial meadow soil (pH 6.2). Plants were grown in a climatic chamber with 22/18 °C day/night temperature, 14-h photoperiod, irradiance of 250 lmol m_2 s_1 and 70 % relative humidity. Tap water was supplied daily sustaining 60 % of full soil moisture capacity.

Leaf injury index (LII) and electrolyte leakage kinetics

For determination of LII 15 leaf pieces (2 cm in length) were cut from plants. The solutions was measured with a conductometer Elwro 5721 (Poland). Finally, samples were boiled for 30 min, cooled at room temperature and conductivity was read again. LII was measured by a modification of the Premachandra formula (Premachandra et al., 1992). The original formula is:

$$I\% = \left[\frac{1 - \left(1 - \frac{t1}{t2}\right)}{1 - \frac{c1}{c2}} \right] \times 100 \quad (1)$$

where t1 and t2 are the first and second (after boiling) conductivity measurements of the solutions in which they are treated samples were immersed and c1 and c2 are the corresponding values of the controls. In our case, t2 = c2, since leaves from untreated plants were used for the experiments. For c1, the conductivity values of ethanol solutions with distilled water, which are close to the values of distilled water, are taken.

Statistical analysis

Two independent experiments were conducted and parameters were measured in at least 3 replications. Data are presented as mean values ± SE

Results

LII of the cynodon (Cynodon dactylon) and of the wheat genotype with low drought tolerance (Slomer) treated with a series of ethanol concentrations. At concentrations of 5 and 10% of the cynodon leaves, no significant electrolyte leakage was recorded until the end of the experiment (Fig. 1).

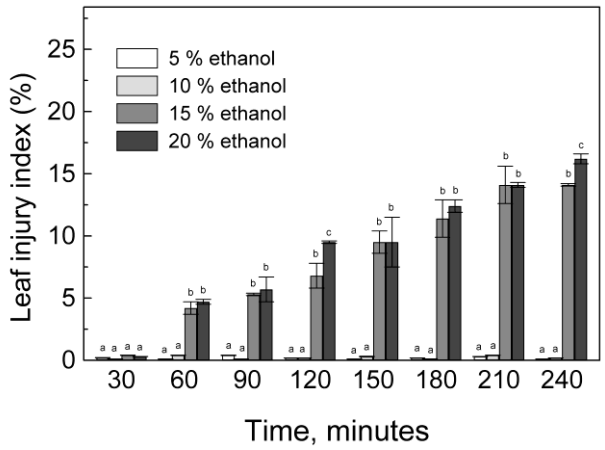

Fig. 1. Leaf injury index of cynodon (Cynodon dactylon) treated with ethanol solutions

At the higher concentrations (15 and 20 %) from the 60th to the 240th minute, a gradual increase in electrolyte leakage up to about 15% was reported (Fig. 1). After the 120th minute, the lowest concentration of ethanol that causes an increase in the electrolyte leakage from the membranes of the leaf cells of the Slomer wheat genotype is 5% (Fig. 2). At the higher concentrations of ethanol, the electrolyte leakage increased from the 30th minute and reached about 20% at the 240th minute at all three concentrations (Fig. 2).

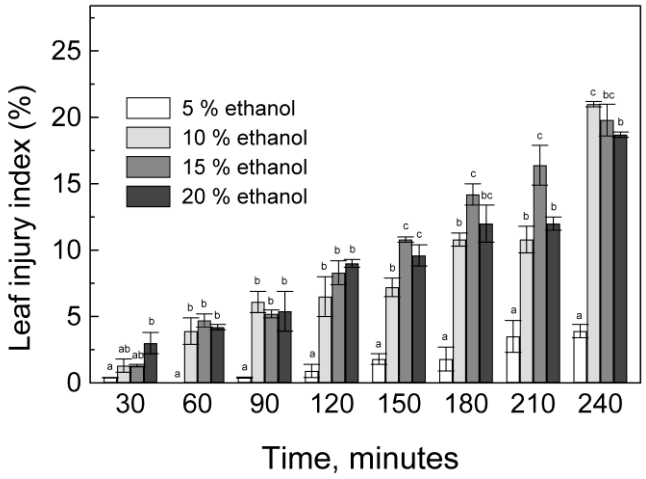

Fig. 2. Leaf injury index of the leaves of the Slomer wheat genotype treated with ethanol solutions

LII of 4 wheat genotypes treated with 15% ethanol.

By the 90th minute, electrolyte leakage increased to the same extent in all four genotypes. After the 120th hour, electrolyte leakage has higher values in the Slomer and Prelom genotypes (Fig. 3). At the end of the experiment, the electrolyte leakage was the highest in the Prelom genotype (Fig. 3). The electrolyte leakage of the Slomer genotype has a lower value (Fig. 3). The electrolyte leakage is the lowest in the Katya and Enola genotypes (Fig. 3).

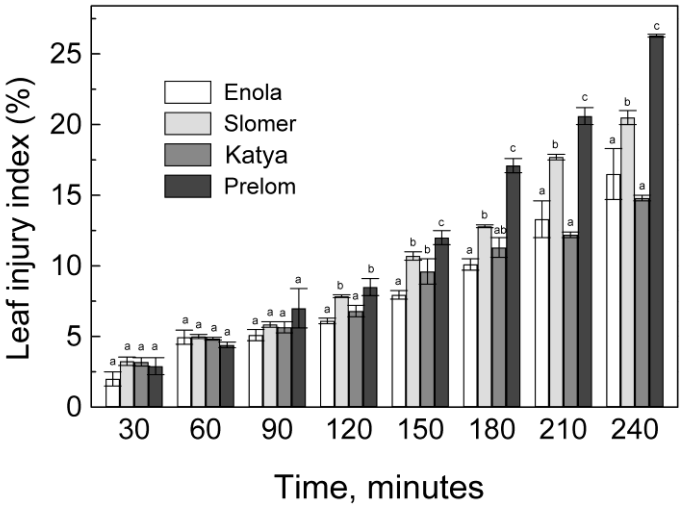

Fig. 3. Leaf injury index of 4 wheat genotypes treated with 15% ethanol solutions

Rates of damage to leaf cell membranes

The rates of damage to the cell membranes of cynodon leaves treated with 15 and 20% ethanol solutions were constant (Fig. 4).

The rates of damage to the leaf cell membranes of the Enola, Slomer and Prelom genotypes treated in 15% increased exponentially (Fig. 4). In the Katya genotype, the rates of leaf cell membrane damage are again constant (Fig. 4).

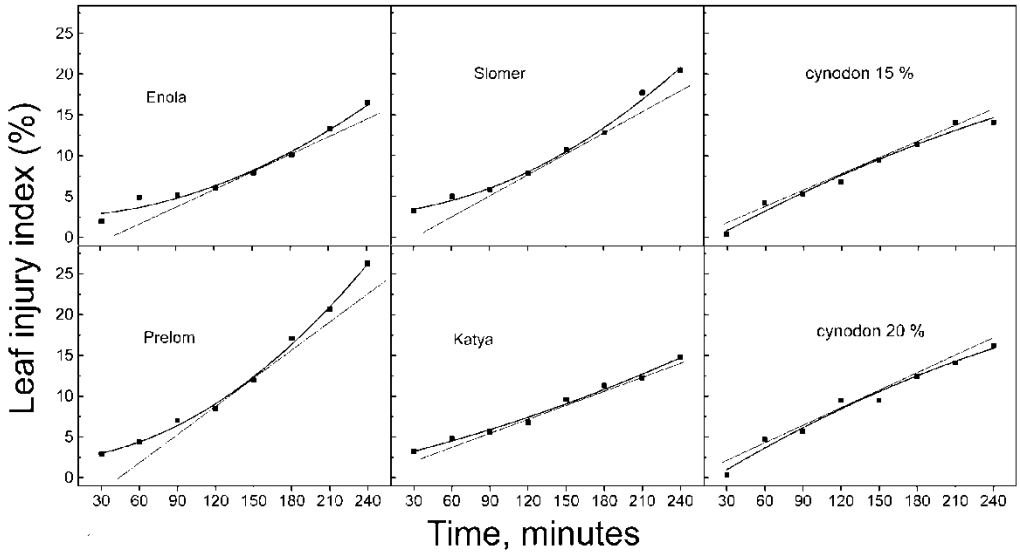

Fig. 4. Rates of damage to cell membranes of leaves of 4 wheat genotypes treated with 15% ethanol solution and cell membranes of cynodon (Cynodon dactylon) leaves treated with 15 and 20% ethanol solutions

Discussion

LII of cynodon to ethanol

The minimum concentration of ethanol at which significant damage to the cynodon cell membranes is observed is 15% (Fig. 1), while it is 10% for the Slomer wheat genotype (Fig. 2). As a rule, wild plants are more viable than cultivated ones, which was also observed in our research. These results confirm the workability of our approach.

Optimization of ethanol concentration for LII testing of wheat genotypes

For the tests, a concentration of ethanol was chosen that was not too low (causing no or little damage to the membranes) or too high (causing strong and equal damage to the membranes of all genotypes). Slomer is a wheat genotype of old Bulgarian selection. Our previous research showed that the genotype has a low degree of drought resistance and labile membranes of leaf cells (Petrov et al.,

2018). Slommer leaves were treated with a series of ethanol concentrations in order to select an appropriate concentration to compare the LII of wheat genotypes (Fig. 2). At the end of the experiment, at all three concentrations (10, 15 and 20%), the damage to the leaf cell membranes was significant (about 20% - Fig. 2). The lowest concentration at which there is significant damage to cell membranes is 10% (Fig. 2). Taking into account the sensitivity of the Slomer genotype and the lack of response of the cynodon to an alcohol concentration of 10% (Fig. 1), the next strongest concentration - 15% - was chosen to compare the membrane stability of the wheat genotypes. An approach similar to ours (via ethanol solutions) has been used to study the ethanol resistance of brewer's yeast (Jones et al., 1987). According to research by Pratt et al. (2003) 10% ethanol inhibited yeast growth and 20% also inhibited their fermentation capacity. The concentrations of ethanol that affect the life processes of the yeast (between 10 and 20%) are same to the test concentration we used (15%).

LII of 4 wheat genotypes treated with 15% ethanol solution

Differences in electrolyte leakage between genotypes were clearly evident at 210 minutes and persisted at 240 minutes (Fig. 3). The maintenance of differences between genotypes in the last two measurements indicates that a treatment period of 4 hours is sufficient to obtain stable results. The average level of electrolyte leakage at the end of the experiment (about 20 %) indicates a strong but not complete damage to the membranes of the leaf cells and allows the achievement of contrasting differences between the genotypes in terms of their membrane stability (Fig. 3). Genotype Slomer is an old selection, while genotypes Enola, Prelom and Katya are new selection wheat.

The Katya genotype known for its drought resistance (Vassileva et al., 2011; Vassileva et al., 2009; Petrov et al., 2018; Doneva et al., 2021; Kocheva et al., 2013) is expected to have stable membranes. Our studies confirm the membrane resistance of this genotype, i.e. the degree of damage to its membranes after exposure to ethanol is weak (Fig. 3). Unexpectedly, ethanol damaged the leaf cell membranes of the new selection genotype Prelom to a significantly greater extent than the leaf cell membranes of the drought-sensitive genotype old selection Slomer (Fig. 3).

Rates of damage to leaf cell membranes

The exponential increase in the rate of leaf cell membrane damage in the Prelom, Slomer and Enola genotypes indicates that ethanol causes a time-progressive destruction of the leaf cell membranes largely beyond the control of the cellular mechanisms for maintaining membrane stability (Fig. 4). The constant rate of electrolyte leakage from the leaf cells of the cynodon after exposure to ethanol (with concentrations of 15 and 20% - Fig. 4) leads to the assumption that the cellular mechanisms for maintaining membrane stability work significantly more efficiently in it. The constant rates of electrolyte leakage after exposure to ethanol and in the Katya genotype suggest that cellular mechanisms maintain the stability of cell membranes in it with an efficiency close to that of the cynodon.

Conclusion

LII of cynodon and 4 wheat genotypes was evaluated by conductometric study with 4 hourly experiments. 15% ethanol was chosen as the test concentration. The leaf cells of the cynodon have the most resistant membranes. Among the wheat genotypes with the highest resistance are the membranes of the leaf cells of the Katya and Enola genotypes. The approach we used allows with a simple protocol and in a short time to successfully compare the membrane resistance of the leaf cells of the wheat genotypes.

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